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Physical Exercise–Induced DNA Methylation in Disease-Related Genes in Healthy Adults—A Systematic Review With Bioinformatic Analysis

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Abstract

This study aimed to systematically review the existing literature regarding physical exercise (PE) and DNA methylation (DNAm) in healthy adults. Specific goals were to (a) identify differently methylated genes (DMGs) after PE intervention, their imprinting status, chromosome and genomic location, function, and related diseases; and (b) to screen for core genes and identify methylation changes of the core genes that can be modified by PE intervention. Our search identified 2,869 articles from which 8 were finally included. We identified 1851 DMGs ($p<0.05$) after PE intervention, although 45 of them were imprinted. Aerobic exercise (AE) seems to induce more DNA hypermethylation rather than hypomethylation, whereas anaerobic exercise (AN) seems to induce more DNA hypomethylation rather than hypermethylation. Aerobic exercise induced highest % of methylation changes on chromosome 6, whereas AN and mixed type (MT) on chromosome 1. Mixed type induced higher % of methylation changes close to transcription start site in comparison to AE and AN. After PE intervention, DMGs were mainly involved in fat metabolism, cell growth, and neuronal differentiation, whereas diseases regulated by those genes were mainly chronic diseases (metabolic, cardiovascular, neurodegenerative). Finally, 19 core genes were identified among DMGs, all related to protein metabolism. In conclusion, our findings may shed some light on the mechanisms explaining PE-induced health benefits such as the potential role that PE-induced DNAm may have in disease prevention and disease treatment.

Key Words: aerobic exercise, anaerobic exercise, mixed type of exercise, gene enrichment analysis, diseases.

Introduction

Physical exercise (PE) is defined as a physical activity consisted of planned, structured, and repetitive bodily movements produced by the skeletal muscles and is performed to improve or maintain the components of physical fitness and health (57). Based on energy modality used during exercising, PE is categorized as follows: (a) aerobic exercise (AE)—endurance efforts; (b) anaerobic exercise (AN)—explosive efforts; and (c) mixed type (MT)— moderate-intensity to high-intensity efforts (11). Aerobic exercise type of PE uses large muscle groups, is maintained continuously, and relies on aerobic metabolism to extract energy (57). Anaerobic exercise type is an intense PE of very short duration fueled by the energy sources within the contracting muscles, independent of the use of inhaled oxygen as an energy source (18,54,56). Mixed type is considered any PE that relies simultaneously on both (aerobic and anaerobic) metabolic pathways to provide energy (34,73).

Physical exercise has been found to induce changes in DNA methylation (DNAm) levels and expression of genes in response to exercise training (8,52). DNA methylation is defined as a process of adding methyl group to the cytosine bases of the DNA strand, thus influencing gene expression (8). Methylation patterns can be inherited in a parent-of-origin-specific manner, suggesting that DNAm may play a role in imprinted genes (8). Imprinted genes are genes whose expression in the offspring is determined by the parental origin (8). In paternally imprinted genes, the paternal copy of the gene is silenced, whereas the maternal copy is

expressed in the offspring. In maternally imprinted genes, the maternal copy of the gene is silenced, whereas the paternal copy of the gene is expressed (8). Because methylation changes in imprinted genes are maintained during fertilization (61,71), genes with methylation changes in germ cells (in both—ova and sperm) that are paternally or maternally imprinted are key candidates for transgenerational transmission of new methylation patterns to the next generations (71,80,87). Moreover, previous evidence suggests that PE may induce changes inDNAmlevels of imprinted genes (8,19).

Differently methylated genes (DMGs) are genes with changes in DNAm levels, i.e., different methylation percentage between 2 different time points (8). Among genes whose methylation levels were found to be significantly changed after exercise training are genes involved in metabolism, muscle growth, hematopoiesis, and inflammation (64). Core genes are defined as the most essential genes within the genome, i.e., genes that are strongly conserved at the nucleotide sequence level, which are of utmost importance for the biological processes undergoing in a living organism (69). Systematic methodologies widely accepted and applied in bioinformatics field for core genes screening are mainly based on the accomplishment of the following 3 criteria: (a) genes' participation in the enriched pathways; (b) genes' degree of connectivity; and (c) target genes of key transcription factors (84). Chromosomal location of DMGs plays a significant role in understanding how organisms' traits vary and evolve (62). According to the evolution theory, it is less important what a physical trait is, but more important where the genes that affect that trait reside in the genome (62). Moreover, the genomic location of methylation changes within a gene is also important. Methylation changes around the transcription start site (TSS) are highly influencing gene expression (7,29,31,63,74). Hypermethylation in the upstream promoter region, as well as downstream in the region of the first exon, induces transcriptional silencing (7) which leads to gene repression (29,31,63,74). Gene repression is especially relevant for genes whose overexpression leads to a certain disease (44).

Methylation studies that are studying the effects of PE intervention on DNA methylation reported PE-induced DNA hypermethylation (12,63), as well as DNA hypomethylation in DMGs (18,68). Identification of hypermethylated and hypomethylated DMGs by different types of PE, as well as their function and related diseases, may contribute to better understanding of the epigenetic mechanisms that are explaining the health benefits induced by PE. This may have a significant impact in the field of personalized medicine, where specifically tailored PE interventions are applied to treat certain diseases.

Therefore, our objective is to systematically review the existing literature regarding PE andDNAm in healthy adults and to assess whether DNAm induced by PE may potentially show different methylation patterns according to PE type. Specific goals were to (a) identify DMGs after PE intervention, their imprinting status, chromosome and genomic location, function, and related diseases; and (b) to screen for core genes and identify methylation changes of the core genes that can be modified by PE intervention.

