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Original article

Antiaging phenotype in skeletal muscle after endurance exercise is associated with the oxidative phosphorylation pathway

Wasin Laohavinij[a], Apiwat Mutirangura[b,c]
[a]*Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*
[b]*Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*
[c]*Center of Excellence in Molecular Genetics of Cancer and Human Diseases, Chulalongkorn University, Bangkok 10330, Thailand*

Background: Performing regular exercise may be beneficial to delay aging. During aging, numerous biochemical and molecular changes occur in cells, including increased DNA instability, epigenetic alterations, cell-signaling disruptions, decreased protein synthesis, reduced adenosine triphosphate (ATP) production capacity, and diminished oxidative phosphorylation.
Objectives: To identify the types of exercise and the molecular mechanisms associated with antiaging phenotypes by comparing the profiles of gene expression in skeletal muscle after various types of exercise and aging.
Methods: We used bioinformatics data from skeletal muscles reported in the Gene Expression Omnibus repository and used Connection Up- and Down-Regulation Expression Analysis of Microarrays to identify genes significant in antiaging. The significant genes were mapped to molecular pathways and reviewed for their molecular functions, and their associations with molecular and cellular phenotypes using the Database for Annotation, Visualization and Integrated Discovery and Kyoto Encyclopedia of Genes and Genomes informatics resources, and GeneCards databases, respectively.
Results: The results showed that endurance exercise has an antiaging potential (Pearson χ^2, $P < 0.01$) by upregulating genes coding for components of the oxidative phosphorylation pathway (Benjamini false discovery rate $Q < 0.05$). We found that numerous genes coding for components of other pathways were also upregulated (Pearson χ^2 $P < 0.01$) as a chronic adaptation to endurance exercise, including *ATP5C1*, involved in ATP synthesis, *CYCS*, involved in electron transfer in the mitochondrial respiratory chain, and *GSTK1*, involved in cellular detoxification.
Conclusions: Endurance exercise may be the best type to promote antiaging phenotypes by increasing mitochondrial biogenesis and ATP production capacity.

Keywords: Aging, endurance exercise, mitochondrial biogenesis, mitochondrial dysfunction, oxidative phosphorylation, skeletal muscle

Abbreviations

ATP = adenosine triphosphate
CU-DREAM = Connection Up- and Down-Regulation Expression Analysis of Microarrays
DAVID = Database for Annotation, Visualization and Integrated Discovery
GEO = NCBI Gene Expression Omnibus repository
GeneCards = A human gene database maintained by the Crown Human Genome Center at the Weizmann Institute of Science
GSE = GEO Series record
GSM = GEO Sample number record
KEGG = Kyoto Encyclopedia of Genes and Genomes
OXPHOS = Oxidative phosphorylation
NADH = nicotinamide adenine dinucleotide reduced form

Correspondence to: Apiwat Mutirangura, Center of Excellence in Molecular Genetics of Cancer and Human Diseases, Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. E-mail: mapiwat@chula.ac.th

Physical activity can be described as a situation in which the skeletal muscles are used for any purpose that results in an increase in energy expenditure compared with the resting state [1]. Long-term physical activity is associated with a reduction of the morbidity and mortality rates in humans [2, 3]. Numerous meta-analyses have shown that regular

physical activity has a significant benefit by reducing the risk of mortality from all causes and from cardiovascular causes by 33% and 35% respectively in people without cardiovascular disease [4]; people with cardiovascular disease received similar benefits from regular physical activity [5, 6].

Exercise is a well-planned, structured, and repetitive activity that has the primary objective of improving or maintaining health and fitness, and is one of the most well-known and best-studied types of physical activity. Exercise is used in numerous guidelines for the primary and secondary prevention of numerous diseases, such as cardiovascular disease, diabetes, sarcopenia, dementia, osteoporosis, and some types of cancer [5-15]. It can be divided into 2 main types, aerobic and anaerobic, based on energy metabolism. Aerobic or endurance exercise is based on improving the cardiorespiratory system by maintaining the intensity level of activity in a low to moderate range for an extended period of time, using oxygen as a primary energy source [16]. By contrast with aerobic exercise, anaerobic exercise induces muscle contractions in a limited amount of time, stimulating a greater intensity of activity. The primary energy sources for this metabolism are the high-energy phosphates adenosine triphosphate (ATP) and creatine phosphate (CP), and anaerobic glycolysis, which generates lactic acid [16].

Aging is a degenerative physiological process influenced by many factors that can be categorized into 2 groups, intrinsic (genetic factors) and extrinsic (environmental and psychosocial factors), which affect numerous organ systems [1, 17]. A decrease in cardiorespiratory fitness, age-associated cognitive impairment, muscle and flexibility loss, reduction of stem-cell maintenance and proliferation, hormonal dysregulation, osteoporosis, depression, dementia, sarcopenia, diabetes, and cancers are some examples of physiological and pathological changes that occur during aging [5-13,17-20]. Although the consequences of aging have been identified in terms of pathologies and physiologies, the overall mechanisms leading to this event remain unknown. Some hypothesize that genomic instability, epigenetic alterations, errors in proteostasis, telomere shortening, and mitochondrial dysfunction are characteristics of aging [17-25]. Mitochondrial dysfunction is a significant process of aging because of the reduced biogenesis of new mitochondria and clearance of defective mitochondria, combined with the mutation and deletion of mitochondrial DNA (mtDNA) because of ineffective repair mechanisms. This results in the impairment of oxidative phosphorylation that is the primary energy-generating metabolic process in cells, the disruption of cellular signaling and interorganelle crosstalk at the interface between the outer mitochondrial membrane and the endoplasmic reticulum, and the initiation of inflammatory processes and activation of inflammasomes [17-22].

In an effort to slow the aging process, various interventions have been used. One of the most promising interventions is regular physical activity combined with a healthy diet and psychosocial well-being, as a holistic approach to maintaining a healthy lifestyle [1]. Although the physical appearances and benefits of each type of exercise have been shown in numerous sources, the similarities and differences in the molecular phenotypes among them remain unknown. The purpose of this study was to identify the transcriptome of the exercises that have the potential to delay aging using publicly available data obtained from online sources to screen for genes that are associated with aging and exercise. In particular, we extracted expression profiles from the Gene Expression Omnibus repository (GEO datasets; http://www.ncbi.nlm.nih.gov/gds/) [26-28]. The Connection Up- and Down-Regulation Expression Analysis of Microarrays (CU-DREAM) software package (http://pioneer.netserv.chula.ac.th/~achatcha/cu-dream/) [29] and the Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/) [30, 31] were used to conduct various statistical tests, including Student *t*, Pearson χ^2, and Benjamini false discovery rate (FDR) tests, to identify the genes and the molecular pathways associated with the aging process and various types of exercise. The official names of significant genes and their molecular functions, and their molecular and cellular phenotypes, were evaluated by GeneCards, a human gene database maintained by the Crown Human Genome Center at the Weizmann Institute of Science (http://www.genecards.org/).

Methods

Overall methodical framework is shown in **Figure 1**.

