Received Date : 30-Nov-2016
Revised Date : 20-Mar-2017
Accepted Date : 23-Mar-2017
Article type : Review Article

Exercise and epigenetic inheritance of disease risk

Short title: Exercise and epigenetic inheritance

Joshua Denham[1*]

[1]School of Science and Technology, University of New England, Armidale, NSW, Australia.

[*]Corresponding author details: Room 259, Botany Building, School of Science and Technology, University of New England, Armidale, NSW, Australia, 2351. Email: jdenham2@une.edu.au; phone: +612 6773 5860; fax: +612 6773 3283.

Abstract

Epigenetics is the study of gene expression changes that occur in the absence of altered genotype. Current evidence indicates a role for environmentally induced alterations to epigenetic modifications leading to health and diseases changes across multiple generations. This phenomenon is called intergenerational or transgenerational epigenetic inheritance of health or disease.

Environmental insults, in the form of toxins, plastics and particular dietary interventions, perturb the epigenetic landscape and influence the health of F1 through to F4 generations in rodents. There is, however, the possibility that healthy lifestyles and environmental factors,

such as exercise training, could lead to favourable, heritable epigenetic modifications that augment transcriptional programs protective of disease, including metabolic dysfunction, heart disease and cancer. The health benefits conferred by regular physical exercise training are unquestionable, yet many of the molecular changes may have heritable health implications for future generations. Similar to other environmental factors, exercise modulates the epigenome of somatic cells and researchers are beginning to study exercise epigenetics in germ cells. The germ cell epigenetic modifications affected by exercise offer a molecular mechanism for the inheritance of health and disease risk.

The aims of this review are to: 1) provide an update on the expanding field of exercise epigenetics; 2) offer an overview of data on intergenerational/transgenerational epigenetic inheritance of disease by environmental insults; 3) to discuss the potential of exercise-induced intergenerational inheritance of health and disease risk; and finally, outline potential mechanisms and avenues for future work on epigenetic inheritance through exercise.

Key words: Epigenome, DNA methylation, exercise training, sperm, microRNA, disease prevention

Introduction

The health benefits of physical activity and exercise training are well established. However, the molecular mechanisms governing the health benefits generated from exercise training remain incompletely understood.[1, 2] Within the last decade epigenetic modifications have gained considerable attention.[3] Profound changes to epigenetic landscapes across numerous tissues are observed in response to a single bout of aerobic and resistance exercise, and with exercise adaptations from chronic exercise training.[4, 5] Some exercise-responsive genes,

including those important for immune,[6] muscle[7-9] and brain function,[4, 10, 11] are regulated by rearrangement of chromatin caused by epigenetic modifications.

Epigenetics is defined as changes to gene expression independent of sequence changes that are mitotically stable and, in some instances, heritable. DNA methylation and hydroxymethylation, acetylation of histone proteins, and microRNAs, are well-documented epigenetic mechanisms capable of influencing gene expression and are also controlled by exercise. The aforementioned epigenetic processes and the enzymes regulating these modifications will be the focus of this review (definitions in Table 1) and are discussed in detail elsewhere.[12-15] It is possible that exercise training prevents and manages the symptoms of many chronic diseases through the reprogramming of somatic cell epigenomes. More recently, data has indicated that exercise training modifies the epigenome of mouse and human spermatozoa.[16-18]

Unlike genetic (hard) inheritance, epigenetic (soft) inheritance is restricted to certain genes and other regions throughout the genome – in locations responsive to environmental perturbations – and occurs due to the incomplete epigenetic erasure and gamete reprogramming during fertilisation and embryogenesis.[19-21] If inherited, exercise-induced germ cell epigenetic modifications could influence embryo development and program neonatal transcriptomes, and ultimately influence offspring health and disease risk. Epigenetic inheritance through exercise could have an unprecedented affect on the health of future generations. Conversely, epigenetic inheritance of disease offers a mechanism for the growing incidence of physical inactivity-related diseases (e.g. obesity) plaguing modern society.