Methods

This systematic review follows the protocol reported in the PRISMA statement for reporting systematic reviews and metaanalyses (53) and the PICOS model (22) for the definition of the inclusion criteria as follows: (a) population—healthy adults; (b) intervention—PE training intervention; (c) comparators—DNAm levels before and after PE intervention; (d) outcomes—DNAm levels assessed by the Illumina Infinium Human Methylation 450 Bead Chip Assay and HiSeq 2,500 system Illumina. (e) study design—interventional studies. Study's protocol was registered in PROSPERO (CRD42022335307).

Search Strategy

FV and AP-P performed independent systematic electronic searches of the scientific literature in Web of Science, Scopus, Cochrane, and PubMed. The search included a combination of the

following keywords: “physical exercise,” “sport,” and “DNA methylation” that were decided upon expert opinion, scientific literature review, and controlled vocabulary (Medical Subject Headings). Search was not restricted in terms of publication date, but it was filtered to retrieve only human studies written in English. The last search was performed on May 12, 2022. The initial selection was based on title, abstract, and keywords review. In the following stages, (a) reviews, meta-analyses, animal studies, in vitro studies, interventions including diets or supplementation, and studies including elderly or nonhealthy population were excluded; (b) duplicates were eliminated, and (c) studies potentially relevant for full-text review were selected. To analyze and discuss the most relevant articles in the field, articles from the reference lists of articles retrieved in the search were also examined by a snowballing strategy (33).

Inclusion and Exclusion Criteria

Inclusion/exclusion criteria were applied by FV and AP-P. Inclusion criteria were (a) studies published in peer-reviewed journals; (b) full-text available; (c) genome-wide DNAm array studies; (d) DNAm is assessed before and after the PE intervention; (e) controlled PE-interventional studies; (f) performed in healthy adults who are not regularly engaged in institutionalized sports activities nor professional athletes; (g) PE interventions employed endurance, resistance training, or a combination of both; and (h) details of the PE intervention such as type, duration, intensity, and frequency of PE were provided. Exclusion criteria were (a) reviews and meta-analyses; (b) animal studies; (c) in vitro studies; (d) observational studies; (e) studies including only acute PE; (f) habitual or lifelong physical activity assessed with questionnaires, armbands, or accelerometers; (g) multicomponent interventions, including diets, nutritional supplementation, pharmaceutical treatment, and cryostimulation protocols; (h) elderly or nonhealthy population; (i) global or candidate-locus DNAm; and (j) no data available.

Text Screening

Titles, abstracts, and keywords were screened independently by FV and AP-P based on the exclusion/inclusion criteria previously established. Subsequently, potential studies for inclusion were reviewed at a full-text level to determine their eligibility for inclusion.
There were no disagreements between FV and AP-P regarding the study eligibility.

Data Extraction

Data were extracted by FV and AP-P: information on authors, year of publication, number of subjects, study demographics (age and sex), PE intervention (type, duration, intensity and frequency of PE), tissue sample, and main findings regarding DNAm such as genes name/symbol and methylation change (hypo/hypermethylation). After data extraction, selected studies were clustered by PE type, and study groups were created. Physical exercise type was determined based on the following criteria: (a) energy modality used to perform exercise (11) and (b) intensity of PE according to the American College of Sports Medicine (24). To minimize errors, data extraction and PE-type categorization were discussed with the research group until a final consensus was reached (AP-P, RH, RF-L, GG, and VL-R). Finally, 3 groups were created: (a) AE—predominance of oxidative phosphorylation with a very light to light intensity; (b) AN—predominance of phosphagens pathway with very high to maximal intensity; and (c) MT—phosphagens pathway 1 glycolytic pathway 1 oxidative phosphorylation with a moderate to high intensity.

Methodological Quality and Risk of Bias Assessment

FV and AS independently assessed methodological quality and risk of bias for each study by applying Cochrane’s single case design risk of bias tool (60). Each study was evaluated for

selection bias (sequence generation and subject selection), performance bias (blinding of subjects and personnel, and procedural fidelity), and detection bias (blinding of outcome assessors, selective outcome reporting, dependent variable reliability, and data sampling). In accordance with the Cochrane's handbook, a low, high, or unclear risk rating was assigned for each domain as follows: (a) low risk when the domain criterion was met, and the bias was unlikely to affect the study's internal validity; (b) high risk when the specific domain criterion was not met raising concerns over the study validity; and (c) unclear risk when there was insufficient information provided to determine whether the noted risk would influence interpretability of results (81).

Methods of Analysis

Identification of Differently Methylated Genes. Eight independent gene methylation profile data sets from PE-interventional studies were obtained either from Gene Expression Omnibus database, GSE109478 and GSE60655 (37), or from the original article: Tables 2 and 3 (63), Supplementary Data set S13 (45), Supplementary Table 1 (18), Tables 4 and 5 (20), Tables 3 and 5 (19), Supplementary file 2B loading (68), Supplementary Table S6 (38), and Supplementary Table 8 (23). All data sets were obtained from the Illumina Infinium Human Methylation 450 Bead Chip Assay and HiSeq 2500 system Illumina. The probes were transformed into the corresponding gene symbol according to the annotation information in the articles or platform. The false discovery rate provided by the authors was applied. The criteria applied to consider DMGs was set up based on the minimum absolute value of fold change identified in the included studies (0.80%, $p<0.01$). Imprinting status among the identified DMGs after PE intervention was checked in the Geneimprint database (27).