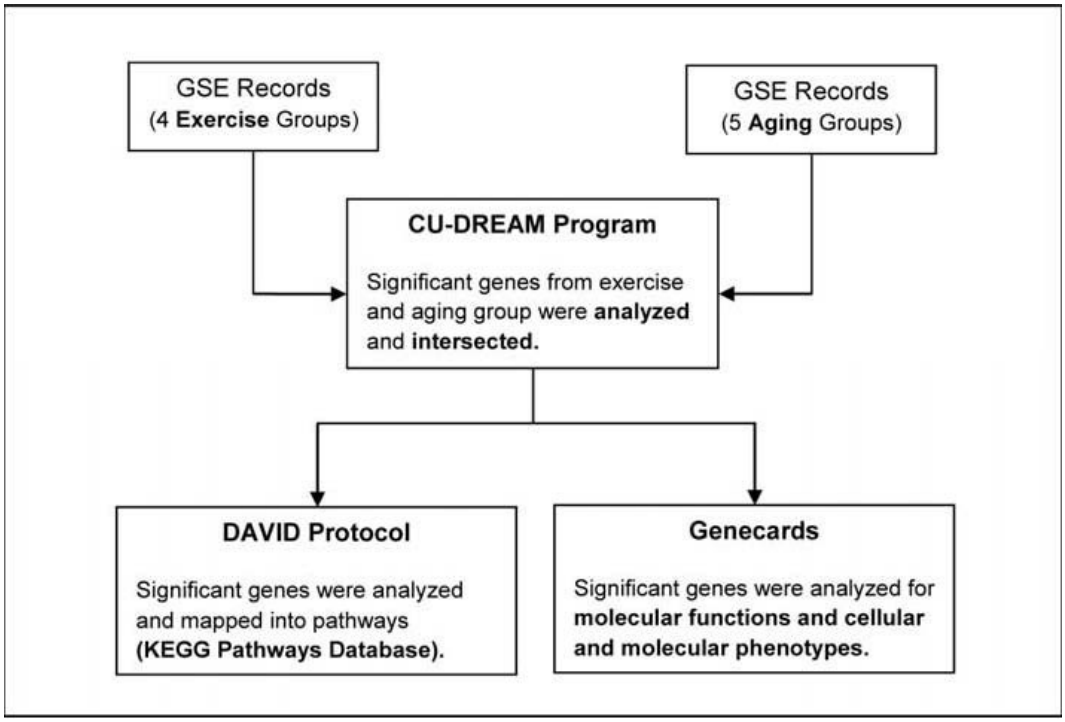

Figure 1. The overall methodological framework for the present study. Significant genes common to the exercise and aging groups were analyzed.
CU-DREAM = Connection Up-and Down-Regulation Expression Analysis of Microarrays, DAVID = Database for Annotation, Visualization and Integrated Discovery, GSE = Gene Expression Omnibus Series record, KEGG = Kyoto Encyclopedia of Genes and Genomes

Data collection and template preparation

Endurance exercise and physical activity groups. The keywords "endurance", "aerobic", "physical activity", and "skeletal muscle" were used to identify the expression profiles from microarray experiments that were related to the topics of interest and were published between July 2007 and April 2013. All the supplementary information, including series matrix files and related platforms, that was freely available from the Gene Expression Omnibus repository (GEO datasets; http://www.ncbi.nlm.nih.gov/gds) [26-28] was downloaded. Subsequently, all of the GEO Sample numbers (GSMs) were extracted for template preparation. In the template preparation process, the "control" samples included the samples labeled "sedentary" and "inactive", while the "experimental" samples included the samples labeled "trained" and "physically active." The threshold parameter was set to a significance level of 0.01 for each regulation.

Resistance exercise and power training groups.

The keywords "resistance" and "skeletal muscle" were used to identify the expression profiles from microarray experiments that were related to the topics of interest and were published between July 2007 and April 2013. All the supplementary information, including series matrix files and related platforms, that was freely available from the GEO datasets [26-28] was downloaded. Subsequently, all of the GSMs were extracted for template preparation. In the template preparation process, the "control" samples included the samples labeled "before resistance exercise" and "before power training", while the "experimental" samples included the samples labeled "after resistance exercise" and "after power training." The threshold parameter was set to a significance level of 0.01 for each regulation.

Four Gene Expression Omnibus Series (GSE) records (115 expression microarrays) were collected for 4 different types of exercises, and 5 GSE records (150 expression microarrays) were collected for the aging group. These data were used for analysis by the CU-DREAM software program. The program analyzed each exercise experimental group to identify which gene expression was significantly up- or downregulated in exercise populations compared with sedentary populations. We also used the program to analyze the aging experimental group to identify which genes were significantly up- or downregulated compared with the younger populations. The significant genes from each analysis were cross-referenced with

each other and categorized into 4 groups: exercise–aging up–up, up–down, down–up, and down–down regulations. After obtaining cross-referenced genes from the CU-DREAM experiments, we categorized the genes into groups according to their exercise type and then mapped the significant pathways from the cross-referenced genes in each GSE record using DAVID and selecting the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways informatics resources. We also analyzed the molecular and cellular phenotypes and specific functions of each gene using GeneCards.

Aging group. The keywords "aging" and "skeletal muscle" were used to identify the expression profiles from microarray experiments published between August 2002 and April 2013 that were related to the topics of interest. All the supplementary information, including series matrix files and related platforms, that were freely available from the Gene Expression Omnibus repository (GEO datasets; http://www.ncbi.nlm.nih.gov/gds) [26-28] were downloaded. Subsequently, all of the GSMs were extracted for template preparation. In the template preparation process, the "control" samples included the samples labeled "young," while the "experimental" samples included the samples labeled "old." The threshold parameter was set to a significance level of 0.01 for each regulation.

Statistical analysis

First, the total RNA levels in the experimental and control samples from the exercise and aging groups were evaluated. Using the prepared templates from the microarrays, series matrix files and platforms, a Student *t* test was performed for each related gene to compare the means of the control and experimental groups for each examined experiment. Each gene was then determined to be downregulated, upregulated, or unchanged, based on the obtained *P*-value. Subsequently, the assessed genes common to the exercise and aging groups were identified. Genes were classified into 4 groups, A through D. The genes common the exercise and aging groups, which were significantly up- or downregulated (depending on the experiment), were included in group A. The remaining significant exercise-group genes were included in group B, while the remaining significant aging-group genes were included in group C. The remaining genes (nonsignificant genes of the exercise and aging groups) were included in group D. The values for the odds ratios (OR), *P*s, and the lower and upper 95% confidence intervals (CIs) of the genes in groups A through D were arranged in an Microsoft Excel format. All of the statistical analyses were performed using the CU-DREAM program (http://pioneer.netserv.chula.ac.th/~achatcha/cu-dream/) [29]. Subsequently, the significant genes (Group A) for each GSE from each exercise type, which showed a significant delay or promotion of the aging process, through downregulation of aging phenotype, were used for further molecular pathway analysis. Using the prepared gene lists, numerous molecular pathways were identified using DAVID (http://david.abcc.ncifcrf.gov/) [30, 31] and selected using the KEGG pathway database. A Pearson χ^2 test and the Benjamini FDR tests were used to determine whether the pathways were dependent on the presence of the gene lists.