Exercise modifies epigenomes and controls health and performance adaptations

Skeletal muscle

The demands placed on skeletal muscle during exercise are responsible for intercellular signalling crucial for exercise adaptation.[22] Increased mitochondrial density and oxidative function is an established adaptation to exercise training and is facilitated by increased peroxisome proliferator-activated receptor gamma, co-activator 1 alpha (*PPARGC1A*) expression.[23] A single bout of cycling exercise demethylated promoter regions and increased the mRNA expression of key metabolic genes (*PPARGC1A*, *TFAM*, *PPARD* and *PDK4*) in humans.[8] Much like training adaptations, the epigenetic response to exercise is intensity dependent, where higher intensity exercise leads to a more pronounced demethylation.[8] In mice, the increased expression of *Ppargc1a* mRNA, however, occurred in the absence of promoter DNA methylation and hydroxymethylation changes but with elevated exonic histone-3 lysine-4 tri-methylation (H3K4me3) – a histone modification indicative of transcriptional activation – following exhaustive exercise.[24] The contrasting findings are potentially due to species and assay differences between the studies. The glucose transporter, *Glut4*, is another example of an epigenetically regulated gene influenced by a single exercise bout,[25] and *Glut4* like many others, is possibly facilitated by the nuclear export of class IIa histone deacetylases (HDAC4 and HDAC5).[26]

Non-coding RNA molecules also regulate skeletal muscle adaptations to exercise training, including the genetically conserved microRNAs (miRNA), which are typically 18–25 nucleotides long. The miR-378 family may regulate insulin sensitivity by the post-transcriptional regulation of *PPAR* genes, as the miR-378 gene located in intron 1 of the *PPARGC1B*, has predicted complementary binding to the *PPARGC1A/B* 3' untranslated regions (UTR) and is increased in skeletal muscle after a bout of interval training.[27] The miR-494 inhibits mitochondrial biogenesis by targeting the 3'UTR of the mitochondrial

transcription factor A (TFAM) and Forkhead Box J3 (FOXJ3) genes.[28] Notably, 7 days of endurance exercise increases skeletal muscle mitochondrial content, *Ppargc1a*, *Tfam* and *Foxj3* expression and decreases miR-494 abundance in mice.[28] In humans, a host of miRs are differentially expressed in skeletal muscle following a single bout of exercise and long-term exercise training and control important metabolic adaptations. For example, skeletal muscle miR-1 and miR-133a are increased after a single bout of moderate intensity cycling in healthy men.[29, 30] but the exercise-induced increased expression of these myomiRs is abrogated after 12 weeks of endurance training, followed by lower resting miR-1 and miR-133a expression.[30] A cross-institutional 6-wk aerobic training intervention significantly improved $\dot{V}O_{2max}$ in sedentary men with concomitant transcriptional and post-transcriptional reprogramming in skeletal muscle, demonstrating the coordinated mRNA and miRNA (miR-1, miR-101, miR-133a, miR-455) alterations underlying the exercise-induced gains to cardiorespiratory fitness.[31] The critical role of miR-133a in mitochondrial biogenesis and exercise performance was recently revealed. The expected mitochondrial, metabolic and performance adaptations to exercise training were abolished in miR-133a-deficient mice, with concomitant decreases in *Ppargc1a/g/b* and *Tfam* mRNA expression.[32] Similar to skeletal muscle adaptations to endurance exercise training, the epigenetic programming is reversible, as a 2-week period of exercise cessation reverts miR-1 and miR-133a to pre-exercise levels,[30] emphasising the need for consistent exercise training for metabolic function. Despite a lack of control subjects amongst the aforementioned human trials, these data highlight the roles of miRNAs in skeletal muscle adaptations to exercise.