Genomic Location of Differently Methylated Genes. Differently methylated genes were annotated to the following genomic locations: TSS1500 (1500 bases upstream of the TSS), TSS200 (200 bases upstream of TSS), 59UTR—5 prime, First Exon, Body, 39UTR—3 prime, and Intergenic. Accordingly, 2 groups were created: (a) close to TSS (TSS1500, TSS200, 59UTR, and First Exon) and (b) distant from TSS (Body, 39UTR and Intergenic).

Enrichment Analysis. Enriched ontology terms and pathways indicating the function of genes were identified by Metascape (89). The software first identifies all statistically enriched terms using Gene Ontology and/or Kyoto Encyclopedia of Genes and Genomes and then hierarchically clusters them into a tree, based on Kappa statistical similarities among their gene interactions. Finally, it applies a kappa score of 0.3 as a threshold to cast the tree into clusters. Disease enrichment analysis was performed by Enrichr based on OMIM disease expanded database (40). It uses lists of genes as input and then automatically computes enrichment for the inserted gene set. Enriched terms and diseases are ranked by p value, and the top 20 are presented.

Screening for Core Genes. Core genes were screened among DMGs after the PE intervention and identified using the following methodology proposed by Yang et al. (84): (a) participation in the enriched pathways; (b) calculated to have a high degree of connectivity>10 (identified by Metascape) (89); and (c) a target gene of key transcription factors according to the ENCODE data set (51).

Results

Search Results

A flow chart with a description of the search procedure is presented in Supplemental Digital Content (see Figure S1, http://links.lww.com/JSCR/A450). The initial search yielded 2,866 titles: Web of Science (N=901) Scopus (N=736), Cochrane (N=206), and PubMed (N=1,023). Three additional studies were added to the list after searching the reference lists of articles

previously retrieved, accounting for a total of N=2,869 articles. Finally, after removal of duplicates 1,348 articles were identified. During the initial selection based on title, abstract, and keywords review, 23 studies were deemed eligible to be reviewed at a full text level. Of these 23 studies, 8 were considered appropriate for inclusion after a full-text review, and 15 studies were excluded because they did not meet the complete inclusion criteria. The main reasons for exclusion at this last stage were (a) global DNAm; (b) candidate-locus DNAm; and (c) no full text/data available. A total of 8 studies were finally included in the review.

Studies Characteristics

This systematic review is composed of 8 studies that included PE intervention. Categorization according to the type of PE was 2 AE (45,63), 4 AN (18–20,68), and 2 MT (23,38). According to the duration of PE, interventions were in range of 4–24 weeks, whereas according to tissue sample used to assess DNAm, studies include SkM (m. vastus lateralis), adipose tissue (AT), whole blood, and sperm. In total, data from 114 subjects (103 men and 11 women) were included. Age range of subjects was from 18 to 42 years. All interventions were with a training frequency from 3 to 5 days per week and intensities ranging from light to maximal. Details are presented in Table 1.

Methodological Quality and Risk of Bias Results

There were no disagreements between the assessors regarding the methodological quality and risk of bias for the included studies. Overall, results indicate a low risk of bias in all bias domains, except for the domains of subject selection and procedural fidelity. Five of 8 studies (62.50%) in the subject selection domain were evaluated with an unclear risk, and 1 of 8 studies (12.50%) with a high risk of bias. In addition, 1 of 8 studies (12.50%) was evaluated with an unclear risk for "procedural fidelity" domain. However, considering the nature of the interventions in our review, blinding domain does not affect the study validity, thus items such as blinding subjects, personnel, and outcome assessors were considered as being low risk, even if the domain criterion was not met (43). Detailed review is presented in Supplemental Digital Content (see Table S1, http://links.lww.com/JSCR/A451).

Table 1
Characteristics of the studies included in the review.*

Study	N	Age	Gender	PE type	Duration	Intensity	Frequency	Tissue	Outcome related to DMGs
Ronn et al 63	23	32–42	M	AE (endurance:spinning and aerobics)	24 wk	Light	3 times/ week	AT	Hypermethylation of obesity and T2DM-related genes; hypomethylation of genes involved in lipolysis regulation
Lindholm et al 45	23 (12m and 11f)	26–28	M and F	AE (endurance: supervised 1-legged knee-extension training)	12 wk	Light	4 times/ week	SkM	Hypermethylation of structural remodeling and glucose metabolism–related genes (diabetes mellitus); hypomethylation of inflammatory/immunological and transcriptional regulation–related genes
Denham et al 18	8	19–23	M	AN (resistance training)	8 wk	Very high	3 times/ week	Whole blood	Hypermethylation of cardiomyopathy-related genes; hypomethylation of growth factor genes
Seaborne et al 68	8	25–30	M	AN (resistance training)	7 wk	Very high to maximal	3 times/ week	SkM	Hypermethylation of neuropathy-related diseases; hypomethylation of hypertrophy-related genes
Denham et al 20	12	18–24	M	AN (sprint training)	4 wk	Maximal	3 times/ week	Whole blood	Hypermethylation of cardiomyopathy-related genes; hypomethylation of cardiovascular related genes (angiogenesis and blood vessel cell migration)
Denham et al 19	13	19–29	M	AN (sprint training)	12 wk	Maximal	2 times/ week	Sperm	Hypermethylation of disease-associated genes (migraine, dystonia, ataxia); hypomethylation of genes related to insulin receptor signaling pathway, and cardiac muscle contraction)
Ingerslev et al 38	12	18–35	M	MT (endurance exercise program—spinning sessions)	6 wk	High	5 times/ week	Sperm	Hypermethylation of genes related to encephalopathy, lateral sclerosis; hypomethylation of CNS-related genes (neuronal differentiation)
Fabre et al 23	15	19–27	M	MT (endurance:cycling)	6 wk	High	5 times/ week	AT	Hypermethylation of genes related to autoimmune disease; hypomethylation of genes related to adipogenesis

*AE = aerobic; AN = anaerobic; AT = adipose tissue; CNS = central nervous system; DMGs = differently methylated genes; F = female; M = male; MT = mixed type; N = number of subjects; PE = physical exercise; T2DM = type 2 diabetes mellitus.