Data analysis

The results of the correlations between the exercise and the aging experiments included the number of genes in groups A through D, and the ORs, 95% CIs, and *P*s, which were organized by the direction of regulation. The assessed experiments were then grouped according to their correlation and exercise types. The molecular pathways and the significant genes that affect the aging process were reviewed. The official names of the identified genes and their functions were evaluated.

Results

Datasets from GEO were screened for expression in exercise and aging microarrays published through April 2013. The exercise microarrays from GEO included GSE8479 for resistance exercise [11], GSE9103 for endurance exercise [32], GSE16907 for power training [33], and GSE20319 for physical activity [34]; the aging microarrays from GEO included GSE80 [35], GSE1428 [36], GSE8479 [11], GSE9103 [32], and GSE38718 [37]. These datasets were chosen because endurance exercise and physical activity represent aerobic exercises, by contrast with resistance exercise and power training, which represent anaerobic exercises. Details of the available demographic data for these GSEs are shown in **Table 1**.

Table 1. Demographic data for the exercise and aging groups

Exercise group GSE		**8479**[a]	**9103**[b]	**16907**[a]	**20319**[b]	**Aging group GSE**				
Type		**Resistance exercise**	**Endurance exercise**	**Power training**	**Physically active**					
Duration of exercise		**6 months**	**4 years**	**12 months**	**32 years**	**80**	**1428**	**8479**	**9103**	**38718**
Experimental group[c]	Samples (n)	14	20	8	10	19	12	25	10	8
	Age (y)	69.6 ± 9.4	19–76	50–57	50–74	68.6 ± 8.4	70–80	70.5 ± 13.5	65.1 ± 7.3	65–76
Control group[d]	Samples (n)	25	20	8	10	16	10	26	10	14
	Age (y)	70.5 ± 13.5	18–72	50–57	50–74	24.5 ± 6.5	19–25	21.2 ± 6.8	22.9 ± 4.1	19–28
Sex		Mixed	Mixed	Female	Mixed	Male	Male	Mixed	Mixed	Mixed
Muscle biopsy		Vastus lateralis	Vastus lateralis	Vastus lateralis	Vastus lateralis	Vastus lateralis	Vastus lateralis	Vastus lateralis	Vastus lateralis	Biceps brachii
Extracted molecule		Total RNA	Total RNA	Total RNA	Total RNA	Total RNA	Total RNA	Total RNA	Total RNA	Total RNA
Organization name		Buck Institute for Research on Aging USA	Mayo Clinic and Foundation USA	University of Jyv[a]skyl[a] Finland	University of Jyv[a]skyl[a] Finland	University of Rochester USA	Boston University USA	Buck Institute for Research on Aging USA	Mayo Clinic and Foundation USA	University of Michigan USA

[a]Experimental results were measured before and after training from the same individuals for a specific duration.
[b]Experimental results were measured from 2 defined sample groups (active/inactive).
[c]Experimental groups are the active or after-training groups in exercise GSEs and are the old age groups in the aging GSEs.
[d]Control groups are the sedentary or before-training groups in exercise GSEs and are the young age groups in the aging GSEs.

Comparison of gene expression profiles between exercise types and the aging of muscles

In this study, we screened numerous microarray data from exercise and aging datasets provided in the GEO database. The data were analyzed using CU-DREAM to identify correlations between each type of exercise and the aging process. The analysis found 15 experiments with significant ($P < 0.01$) CU-DREAM results, comprised of 5 GSEs with up–down correlation, 6 with down–up correlation, and 4 with down–down correlation between exercise and aging. These results showed that endurance exercise is the type with the most potential to at least partially delay some specific molecular and physical phenotypes of aging. All of the GSEs comparing endurance exercise and aging in up–down correlation were significant (5/5) (**Figure 2A**). The ranges for the ORs and *P*s for each experiment were from 1.58 to 3.17 and 1.34×10^{-22} to 3.78×10^{-4}, respectively (**Supplementary Table S1A**). Furthermore, we also found that 370 genes were significantly up- and downregulated ($P < 0.01$) in the endurance and aging groups, respectively. In particular, there were 2, 6, 23, and 66 genes in common in 5, 4, 3, and 2 experiments, respectively (**Supplementary Table S2A**), which may represent the key factors in the antiaging processes. Additionally, we found that resistance exercise was also significant in 4/5 GSEs suggesting that it could delay the aging process; however, it gave the opposite correlation to endurance exercise, with a down–up correlation between resistance exercise and aging (**Figure 2B**). The ranges for the ORs and *P* for each experiment were 1.50 to 5.65 and 1.81×10^{-103} to 1.81×10^{-3}, respectively (**Supplementary Table S1B**). There are 1,208 genes that were significantly down- and upregulated in resistance exercise and aging groups, respectively, and there were 1, 12, and 134 genes in common in 4, 3, and 2 experiments, respectively (**Supplementary Table S2B**). Although each type of exercise showed specific adaptations for delaying aging by regulating the expression of numerous genes as chronic adaptations to exercises that result in some specific physical phenotypes, we also found that resistance exercise downregulated numerous genes that were recognized as having a role in the aging process. Our experiments showed that there were 3 experiments that showed a significant down–down correlation between resistance exercise and aging (3/5) (**Figure 3B**). The ranges for the ORs and *P*s between each experiment were 1.93 to 3.63 and 1.24×10^{-67} to 1.72×10^{-5}, respectively (**Supplementary Table S1C**). Furthermore, we found that there were 1,381 genes with significant changes in expression, which shared 8 and 165 genes in common in 3 and 2 experiments, respectively (**Supplementary Table S2C**). However, these results cannot be used to conclude that resistance exercise can promote aging because of the limited data and further experiments needed to support this hypothesis. Moreover, there were no results from other types of exercise that supported the promotion of the aging process by exercise.

Molecular pathways of commonly regulated genes between endurance exercise and aging

Because CU-DREAM showed only all experiments (5/5) comparing endurance exercise and aging to have a significant up–down correlation, further studies of the molecular pathways associated with the antiaging processes associated with endurance exercise were conducted using the DAVID/KEGG database. In the experiments, 6 molecular pathways were upregulated ($P < 0.05$) (**Table 2**).

Oxidative phosphorylation, which had 43 genes coding for pathway components upregulated after endurance exercise, was the pathway most significantly associated with downregulation of genes coding for aging phenotypes, found in 5/5 GSEs. The *P* values for each experiment ranged from 8.8×10^{-44} to 1.6×10^{-2}. Furthermore, cardiac muscle contraction pathway also showed specificity, with significance in 3/5 GSEs (60%), and 14 genes from endurance exercise were involved in this pathway. The *P* values for each experiment ranged from 3.8×10^{-10} to 2.6×10^{-5}. Other molecular pathways were significant in 1 GSE, including the citrate cycle, propanoate metabolism, pyruvate metabolism, and arginine, and proline metabolism.