Genome-wide surveys of skeletal muscle DNA methylation have also revealed coordinated reprogramming reflective of adaptation to exercise. Six months of moderate intensity exercise altered genome-wide DNA methylation status and gene expression in middle-aged individuals with and without a family history of type 2 diabetes, particularly in genes with

roles in pathways for metabolism (retinol metabolism, calcium and MAPK signalling.[7] Reprogramming of the skeletal muscle transcriptome and altered genome-wide DNA methylation in important exercise adaptation pathways – e.g. oxidative phosphorylation and blood vessel development – were observed in young individuals after three months of single leg knee extension exercise.[33] In this robust study, the increased performance (maximum power output), citrate synthase activity, DNA methylation and gene expression changes observed in the exercised leg were absent in the untrained leg, indicating the epigenetic changes were caused by the exercise training.[33] The findings also indicate that circulating factors, such as non-coding RNAs, may not regulate the systemic skeletal muscle epigenetic landscape. Therefore, exercise training – both acute and long term – is a powerful environmental factor responsible for epigenetic reprograming of skeletal muscle in metabolic genes and those with known roles in performance adaptations. While exercise is a favourable environment capable of modifying the skeletal muscle epigenome, inactivity is a harmful one that leads to metabolic dysfunction. Like the health benefits conferred by exercise training, the epigenetic landscape is perturbed by severe inactivity, as aberrant DNA methylation[34] and miRNA expression[35] reprogramming occurs with bed rest in humans, and hind limb suspension in mice.[36]

Heart

Extensive endurance exercise training leads to physiological cardiac hypertrophy, which unlike pathological cardiac hypertrophy is without cardiac dysfunction.[37, 38] Exercise-induced physiological left ventricular hypertrophy (LVH) increases stroke volume thereby improving maximal oxygen uptake and endurance performance. Swimming training leads to LVH with increased left ventricular miR-27a and miR-27b abundance and decreased target mRNA, angiotensin converting enzyme (*Ace*) in rats.[39] The same research group have revealed the

epigenetic control of LVH by modulation to miR-208 after exercise.[40] Rat miR-21, miR-144 and miR-124 are also involved in cardiac growth as they signal exercise-induced LVH through their targeted regulation of phosphatase and tensin homolog (PTEN) and phosphoinositide-3-kinase catalytic alpha (PI3Kα).[41] Increased miR-126 is implicated in cardiac angiogenesis associated with exercise training through its regulation of proteins in the vascular endothelial growth factor pathway (Pi3kr2 and Spred1) in rats.[42] Similarly, the role of miR-222 in cardiac hypertrophy was also revealed. Increased miR-222 induced cardio myocyte growth *in vitro*, and a similar increase was found after 3-wks of exercise (both voluntary wheel running and swimming models) in mouse cardio myocytes and in serum after acute exercise performed by cardiac patients.[43] Additional *in vitro* antagomiR and luciferase experiments confirmed miR-222's role in promoting cardio myocyte growth, proliferation and the prevention of apoptosis by targeting four transcripts (cell cycle inhibitor p27, homeodomain interacting protein kinase 1 and 2, and homeobox containing 1).[43] Finally, cardiac hypertrophy was blocked in exercised mice injected with LNA-anti-miR-222.[43]

Indeed, it seems that exercise is a therapeutic strategy to remediate adverse cardiac function caused by heart disease through regulation of epigenetic modifications. Cardiac dysfunction caused by myocardial infarction is attenuated by aerobic exercise training, and seems to be controlled by miRNAs. The aberrant increased and decreased expression of miR-214 and miR-1, respectively, caused by surgery-induced myocardial infarction (MI) in Wistar rats, was restored to pre-infarct levels after forced swimming exercise.[44] Moreover, exercise training prevented cardiac scarring and cardiac dysfunction after MI by increasing cardiac miR-29a and miR-29c abundance, subsequently reducing the expression of collagen gene (collagen type I alpha 1 and collagen type III alpha I).[45] The increased miR-29a expression

caused by exercise regulated key genes (*Hspg2*, *Sparc*, *Col4a1*, *Lamc1*, *Fbn1* and *Nid2*) and regenerated damaged muscle through the proliferation of myogenic progenitor cells in mice.[46]