Identification of Differently Methylated Genes

Based on the definition criteria applied (fold change >0.80%, p< 0.01), we identified 1851 DMGs after PE intervention. Six hundred and five genes were differently methylated after AE (77.69% hypermethylated and 22.31% hypomethylated), 914 genes after AN (33.81% hypermethylated and 66.19% hypomethylated), and 332 genes after MT of PE (56.93% hypermethylated and 43.07% hypomethylated). Percentages of hypermethylated and hypomethylated genes after PE intervention are presented in Figure 1A. Our results may potentially show different methylation patterns according to the PE type.

Imprinted Genes Identification

We identified 45 imprinted genes (out of 1851DMGs) in SkM, AT, whole blood, and sperm, modified after PE intervention. Fifteen of them were maternally imprinted, whereas 30 were paternally imprinted. Noteworthy, 21 of the paternally imprinted genes were differently methylated in sperm. In brief, of the 45 imprinted genes, 7 were modified only after AE type of PE, 31 only after AN type of PE, and 2 only after MT of PE. However, HOXA3 and RB1 were modified after both AE and AN type of PE; GATA3 and PAOX were modified after AE and MT of PE, whereas KCNQ1 was modified after AN and MT of PE. Imprinted genes modified after PE intervention were mainly related to chronic diseases, such as metabolic, cardiovascular, neurodegenerative, musculoskeletal conditions, cancer, and various syndromes (26). Detailed view of all imprinted genes and related diseases is presented in Supplemental Digital Content (see Table S2, http://links.lww.com/JSCR/A452).

Chromosome Location of Differently Methylated Genes

The percentage of DMGs on each chromosome is shown in Figure 1B. Most of the DMGs after PE are settled on different chromosomes as follows: AE—6 (10.74%), 1 (8.93%), 3 (7.77%), 17 (6.45%), and 10 (6.28%); AN—1 (9.63%), 3 (8.10%), 12 (6.56%), 11 (6.35%), and 6 (5.58%); and MT—1 (12.35%), 19 (7.53%), 2 (6.93%), 3 (6.02%), and 5 (5.72%). Our results suggest that AE type of PE induced highest % of methylation changes in genes on chromosome 6, whereas AN and MT of PE induced highest % of methylation changes in genes on chromosome 1.

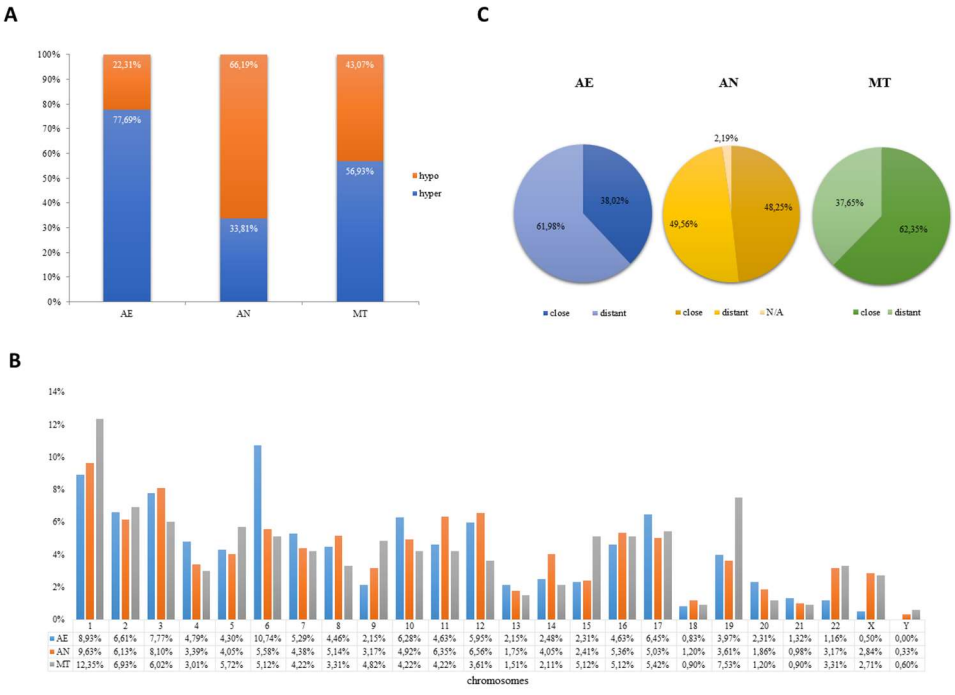

Figure 1. Differently methylated genes (DMGs) after physical exercise (PE). A) Percentage (%) of hypomethylated and hypermethylated genes according to the type of PE: aerobic (AE), anaerobic (AN), and mixed type (MT). B) Percentage (%) of DMGs in each chromosome. C) Genomic location of DMGs in relation to the transcription site: close (TSS1500: 1,500 bases upstream of transcription start site; TSS200: 200 bases upstream of transcription start site; 59UTR: 5 prime; and first exon) and distant (body, 39UTR: 3 prime; and intergenic). N/A 5 not available.