Significant genes responsible for the antiaging phenotype

We reviewed the genes significant in at least 3 of the 5 GSEs in the endurance-exercise–aging correlation from CU-DREAM for their molecular functions and their associations with molecular and cellular phenotypes using GeneCards. We divided the lists of significant genes into 2 groups, the genes associated with the DAVID/KEGG oxidative phosphorylation (OXPHOS) pathway (**Table 3**) and the remainder (**Table 4**).

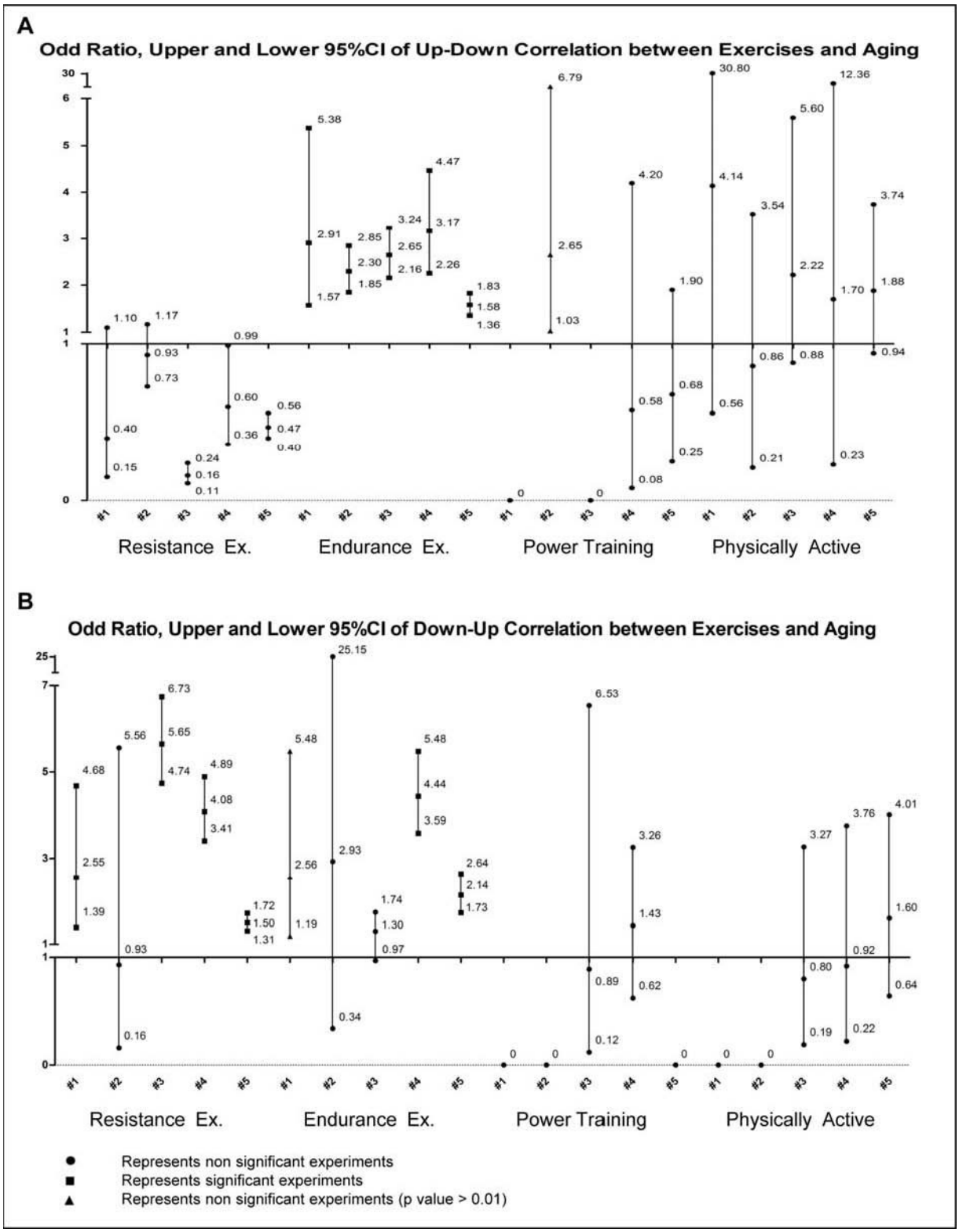

Figure 2. Connection Up- and Down-Regulation Expression Analysis of Microarray (CU-DREAM) results: inverse correlation between exercise and aging. **A:** ORs and 95% CIs for the up–down correlation between exercise and aging. **B:** ORs and 95% CIs for the down–up correlation between exercise and aging.