Human aortic endothelial cells (HAECs) cultured *in vitro* with high-density lipo-protein cholesterol (HDLC) isolated from chronic heart failure (CHF) patients supresses the pro-angiogenic miRNAs, miR-21 and miR-126.[47] After moderate intensity cycling, however, the miR-21 and miR-126 expression was increased almost to a comparable level to that of the healthy controls.[47] Therefore, exercise training may restore angiogenic capacity of endothelial cells that is otherwise deregulated to elicit a pro-atherogenic environment in CHF patients, and circulating proteins (HDLC and IL-6) or molecules (exosomal RNAs) may modulate the epigenome of multiple tissues in response to exercise. The exercise-induced increased expression of miR-29b and miR-455 isolated from cardiac exosomes was associated with decreased cardiac matrix metalloprotease 9 gene expression, with a known role in cardiac fibrosis and myocyte uncoupling, in a mouse model of diabetes (db/db mice).[48] Thus, aerobic exercise training elicits LVH and effectively attenuates the adverse impacts of myocardial dysfunction caused by MI through the modulation of a network of miRNAs. It is, however, currently unknown whether other epigenetic modifications (e.g. DNA methylation, histone modifications) regulate cardiac adaptations conferred by exercise training.

Blood

Leukocyte epigenetic landscapes are implicated as risk factors of diseases, particularly cancer. Losses of global and long interspersed nuclear elements 1 (LINE-1) DNA methylation are indicative of ageing and cancer. Interestingly, self-reported physical activity is generally positively correlated to global DNA methylation in healthy, middle-aged and

older adults.[49, 50] Data is, however, equivocal as other reports present inverse correlations[51] or a lack of statistically significant associations between physical activity and global DNA methylation.[52] The discordant findings are could be explained by different methods of assessing DNA methylation status (LINE-1 versus luminometric methylation assay) and potential type 1 error due to modest statistical power. An issue with blood sampling is that leukocytes are heterogeneous and the epigenetic landscape is affected by the distribution of cell subsets. However, cell-lineage specific DNA methylation accurately estimates cell type distributions of leukocytes, and can be used to adjust statistical models to enable the reliable assessment of differentially methylated immune cells.[53, 54]

Superior cardiorespiratory fitness, a healthy blood lipid profile and improvements to body composition are well known adaptations to regular aerobic exercise training that are likely regulated by epigenetic mechanisms. Four weeks of sprint interval training (SIT) had a pronounced effect on genome-wide leukocyte DNA methylation in healthy young men.[55] Although the transcriptional responses were not quantified, the genome-wide DNA methylation changes occurred in genes enriched for pathways important for cardiovascular health, such as calcium, MAPK and PI3K-Akt signalling pathways, and were associated with the improvement to cardiorespiratory fitness and low-density lipo-protein lowering.[55] The blood epigenome could serve as a biomarker of exercise-related traits and responsiveness to exercise interventions. Five regions in or close to the aquaporin 9 (*AQP9*), dual specificity phosphatase 22 (*DUSP22*), homeodomain interacting protein kinase 3 (*HIPK3*), troponin T type 1 (*TNNT1*) and troponin I type 3 (*TNNI3*) were differentially methylated in overweight adolescent low and high responders to a multidisciplinary weight-loss intervention[56] Others revealed high-intensity walking exercise alleviates the ageing-related DNA methylation decrease in a pro-inflammatory cytokine gene, PYD and CARD domain containing (*ASC*),[57] and that while recombinant interleukin-6 increases protein content of DNMT3A and

DNMT3B *in vitro*, other circulating factors are responsible for acute regulation of epigenetic enzymes and chromatin remodelling elicited by exercise.[58] Many signalling molecules are implicated in initiating epigenetic reprogramming; these include transcription factors, reactive oxygen and nitrogen species, inflammatory cytokines and non-coding RNAs. It will be important to establish their potential roles in regulating epigenetic enzymes, the corresponding tissue-specific epigenetic modifications, corresponding transcriptional reprogramming and phenotypic adaptations after exercise training.