Genomic Location of Differently Methylated Genes According to the applied criteria, (a) 38.02% of the methylation changes induced by AE type of PE were close to the TSS, whereas 61.98% were distant from the TSS; (b) 48.25% of the methylation changes induced by AN type of PE were close to the TSS, 49.56% were distant from the TSS, whereas 2.19% could not be annotated and hence were considered as not available; and (c) 62.35% of the methylation changes induced by MT of PE were close to the TSS, whereas 37.65% were distant from the TSS (Figure 1C). Analysis of genomic location of DMGs has shown that MT of PE induces a higher % of methylation changes that are close to TSS, in comparison to AE and AN.

Enrichment Analysis

Pathways. Pathways enrichment analysis indicating functions of genes showed that (a) DMGs after AE type of PE were mainly related to muscle fiber organization and development, fat cell differentiation, adaptive immune system, hormone secretion, and system regulation; (b) DMGs after AN type of PE were mainly related to cell morphogenesis, protein catabolic process, growth factor signaling pathway, and regulation of growth; and (c) DMGs after MT of PE were mainly related to cellular morphogenesis, myocardial development, and regulation of neuronal and muscle cell differentiation. A detailed view is presented in Figure 2A.

Diseases. Disease enrichment analysis showed that PE intervention induced methylation changes in genes mainly related to chronic diseases such as metabolic, cardiovascular, and neurodegenerative diseases, musculoskeletal conditions, cancer, and various syndromes. A detailed view of diseases regulated by AE, AN, and MT of PE is presented in Figure 2B.

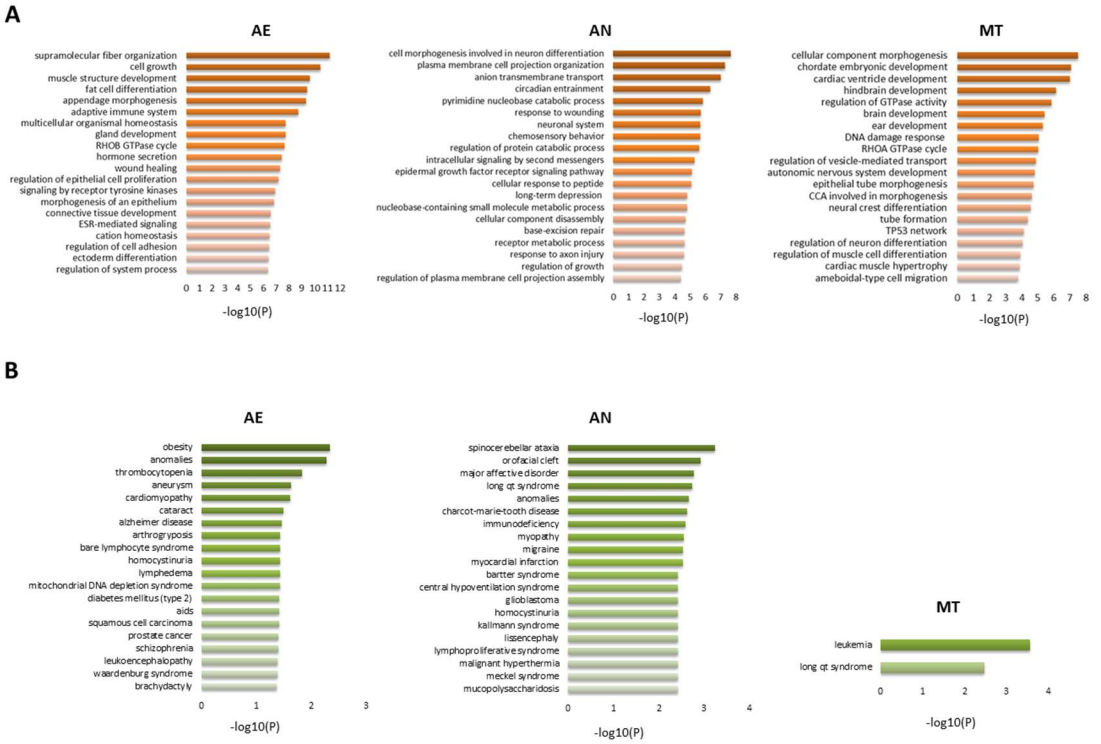

Figure 2. Enrichment analysis of differentlymethylated genes (DMGs) during aerobic (AE), anaerobic (AN), andmixed type (MT) of physical exercise. A) Pathway enrichment analysis of DMGs during AE, AN, and MT. All enriched terms were identified by Metascape. B) Disease enrichment analysis of DMGs during AE, AN, and MT. All diseases were identified by Enrichr based on OMIM disease expanded database. Enriched terms and diseases are ranked by p value, and the top 20 are presented.

Screening for Core Genes

After applying the defined 3-condition criteria, we identified the following core genes in our study: ANAPC7, ANAPC10, ASB2, ASB8, ASB14, ATG7, CUL1, FBXL13, FBXW4, HERC2, KLHL11, KLHL41, NEDD4L, PRKN, RNF220, SMURF1, TRIM71, UBE2C, and UBE2J2 (Table 2). All of them were differently methylated in SkM after AE type of PE and were related to protein metabolism (40). They were involved in the regulation of metabolic diseases, brain disorders,

and neurodegenerative diseases (e.g., dementia, Huntington's disease, Parkinson's disease), myopathy, cancer, and Prader-Willi syndrome (26). Core genes identified in our study were not imprinted. We did not identify any core genes after AE type of PE that were differently methylated in AT (candidate genes did not fulfill the third condition, i.e., target gene of a key transcription factor). Similarly, we did not identify core genes after AN(candidate genes did not fulfill the second condition of the preestablished criteria, i.e., high degree of interconnectivity) and MT of PE (candidate genes did not fulfill the third condition, i.e., target gene of a key transcription factor).