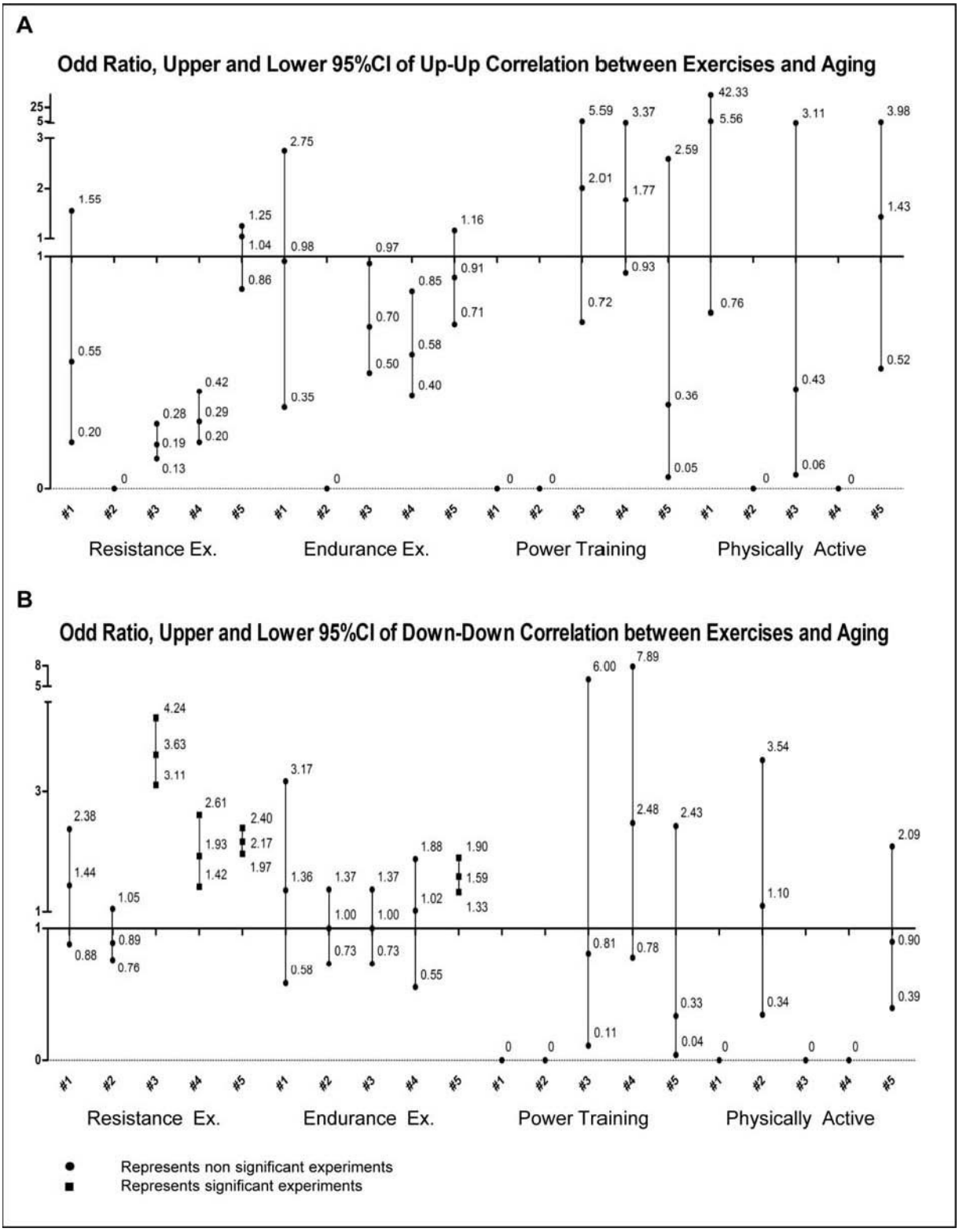

Figure 3. Connection Up-and Down-Regulation Expression Analysis of Microarray (CU-DREAM) results: correlation between exercise and aging. **A:** ORs and 95% CIs for the up–up correlation between exercise and aging. **B:** ORs and 95% CIs for the down–down correlation between exercise and aging. Ex = exercise

Table 2. DAVID/KEGG pathway results for endurance exercise.

Significant molecular pathways	*Q* range	No. of GSEs in common	No. of genes	Lists of genes associated with the pathways
Oxidative phosphorylation	8.8×10^{-44} -1.6×10^{-2}	5/5	43	*ATP5B, ATP5C1, ATP5E, ATP5F1, ATP5G1, ATP5G3, ATP5H, ATP5J, ATP5L, ATP5O, COX4I1, COX5A, COX5B, COX6B1, COX6C, COX7B, COX7C, COX8A, CYC1, NDUFA10, NDUFA4, NDUFA6, NDUFA8, NDUFA9, NDUFAB1, NDUFB1, NDUFB10, NDUFB2, NDUFB8, NDUFB9, NDUFC1, NDUFS1, NDUFS2, NDUFS3, NDUFS8, SDHA, SDHB, UQCR11, UQCRB, UQCRC1, UQCRC2, UQCRFS1, UQCRQ*
Cardiac muscle contraction	3.8×10^{-10} -2.6×10^{-5}	3/5	14	*COX4I1, COX5A, COX5B, COX6B1, COX6C, COX7B, COX7C, COX8A, CYC1, UQCRB, UQCRC1, UQCRC2, UQCRFS1, UQCRQ*
Citrate cycle (TCA cycle)[b]	3.6×10^{-5}	1/5	7	*SDHA, SDHB, SUCLG1, CS, PDHA1, FH, MDH1*
Propanoate metabolism[b]	8.3×10^{-3}	1/5	5	*LDHB, ALDH7A1, ALDH1B1, SUCLG1, PCCB*
Pyruvate metabolism[b]	1.7×10^{-2}	1/5	5	*LDHB, ALDH7A1, ALDH1B1, PDHA1, MDH1*
Arginine and proline metabolism[b]	4.1×10^{-2}	1/5	5	*GOT2, ALDH7A1, GOT1, ALDH1B1, CKMT2*

Q = Benjamini false discovery rate ($Q < 0.05$). The number of genes represents the total numbers of significant genes from every significant GSE that is associated with a particular molecular pathway.
[a]Significant in GSE 80, 1428, and 8479.
[b]Significant in GSE 8479.

Significant genes responsible for the antiaging phenotype during endurance exercise in the oxidative phosphorylation pathway

There were 13 genes significant in at least 3/5 GSEs from the 43 endurance exercise genes that were involved in the OXPHOS pathway (**Table 3**). We subdivided them into 2 groups based on their molecular and cellular phenotypes. Three genes coded for enzyme components responsible for ATP synthesis, and 10 genes coded for proteins involved in electron transfer on the mitochondrial respiratory chain. The most significant gene in the OXPHOS pathway associated with endurance exercise was *ATP5C1*, a gene coding for the ATP synthase, H^+ transporting, mitochondrial F1 complex, γ polypeptide, which is expressed mainly in cardiac muscle and was present in 5/5 GSEs. The main role of the molecular and cellular phenotypes of *ATP5C1* is to catalyze ATP synthesis using an electrochemical gradient of protons, which is generated by electron transport complexes on the mitochondrial respiratory chain, across the inner mitochondrial membrane during OXPHOS. Moreover, *ATP5G3* and *ATP5J*, which code for proteins that play a role in catalyzing ATP synthesis together with *ATP5C1*, also presented in 4/5 GSEs. Furthermore, *COX7B* and *CYC1*, coding for proteins responsible for electron transportation in the mitochondrial respiratory chain, were present in 4/5 GSEs. *UQCRB*, a gene coding for proteins associated with redox-linked proton pumping, was also present in 4/5 GSEs.

Significant genes responsible for the antiaging phenotype after endurance exercise that are not associated with the oxidative phosphorylation pathway

Among the remaining genes, 18 were present in at least 3/5 GSEs (**Table 4**). There were numerous molecular and cellular phenotypes associated with this group, such as electron carrier proteins, proteins related to cellular detoxification, amino acid metabolism, apoptotic regulation, and a key component of the ribosomal subunit. The most significant gene, which was present in 5/5 GSEs, was *CYCS*, coding for cytochrome C. Its molecular and cellular phenotypes

Table 3. Molecular functions and phenotypes of genes significant in endurance exercise from the Kyoto Encyclopedia of Genes and Genomes oxidative phosphorylation pathway

Gene	Number of GSEs in common	*P* range	Molecular function	Molecular and cellular phenotypes
ATP5C1	5/5	6.80×10^{-5} to 8.86×10^{-3}	ATP synthase γ-subunit	Catalyzes ATP synthesis, mainly expressed in the heart
ATP5G3	4/5	4.12×10^{-7} to 2.06×10^{-3}	ATP synthase lipid-binding protein	Catalyzes ATP synthesis
ATP5J	4/5	6.58×10^{-5} to 3.57×10^{-3}	ATP synthase-coupling factor	Catalyzes ATP synthesis
COX7B	4/5	8.81×10^{-5} to 6.36×10^{-3}	Cytochrome C oxidase subunit	Terminal oxidase in the mitochondrial electron transport
CYC1	4/5	1.34×10^{-5} to 6.77×10^{-3}	Cytochrome C	Accepts electrons from the Rieske protein and transfers electrons to cytochrome c in the mitochondrial respiratory chain
UQCRB	4/5	9.80×10^{-6} to 4.15×10^{-3}	Ubiquinol-Cytochrome C reductase complex subunit	Redox-linked proton pumping
COX4I1	3/5	1.69×10^{-4} to 9.69×10^{-4}	Cytochrome C oxidase subunit	Catalyzes the electron transfer from reduced cytochrome c to oxygen
COX7C	3/5	2.20×10^{-4} to 9.60×10^{-3}	Cytochrome C oxidase subunit	Catalyzes the electron transfer from reduced cytochrome c to oxygen
NDUFA4	3/5	7.89×10^{-4} to 8.57×10^{-3}	α-Subcomplex subunit	Transfer of electrons from NADH to the respiratory chain
NDUFB1	3/5	4.95×10^{-5} to 5.58×10^{-3}	β-Subcomplex subunit	Transfer of electrons from NADH to the respiratory chain
NDUFB2	3/5	4.35×10^{-3} to 6.58×10^{-3}	β-Subcomplex Subunit	Transfer of electrons from NADH to the respiratory chain
NDUFB8	3/5	4.03×10^{-7} to 4.14×10^{-3}	β-Subcomplex Subunit	Transfer of electrons from NADH to the respiratory chain
UQCRFS1	3/5	6.28×10^{-6} to 2.61×10^{-3}	Ubiquinol-Cytochrome C reductase complex subunit	Generates an electrochemical potential coupled to ATP synthesis