Circulating – e.g. whole blood, serum, plasma – miRNAs are a mixture of non-coding RNAs derived from peripheral tissues and leukocytes.[59, 60] For this reason, circulating miRNAs could serve as biomarkers of disease and exercise-associated traits because they respond to internal (biological) and external environments, such as lifestyle (reviewed in [61, 62]). Indeed, individuals with comparatively low versus high $\dot{V}O_{2max}$ (ml/kg$^{0.75}$/min) possessed increased serum miRNAs – miR-21, miR-210 and miR-222.[63] A marathon race increased the expression of plasma miR-1, miR-133a and miR-206 and the exercise-induced change in athletes' miRNAs were positively correlated to their $\dot{V}O_{2max}$ (n=14).[64] Muscle-enriched miRNAs are particularly strong candidates for exercise-related biomarkers, as resting whole blood content of miR-1 and miR-486 were positively correlated to $\dot{V}O_{2max}$ (n=123).[65] Furthermore, a host plasma/serum-based miRNAs are regulated by a single bout of aerobic exercise training[66] and long-term exercise training[67-69] (previously reviewed in [4]), and circulating miRNAs are differentially expressed between endurance and strength-trained athletes.[70, 71] Specifically, the endurance athletes possessed increased abundance of plasma miR-21, miR-221, miR-222 and miR-146a relative to strength athletes,[70] demonstrating the different signalling pathways associated with strength and endurance adaptations to particular modes of exercise.

The brain and other tissues

Exercise is an environmental (lifestyle) factor with the potential to systemically modify the epigenetic landscape across numerous tissues. Differentially expressed miRNAs have been found between exercising and non-exercising rodents in liver (miR-378b[72]), kidney (miR-192[73]), spinal cord after spinal cord injury (miR-21, miR-15b and miR-199a-3p)[74, 75] and in various areas of the brain, including the cerebral cortex after traumatic brain injury (miR-21, miR-92a, miR-124, miR-138, miR-874 and let-7c[76]), hippocampus (miR-124) – associated with stress resilience[77] – and the nucleus accumbens (miR-342-5p and miR-466).[78] The brain is responsive to exercise-induced global DNA methylation modifications[79, 80] leading to neurogenesis by demethylating the promoter region of *Bdnf* gene.[10] The brain seems particularly responsive to exercise-induced epigenetic regulation. Histone protein-3 lysine-9 methylation and the abundance of DNA methyltransferase (DNMT)-1 and DNMT3b are reduced following a single bout of exercise,[81] consistent with other data at the transcriptional level.[11] Histone modifications following exercise are also linked to improvements to learning and coping with psychological stress. Supporting the capacity of acute exercise to modulate the proteins responsible for modifying the epigenome, increased phospho-acetylation (on histone 3 at serine-10/lysine-14) – a histone modification reflective of transcriptional activation – was observed in the dentate gyrus of rats after forced swimming and remained elevated above basal levels four hours after the cessation of exercise;[82] an effect observed by others.[83] Decreased and increased activities of enzymes, histone deacetylase (HDAC) and histone acetyltransferase (HAT), respectively, are reasonable factors controlling histone modifications observed in the hippocampus of exercised rats.[84] It, therefore, seems that exercise training modulates the epigenome and miRNome to promote neuroplasticity and neurogenesis, which subsequently improves cognition and psychological resilience.

Collectively, these studies infer epigenetic modifications govern key adaptations to aerobic exercise training across numerous tissues by fine-tuning gene expression and signalling leading to translational reprogramming (Figure 1). Severe sedentary behaviour deregulates epigenetic modifications to facilitate the manifestation of cardio-metabolic risk factors (Figure 2). Exercise could influence epigenetic modifications to restore or remediate many cardio-metabolic diseases, including cardiac dysfunction, atherosclerosis and type 2 diabetes. If like many somatic cells, germ cells, are vulnerable to the epigenetic reprogramming conferred by exercise then these modifications may influence embryogenesis at the transcriptional level. This, in turn, could influence the somatic and germ cell epigenomes of the progeny ultimately provide a transcriptional framework conducive to the prevention of disease and exercise adaptation.