Table 2

Core genes that were modified after physical exercise intervention and diseases/disorders related.*

Gene†	Chr	Gene region	CpG island	Δ (%)	*p*	Disease/disorder related
ANAPC7	12	3′UTR	N/A	5,36	$p < 0.01$	N/A
ANAPC10	4	Body	N/A	7,36	$p < 0.01$	Opitz-Gbbb syndrome
ASB2	14	Body	Island	−5,77	$p < 0.01$	Leukemia
ASB8	12	3′UTR	N/A	6,37	$p < 0.01$	N/A
ASB14	3	TSS1500	N/A	5,01	$p < 0.01$	N/A
ATG7	3	Body	N/A	5,66	$p < 0.01$	Dementia, Huntington's disease
CUL1	7	3′UTR	N/A	6,18	$p < 0.01$	Ovarian cancer, Weaver's syndrome
FBXL13	7	Body	N/A	6,37	$p < 0.01$	ASDP syndrome
FBXW4	10	Body	N/A	5,81	$p < 0.01$	SHFM disorder
HERC2	15	Body	N/A	5,17	$p < 0.01$	Prader-Willi syndrome, mental retardation, breast cancer
KLHL11	17	Body	N/A	5,30	$p < 0.01$	Polyradiculopathy
KLHL41	2	Body	N/A	5,24	$p < 0.01$	Myopathy
NEDD4L	18	TSS200	Shore	−5,82	$p < 0.01$	Hypertension, epilepsy, Liddle's syndrome
PRKN	6	Body	N/A	5,05	$p < 0.01$	Parkinson's disease, ovarian cancer, lung cancer
RNF220	1	TSS1500	Shore	−6,34	$p < 0.01$	MC1 deficiency
SMURF1	7	Body	N/A	5,22	$p < 0.01$	Meningitis, Wolfram's syndrome
TRIM71	3	Body	N/A	6,04	$p < 0.01$	Hydrocephalus, spermatogenic failure
UBE2C	20	Body	Shelf	6,88	$p < 0.01$	CC7 deficiency, gastric cancer
UBE2J2	1	5′UTR	Shelf	7,21	$p < 0.01$	N/A

*ASDP = advanced sleep phase disorder; CC7 = complement component 7; Chr = chromosome; MC1 = mitochondrial complex one; N/A = not available; SHFM = split hand/foot malformation; Δ = methylation change.
†All genes are differently methylated in skeletal muscle after an aerobic type of physical exercise.

A comparative summary between AE, AN, and MT of PE and DNA methylation is presented in Figure 3.

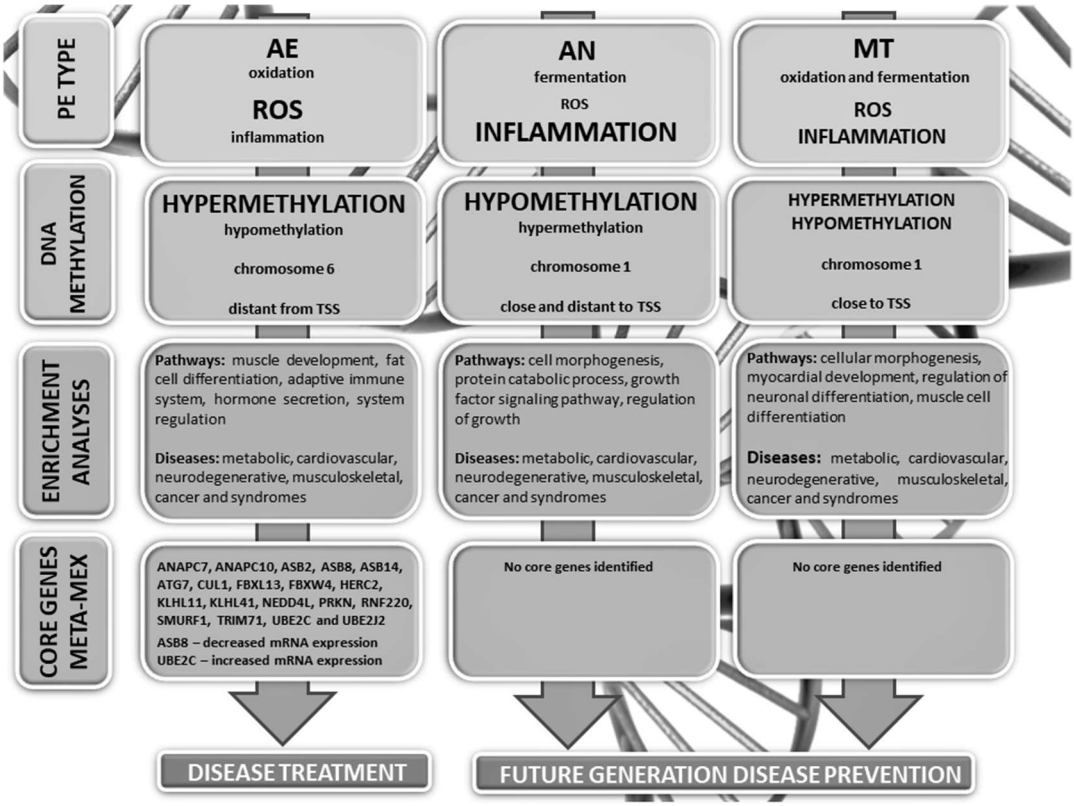

Figure 3. A comparative summary of DNA methylation changes after aerobic (AE), anaerobic (AN), and mixed type (MT) of physical exercise. Imprinted differently methylated genes (DMGs), chromosome and genomic location, function and related diseases of DMGs, and core genes among DMGs are presented. TSS=transcription start site.