ATP = adenosine triphosphate, NADH = reduced form of nicotinamide adenine dinucleotide

are as an electron carrier protein in the mitochondrial respiratory chain, and it is a key factor in the apoptotic processes of cells. Furthermore, *GSTK1*, the gene for glutathione S-transferase κ1, a gene coding for a enzyme that has a role in cellular detoxification, was also present in 4/5 GSEs.

Discussion

The main objective of this study was to find correlative evidence between types of exercise and their ability to at least partially delay the aging process based on changes in the cellular and molecular phenotypes caused by specific molecular pathways and genes. In this study, we evaluated the correlations between 4 exercise groups and 5 aging groups that contained hundreds of microarray experiments obtained from the GEO dataset. Benjamini FDR analysis was used to correct for the false positives as a result of chance from multiple comparisons in the mapping pathways.

The CU-DREAM program was designed to find the genes that correlate between 2 specific subjects and their gene distributions. If the distributions among these genes are higher than normal randomization, we can conclude that there was a relationship between the 2 specific subjects we studied. Furthermore, this program showed the significant advantage of a highly precise statistical significance by identifying the whole genome, which provided reliable results. However, there is a general limitation in using this program

Table 4. Molecular functions and phenotypes of significant remaining genes in endurance exercise

Gene	Number of GSEs in common	*P* range	Molecular function	Molecular and cellular phenotypes
CYCS	5/5	6.96×10^{-5} to 4.85×10^{-3}	Cytochrome C	Electron carrier protein, important apoptotic role
GSTK1	4/5	3.22×10^{-5} to 7.78×10^{-3}	Glutathione S-transferase subunit	Cellular detoxification
BHLHE41	3/5	1.34×10^{-6} to 9.60×10^{-3}	Helix-loop-helix protein	Transcriptional repressor
C14orf2	3/5	7.06×10^{-5} to 4.83×10^{-3}	Mitochondrial proteolipid	Chromosome 14 open reading frame
CKMT2	3/5	7.66×10^{-9} to 5.91×10^{-3}	Mitochondrial creatine kinase	Reversibly catalyzes the transfer of phosphate between ATP and phosphagens
COQ3	3/5	7.39×10^{-5} to 7.27×10^{-3}	COQ3 methyltransferase	Coenzyme Q biosynthesis
EIF3K	3/5	1.34×10^{-4} to 5.21×10^{-3}	Translation initiation factor	Prevents premature joining of the 40S and 60S ribosomal subunits prior to initiation
GOT2	3/5	5.26×10^{-6} to 6.07×10^{-3}	Fatty acid-binding protein	Amino acid metabolism, facilitates cellular uptake of long-chain free fatty acids
MRPS12	3/5	5.51×10^{-4} to 3.48×10^{-3}	Mitochondrial ribosomal protein	Key component of the small ribosomal subunit
NEDD1	3/5	2.64×10^{-4} to 3.45×10^{-3}	Neural precursor cell protein	Mitosis progression, promotes the nucleation of microtubules from the spindle
PDE4A	3/5	2.16×10^{-4} to 2.53×10^{-3}	Phosphodiesterase isozyme	Regulating the cellular concentration of cAMP
PPIF	3/5	3.69×10^{-5} to 6.88×10^{-3}	Mitochondrial cyclophilin	Antiapoptotic activity
PRDX2	3/5	4.61×10^{-4} to 9.86×10^{-3}	Thiol-specific antioxidant	Eliminating peroxides generated during metabolism
SIRT5	3/5	2.03×10^{-4} to 4.98×10^{-3}	Regulatory protein	Regulation of transcription and apoptosis
SLC16A1	3/5	6.38×10^{-5} to 9.09×10^{-3}	Solute carrier	Catalyzes the movement of monocarboxylates across the plasma membrane
SLC38A1	3/5	3.49×10^{-5} to 9.75×10^{-4}	Solute carrier	Mediates cotransport of glutamine and sodium ions
ST8SIA5	3/5	1.04×10^{-5} to 1.97×10^{-3}	Sialyltransferase	Synthesis of gangliosides
TPI1	3/5	3.33×10^{-5} to 9.54×10^{-3}	Triosephosphate isomerase	Catalyzes the isomerization of G3P and DHAP in glycolysis and gluconeogenesis

ATP = adenosine triphosphate, COQ3 = 3-demethylubiquinone-9,3-O, DHAP = Dihydroxyacetone phosphate, G3P = glyceraldehyde-3-phosphate dehydrogenase, NADH = nicotinamide adenine dinucleotide reduced form

because it usually provides less significant results, lower ORs (in the case of OR>1) and higher *P* values than the actual results, because of the different platforms for each specific GSE. For example, there are no significant genes associated between aging and the physical activity and power training groups. This may be because some specific genes that had the potential to delay aging did not cross-match from different platforms during the analysis.

This may result in low significance or no significant difference between the experiments. Furthermore, the heterogeneities of tissues between the GSEs can interrupt the results because some genes may be ignored or marked as nonassociated genes. In conclusion, the CU-DREAM program can be a reliable source for performing statistically significant tests, but the results must be interpreted with caution when the nonsignificant test results have been reported.

In the present study, we found that endurance exercise could potentially delay aging by upregulating numerous sets of genes, which can be mapped using the DAVID/KEGG database into the OXPHOS

pathway, a primary energy metabolism process of cells. Numerous studies about the molecular effects of endurance exercise on antiaging demonstrated that endurance exercise produced higher levels of ATP production capacities, mtDNA abundance, protein expression of mitochondrial biogenesis, and the upregulation of mitochondrial and oxidative metabolism pathways than sedentary activity [9, 25, 38, 39]. Increasing OXPHOS can be interpreted as an increase in mitochondrial biogenesis, improved ATP production capacity and an increase in numerous proteins associated with the metabolic process as a result of the chronic adaptations of a cell to endurance exercise. These molecular changes may be the primary mechanism for slowing the aging process.

Furthermore, we trawled the gene lists associated with endurance exercise and aging to identify the potential antiaging genes and provide a better understanding of the genetic regulation during OXPHOS. We found that the majority of genes were significantly involved in the OXPHOS pathway, directly and indirectly. For example, *ATP5C1*, the most in common gene of the OXPHOS group, is responsible for ATP synthesis, which is an important step for energy production during OXPHOS. Gene expression profiles showed a similar result in that there was upregulation of *ATP5F1*, a gene coding for ATP synthase, and increased expression of other genes associated with energy metabolism and mitochondrial proteins in endurance athletes compared with healthy controls [40]. Moreover, numerous genes upregulated after endurance exercise are also involved in OXPHOS, such as *COX7B*, *CYC1*, *UQCRB*, and *NDUFA4*, which code for molecules that play a role in electron transfer at their specific sites in the mitochondrial respiratory chain. In addition, we found that some genes that were responsible for the antiaging phenotypes, but not associated with OXPHOS as categorized by the DAVID/KEGG database, were also directly involved in OXPHOS. For example, *CYCS*, the gene for cytochrome C, the most common gene in the non-OXPHOS group, is an electron carrier protein in the OXPHOS pathway; however, for reasons we could not recognize, the DAVID/KEGG database did not categorize it as such.

Although numerous significant genes associated directly with OXPHOS had been indicated, numerous genes in the non-OXPHOS group were indirectly involved in OXPHOS, for example, genes associated with antioxidants. Antioxidant enzymes and heat shock proteins are significantly increased in trained subjects as an adaptation to the increase in reactive oxygen species, which are the by-products of the increased oxygen consumption occurring during exercise [41]. This finding is consistent with our present findings. We found that numerous genes, such as *GSTK1* and *PRDX2*, which are responsible for cellular detoxification and the elimination of peroxides generated during metabolism, respectively, were upregulated in the endurance exercise group. These 2 genes are an example of antioxidants that help to reduce reactive oxygen species, which are the byproducts of OXPHOS during endurance exercise.

Conclusion

Our study indicates that there is an association between certain types of exercise and aging. Endurance exercise showed the strongest association with downregulation of expression of genes related to the aging phenotype. Increased activity of the oxidative phosphorylation pathway, combined with specific molecular and cellular phenotypes, such as ATP synthesis, electron transfer in the mitochondrial respiratory chain, and cellular detoxification, resulted from the chronic adaptation to endurance exercise. This has the potential to slow mitochondrial dysfunction, one of the hallmarks of aging, by increasing mitochondrial biogenesis and increasing ATP production capacity.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

Supplementary Table S1A. CU-DREAM comparing upregulation in exercise and downregulation in aging

Aging group GSEs	Exercise group GSEs (types)	8479 (Resistance)	9103 (Endurance)	16907 (Power training)	20319 (Physically active)
80	*P*	6.73×10^{-2}	3.78×10^{-4}	6.58×10^{-1}	1.32×10^{-1}
	OR	0.40	2.91	0	4.14
	95% CI	0.15–1.10	1.57–5.38	–	0.56–30.80
1428	*P*	5.29×10^{-1}	9.73×10^{-15}	3.54×10^{-2}	8.29×10^{-1}
	OR	0.93	2.30	2.65	0.86
	95% CI	0.73–1.17	1.85–2.85	1.03–6.79	0.21–3.54
8479	*P*	5.07×10^{-24}	1.34×10^{-22}	1.20×10^{-1}	8.31×10^{-2}
	OR	0.16	2.65	0	2.22
	95% CI	0.11–0.24	2.16–3.24	–	0.88–5.60
9103	*P*	4.13×10^{-2}	2.76×10^{-12}	5.90×10^{-1}	5.97×10^{-1}
	OR	0.60	3.17	0.58	1.70
	95% CI	0.36–0.99	2.26–4.47	0.08–4.20	0.23–12.36
38718	*P*	2.85×10^{-18}	2.35×10^{-9}	4.65×10^{-1}	6.82×10^{-2}
	OR	0.47	1.58	0.68	1.88
	95% CI	0.40–0.56	1.36–1.83	0.25–1.90	0.94–3.74

*Significant experiments noted in bold, OR = odds ratio, CI = confidence interval

Supplementary Table S1B. CU-DREAM comparing downregulation in exercise and upregulation in aging

Aging Group GSEs	Exercise group GSEs (types)	8479 (Resistance)	9103 (Endurance)	16907 (Power Training)	20319 (Physically active)
80	*P*	1.81×10^{-3}	1.25×10^{-2}	7.93×10^{-1}	6.39×10^{-1}
	OR	2.55	2.56	0.00	0.00
	95% CI	1.39–4.68	1.19–5.48	–	–
1428	*P*	9.35×10^{-1}	3.03×10^{-1}	9.34×10^{-1}	8.91×10^{-1}
	OR	0.93	2.93	0.00	0.00
	95% CI	0.16–5.56	0.34–25.15	–	–
8479	*P*	1.81×10^{-103}	7.43×10^{-2}	9.05×10^{-1}	7.52×10^{-1}
	OR	5.65	1.30	0.89	0.80
	95% CI	4.74–6.73	0.97–1.74	0.12- 6.53	0.19–3.27
9103	*P*	9.14×10^{-61}	3.04×10^{-51}	3.98×10^{-1}	9.03×10^{-1}
	OR	4.08	4.44	1.43	0.92
	95% CI	3.41–4.89	3.59–5.48	0.62-3.26	0.22–3.76
38718	*P*	9.22×10^{-9}	8.59×10^{-13}	2.32×10^{-1}	3.09×10^{-1}
	OR	1.50	2.14	0.00	1.60
	95% CI	1.31–1.72	1.73–2.64	–	0.64–4.01

*Significant experiments noted in bold, OR = odds ratio, CI = confidence interval

Supplementary Table S1C. CU-DREAM comparing downregulation in exercise and downregulation in aging

Aging group GSEs	Exercise group GSEs (types)	8479 (Resistance)	9103 (Endurance)	16907 (Power Training)	20319 (Physically active)
80	*P*	1.47×10^{-1}	4.72×10^{-1}	7.60×10^{-1}	5.84×10^{-1}
	OR	1.44	1.36	0.00	0.00
	95% CI	0.88–2.38	0.58–3.17	–	–
1428	*P*	1.81×10^{-1}	9.97×10^{-1}	3.44×10^{-1}	8.72×10^{-1}
	OR	0.89	1.00	0.00	1.10
	95% CI	0.76–1.05	0.73–1.37	–	0.34–3.54
8479	*P*	1.24×10^{-67}	9.92×10^{-1}	8.39×10^{-1}	9.69×10^{-2}
	OR	3.63	1.00	0.81	0.00
	95% CI	3.11–4.24	0.73–1.37	0.11–6.00	–
9103	*P*	1.72×10^{-5}	9.47×10^{-1}	1.11×10^{-1}	4.09×10^{-1}
	OR	1.93	1.02	2.48	0.00
	95% CI	1.42–2.61	0.55–1.88	0.78–7.89	–
38718	*P*	5.21×10^{-54}	2.10×10^{-7}	2.50×10^{-1}	8.04×10^{-1}
	OR	2.17	1.59	0.33	0.90
	95% CI	1.97–2.40	1.33–1.90	0.04–2.43	0.39–2.09