Discussion

This systematic review summarized the existing literature regarding PE and DNAm in healthy adults. We also tried to assess if DNAm induced by PE may potentially show different methylation patterns according to the PE type. We identified the imprinting status of DMGs after PE intervention, their chromosome and genomic location, and their function and related diseases. Finally, we identified the core genes that were modified by PE intervention. This systematic review included 8 interventional studies, i.e., 1851 DMGs. In general, selected studies presented a low risk of bias, except the domains for subject selection (5 studies with an unclear and 1 study with a high risk of bias) and procedural fidelity (1 study with an unclear risk of bias).

PE-induced DNA methylation seems to be mediated by the oxidative stress and the inflammatory response during PE (10,77). It is well known that PE induces oxidative stress by the accumulation of reactive oxygen species (77). The reactive oxygen species can act as catalysts of DNAm through the mechanism of activating and increasing DNA methyl trasferase expression, leading to DNA hypermethylation (13). Aerobic exercise has been reported to induce higher rate of oxidative stress and reactive oxygen species than AN because of the oxidation of fat and carbohydrates (77). Conversely, a systematic review that compared the reactive oxygen species production and oxidative stress in AE and AN studies reported lower rate of reactive oxygen species in AN studies (35). In addition, PE induces an inflammatory response because of the mobilization of leukocytes and the increase in circulating inflammatory mediators (10). This inflammatory process is regulated and resolved by teneleven-translocation (TET) proteins through the mechanisms of inducing immune cell development, stem cells self-renewal, and terminal differentiation (88). Worthy to note is that TET proteins take part and induce the process of DNA hypomethylation (28,47,83). A recent review reported an increased number of white blood cells and proinflammatory cytokines, which was more evident after PE with higher intensity compared with lower-intensity PE (10).

The results of this review are also indicative of different methylation patterns that may be potentially induced by AE, AN, and MT of PE. However, because of the limited number of studies, heterogeneity in terms of duration of PE intervention, and tissue-sample used to assess DNAm, these findings must be interpreted with caution. To the best of our knowledge, PE type–specific DNAm in humans has not been previously reported. Nevertheless, few studies have found that PE-induced DNAm might be intensity dependent (2,9), whereas higher-intensity exercise induced DNA hypomethylation (2). According to the results we obtained, AE seems to induce more DNA hypermethylation rather than hypomethylation, whereas AN seems to induce more DNA hypomethylation rather than hypermethylation. In line with our results, a recent study reported a significant increase in the percentage of global DNAm after endurance intervention in healthy adults, evident after the intervention with lower intensity (42). In addition, animal studies are also in accordance with the results we obtained. A study in mice showed increased DNAm of NR4A1 gene after running wheels training (39). Aerobic exercise training induced DNA hypermethylation of genes related to arterial function in hypertensive rats (12). A genome-wide DNA methylation study in horses reported DNA hypermethylation of genes related to cell division, signaling, adhesion, and transport after trotting exercise (30). Conversely, high-intensity resistance training in young rats induced SkM remodeling and DNA hypomethylation, especially in the cellular stress response genes (59).

Although genome-wide methylation studies after PE in humans are scarce, and the results obtained in this review are based on limited number of studies and numerous limitations, present findings seem to be in line with previous findings in animal studies. This may serve as a starting point, which should raise the need for designing further interventional studies that will examine genome-wide DNAm according to the type of PE.

It has been reported that PE induces changes in DNAm levels of imprinted genes (19). In this line, we identified 45 imprinted genes out of 1851 DMGs in SkM, AT, blood, and sperm

that were modified after PE. Considering thatmethylation changes in imprinted genes are maintained during fertilization (61), it is suggested that these genes may be key candidates for transgenerational transmission of new methylation patterns to the next generations (80). This is especially relevant if methylation changes are in germline cells (17). Accordingly, in this study, we identified 21 paternally imprinted genes that were differently methylated in sperm after PE intervention. Because these genes were involved in disease regulation, it may be herein suggested that potential diseases in the offspring may be prevented by means of PE-inducing specific DNAm changes in sperm (19).

Furthermore, we have identified DNAm changes in imprinted genes in SkM, AT, and blood in this study. Fifteen genes were maternally imprinted, whereas 9 were paternally imprinted. DNA methylation changes in somatic tissues may also have a role in the transgenerational transmission of PE-induced effects (32,66,82). It is known that DNAm is controlled and regulated by miRNAs (14). miRNAs are transferring information among the cells mediating soma to germline communication (60,65,82). Soma to germline communication mediated by miRNAs has been proposed as another mechanism for transgenerational transmission of the new methylation marks from soma cells (66,82). In this line, previous studies have reported transgenerational transmission of PE-induced effects through miRNAs (5,32,49,72,85,86).

AE type of PE induced highest % of methylation changes in genes on chromosome 6, whereas AN and MT induced higher % of methylation changes in genes on chromosome 1. In comparison to AE and AN, MT of PE induced higher % of methylation changes that are close to TSS. Methylation changes that are close to TSS have been reported to increase the probability of transcriptional silencing that leads to gene repression (1,29,31,66). Gene repression is especially relevant for genes whose overexpression leads to certain disease (44).