*Significant experiments are noted in bold, OR = odds ratio, CI = confidence interval

Supplementary Table S2A. Lists of in common genes from significant CU-DREAM results comparing upregulation in endurance exercise and downregulation in aging

In common	5 (n= 2)	4 (n= 6)	3 (n= 23)	2 (n= 66)
Genes	*ATP5C1 CYCS*	*ATP5G3, ATP5J, COX7B, CYC1, GSTK1, UQCRB*	*BHLHE41, C14orf2, CKMT2, COQ3, COX4I1, COX7C, EIF3K, GOT2, MRPS12, NDUFA4, NDUFB1, NDUFB2, NDUFB8, NEDD1, PDE4A, PPIF, PRDX2, SIRT5, SLC16A1, SLC38A1, ST8SIA5, TPI1, UQCRFS1*	*ACSL6, ALDH1B1, ALDH7A1, ATP5E, ATP5F1, ATP5G1, ATP5L, ATP5O, BCAP29, BOLA3, C1orf151, C21orf33, C2orf88, CALU, CHCHD10, COX5A, COX5B, CS, DECR1, DPH5, DTNA, DUS4L, FAM162A, FH, FLJ11292, FRMD3, FYN, GNPTAB, GOSR2, GOT1, GPCPD1, GULP1, MIPEP, MRPL15, MRPL35, MRPL41, NDUFA6, NDUFA9, NDUFAB1, NDUFC1, NDUFS3, NDUFS8, NUDT6, OXNAD1, OXSM, PARVB, PDHA1, PHC3, POU6F1, PTCD3, PTPRO, RUNX2, SH3KBP1, SLC25A11, SLC25A4, ST6GALNAC2, SUCLG1, TMEM164, TNFAIP2, TOR3A, TUB, UQCRC1, UQCRC2, UQCRQ, WDR45, ZNF252*

Supplementary Table S2B. Lists of in common genes from significant CU-DREAM results comparing downregulation in resistance exercise and upregulation in aging

In common	4 (n= 1)	3 (n= 12)	2 (n= 134)
Genes	*CRIM1*	*FEZ2, H3F3B, HNRNPM, LAMP1, METTL7A, NKTR, RBM5, SRRM2, TRMT112, TSC22D1, XRCC6, ZFHX3*	*ADAMTS5, ALDH2, ALDH9A1, APBB3, ARFGAP2, ARL6IP5, ATG12, ATP1B4, ATP6V0A1, BCL6, BTG2, C1orf123, C21orf7, CALM1, CBLB, CEBPB, CELF2, CEP350, CFLAR, CLK4, COL4A3BP, CREB1, CREBBP, CXXC1, DDIT4, DHRS7, DMD, DPF2, DYNC1LI2, EFHD1, EIF1AD, ERRFI1, FAM107A, FAM8A1, FBLN1, FIGF, FILIP1, FKBP5, FOXN3, FOXO3, GGNBP2, GLG1, GLUL, GNPDA1, H1FX, HAUS2, HNRNPA1, HNRNPC, HSD17B7, HSF2, IGFBP6, IK, ING5, JDP2, KARS, KHDRBS1, KIAA0494, KLF10, KLF5, KLF9, KLHL28, LAMA2, LGR5, MBNL1, MCM7, MORC2, MYC, MYH11, NFAT5, NPHP3, NT5C2, PAM, PCM1, PDLIM5, PERP, PEX16, PHIP, PPDPF, PRDM2, PRDX6, PRKRIP1, PRNP, PTGES3, RASEF, RHEB, RNF103, RPL10A, RPL12, RPL13, RPL22, RPL23A, RPL4, RPL41, RPL7A, RPS4X, RPS6KA5, RRAD, RUNX1T1, SELM, SH3GLB1, SKAP2, SLBP, SLC7A6, SLIT2, SLPI, SMAD2, SOD3, STAG2, SUV420H1, SYNPO2, TANC1, TGFBR3, THUMPD1, TM2D3, TMEM43, TOP1, TP63, TPM3, TSR1, UACA, UBE2L3, UPF3A, USP54, VIT, WTAP, XPC, XPNPEP3, XRCC2, ZBTB16, ZMAT3, ZNF451, ZNF549, ZNF627, ZNF652*

Supplementary Table S2C. Lists of in common genes from significant CU-DREAM results comparing downregulation in resistance exercise and downregulation in aging

In common	3 (n= 8)	2 (n= 165)
Genes	*ATP5C1, CHCHD3, CYCS, DCUN1D5, DLAT, FAM171A1, NEDD1, RXRG*	*ACAA2, ACADM, ACADSB, AGPAT1, AIMP1, AIMP2, ANGEL2, ANKH, ASB12, ASPH, ATP2C1, ATP5F1, ATPAF1, BHLHE41, BLMH, BPGM, C11orf74, C14orf142, C16orf72, C21orf33, C6orf136, CALU, CCM2, CD36, CENPV, CHCHD4, CISD1, CMC1, COQ3, COX10, CRADD, CSPP1, CWC27, CYC1, DEXI, DNM1L, DPH2, DPH5, DTNA, DUS4L, DUSP3, DZIP3, EPM2A, ETV6, FAM100B, FAM110B, FAM190B, FAM82B, FBXO3, FH, FKBP1A, FRMD3, FSD2, FZD7, GBAS, GFM1, GFM2, GNPTAB, GOLGA2, GOLGA4, GOSR2, GOT1, GOT2, GRSF1, GTPBP8, HIBADH, HOOK2, HSPA4, IFRD2, ISCA1, KBTBD2, KCTD9, KPNA6, LANCL1, LPIN1, LYPLA1, LYRM5, MAP2K6, MAPKAP1, MFF, MKKS, MLEC, MOCS2, MRPL35, MRPL48, MRPS30, MRS2, MSRB2, MTRF1L, MTX2, MYOZ3, MYPOP, NAMPT, NDUFAF2, NDUFAF4, NEURL2, NMNAT1, NNT, NUDT6, OSBPL3, OXSM, PDHA1, PDSS1, PEX2, PGK1, PIAS2, PPARGC1A, PPIF, PPP2R3C, PRDX3, PRKAR2A, PSME4, PTCD3, PTPLA, PYROXD1, RABEP1, RABGGTB, RAVER2, RCHY1, RGS3, RNF144A, RPS6KC1, RWDD1, S100A1, SCYL2, SDHC, SDHD, SHISA4, SIRT5, SLC25A15, SLC25A4, SLC25A44, SLC25A46, SMAP1, SNTB1, SOBP, SRF, STAMBP, STRADB, SUCLA2, SYNGR1, TATDN1, TBC1D14, TEF, TFRC, TMTC4, TOMM40L, TOR3A, TPI1, TTN, TUBA3C, UBE2V1, UBP1, UCHL3, ULK2, UQCRB, UQCRH, USP38, UXS1, VLDLR, WIPI1, WSB2, WWP1, ZC3H8, ZNF438*

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