It is well known that PE in general, AE or AN, has been recognized as an effective strategy to induce health-beneficial effects and maintain health (16). However, recent studies have been
promoting combined interventions relying on both aerobic and anaerobic mechanisms for energy production as more health beneficial (15,36,46,48,55,67,79,82). This seems to be in line with our results, suggesting that MT of PE induced higher % of methylation changes close to the TSS, in comparison to AE and AN. These methylation changes close to TSS may potentially lead to higher probability of gene repression in disease-related genes (29,31,66). Therefore, we suggest that present findings may contribute to the mechanistic explanation of why the previous literature has been promoting combined interventions or MT of PE as more health beneficial.

In general, genes that were differently methylated after PE intervention were involved in fat metabolism, immune system adaptations, protein catabolic process, muscle fiber organization, muscle development, cell growth, hypertrophy, hormone secretion, and neuronal differentiation. In addition, diseases regulated by genes that were modified after PE intervention are metabolic, cardiovascular, neurodegenerative diseases, musculoskeletal disorders, cancer, and various syndromes.

It is known that one of the reasons that might contribute to diseases development and progression is the overexpression of genes related to certain diseases, which is mediated by abnormal change in DNAm levels (21). Conversely, it is reported that aberrant methylation might be reversed to some extent with a specifically designed and tailored PE intervention (56). Accordingly, results from this review are suggesting that PE intervention may induce DNA methylation changes in genes that are regulating certain diseases. These PE-induced methylation changes may contribute to the explanation of the health-beneficial effects induced by PE. In linewith this, clinical studies are suggesting PE as a more effective treatment for metabolic (41,50), cardiovascular (76,78), and neurodegenerative diseases (70), osteoporosis (75) and cancer (6,8) in addition to the traditional therapy. Also, PE has been

reported to improve the quality of life in patients with Down's syndrome (58) and Prader-Willi syndrome (3).

Core genes are especially important for the biological processes undergoing in a living organism (69). Among the 1851 genes that were modified after PE intervention, we identified 19 core genes: ANAPC7, ANAPC10, ASB2, ASB8, ASB14, ATG7, CUL1, FBXL13, FBXW4, HERC2, KLHL11, KLHL41, NEDD4L, PRKN, RNF220, SMURF1, TRIM71, UBE2C, and UBE2J2. All of them were related to protein metabolism (40) and differently methylated in SkM after AE type of PE. However, no core genes were identified after AE type of PE from the DMGs in AT. Similarly, we did not identify any core genes after MT of PE (sperm and AT), neither after AN type of PE (whole blood, sperm, and SkM). Because PE is planned, structured, and repetitive bodily movement produced by the SkM (57), it is reasonable that core genes are identified in SkM as the most affected tissue by PE. Surprisingly, even if we found DMGs in SkM afterANtype of PE, we identified no core genes in AN because of the lack of interconnectivity networks among the genes. This indicates that a higher degree of entropy might be present in AN, thus resulting in low genes interconnectivity. In this line, previous study has reported that entropy was significantly higher at higher intensity of PE, in comparison to lower-intensity PE (25).

Finally, present findings indicate that PE can modify some of the most essential genes within the human genome related to protein metabolism by inducing DNAm changes. This may contribute to the understanding of the physiological and epigenetic mechanisms that may be underlying the health-beneficial effects induced by PE.

The limited number of studies and the heterogeneity in terms of duration of PE interventions, as well as tissue sample used to assess DNAm, are major limitation of this review. In general, heterogeneity of different aspects (including the heterogeneity of individual response and the time scale–dependent covariation) may be a limitation for each study that aims to generalize group pooled data results to individual effects in science. New statistical methods are being recently proposed to deal with the issues of interindividual and intraindividual (time- dependent) covariation (4). However, these methods require different study designs than the ones that have been reported in the scientific literature so far. A thorough change in the traditional experimental designs, as well as transition to the analysis of multivariate intensive longitudinal data will be needed in the future to overcome the issues related to interindividual and intraindividual covariation.

Additional limitation may be the biopsy sampling time. In the studies included in this review, as well as in other methylation studies that are assessing the effects of PE intervention, biopsy sampling time is usually up to 24 h after the last training session. Thus, we cannot exclude the potential bias that may be caused by the acute effects of PE. Future studies should be designed to overcome such limitation, maybe by including multiple time points of biopsy sampling and comparing thereafter the effects induced by acute PE and PE intervention. Including training and detraining phases may also help in overcoming this limitation.

Despite the numerous limitations of this review because of which our findings must be interpreted with caution, our results seem to be consistent with previous findings in animal studies. Moreover, present findings shed light on the epigenetic mechanisms that may potentially contribute to explain PE induced health benefits. This should raise the need for designing future genome-wide methylation studies that will examine the effects of different types of PE on DNAm.

Practical Applications

Main findings indicate that PE intervention may induce changes in DNAm of genes involved in fat metabolism, immune system adaptations, protein catabolic process, muscle fiber organization, muscle development, cell growth, hypertrophy, hormone secretion, neuronal differentiation, and in some disease-related genes. Moreover, PE intervention induced methylation changes in imprinted genes, as well as in some of the most essential genes within the genome, such as the core genes related to protein metabolism. These

findings may have a significant impact on the field of personalized medicine because they provide a better understanding of the mechanisms that are explaining the health benefits induced by PE, as well as its potential role in disease treatment and prevention. This may help the professional in exercise medicine in the process of designing appropriate PE interventions that will promote health and contribute in the treatment of a certain disease.

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