Epigenetic inheritance of health and disease risk: a focus on the affects of environmental insults, paternal and maternal exercise in mammals

Environmental insults

Evidence supporting the inheritance of health and disease risk is beginning to emerge, particularly in context with unfavourable, environmental insults. The seminal data supporting the premise that environmental reprogramming of DNA methylation leads to transgenerational inheritance of disease (male infertility) in F1 to F4 generations, was caused by endocrine disruptor – vinclozolin – treatment to gestating female rats.[85] The detrimental health consequences of vinclozolin, other toxins (e.g. bisphenol A, DDT and jet fuel) and environmental insults (e.g. stress, smoking and alcohol) treatment to gestating rodents include obesity, infertility, ovarian disease and behavioural consequences (stress response and mate preference) in subsequent offspring and are reviewed elsewhere.[86, 87]

Non-Mendelian inheritance of health and disease risk is not a new phenomenon. It presented by Jean-Baptiste Lamarck over 200 years ago in his Philosophie Zoologique (Zoological Philosophy) in 1809, when the molecular and cellular mechanisms were unthought-of, but which are now beginning to be uncovered. One of the earliest published observations possibly involving epigenetic inheritance of disease was by the physiologist, Charles-Édouard Brown-Séquard, in 1859. He found certain injuries to the spinal cord of guinea-pigs elicited convulsions and that some of their offspring displayed symptoms of the disease – a very rare condition in guinea-pigs.[88] Now, molecular mechanisms such as circulating non-coding RNAs and other epigenetic modifications offer explanations for the observed inheritance of disease.

More recently, maternal under nutrition during pregnancy is linked to numerous conditions in humans and mice, and acts through epigenetic mechanisms.[89-91] For instance, *in utero* embryo undernourishment lead to locus-specific differential DNA methylation, with retention of nucleosomes, in the F1 adult mouse sperm and was associated with transcriptional changes and metabolic disease in the F2 offspring (less muscle mass, more adiposity and glucose intolerance).[89] Notably, DNA methylation analyses were experimentally validated by two techniques (MeDIP-seq and bisulfite sequencing) and four to five litters were used to target the environmental impact on DNA methylation, rather than the influences of genetic variants. DNA methylation is not the only epigenetic modification implicated in this process. Small non-coding RNAs, targeting kinase activity, nervous system and early development pathways are deregulated in F3 sperm from vinclozolin-treated maternal ancestry.[92] Sperm non-coding RNAs have a distinct role in environmentally-induced multigenerational and transgenerational heritability of health and disease risk. Paternal psychological stress[93-95] and low protein diet[96] perturb sperm non-coding RNAs and transmit intergenerational health consequences in mouse F1 offspring. Offspring histone[96, 97] and DNA methylation

modifications[89, 90] are also affected by the paternal environment (low protein diet, cocaine abuse and *in utero* undernourishment). In humans, the sperm epigenome is modified by ageing[98, 99] and obesity.[100] Interestingly, the age-associated sperm DNA de-methylation of the *FOXK1* promoter is associated with offspring foetal cord blood methylation and accelerated gene demethylation in autistic youth.[98]

It is important to note the difference between transgenerational inheritance and environmental impacts leading to intergenerational or multigenerational inheritance (see Figure 3). It is currently unknown as to whether exercise training induces transgenerational epigenetic inheritance of health and disease risk. Current literature, however, suggests exercise leads to intergenerational inheritance of specific traits and these are discussed in the next section.

Paternal exercise and the inheritance of health and disease risk

Although it is unclear as to whether exercise alone influences the progenies' epigenomes and health status, preconceptional paternal exercise training (Figure 4) attenuates the aberrant sperm miRNA and DNA methylation alterations associated with high-fat diet-induced obesity and metabolic dysfunction. Specifically, a pre-conception swimming exercise or diet intervention (8 weeks) normalised body weight, glucose intolerance, plasma leptin and C-reactive protein concentration in obese sires initially fed a high fat diet.[18] The researchers also found high-fat diet lead to aberrantly expressed X-chromosome-associated sperm miRNAs, involved in cell cycle regulation, apoptosis and embryo development pathways (miR-503, miR-542-3p and miR-465b-5p, respectively), which were also restored to control levels by an 8-wk diet or exercise intervention[18] Finally, although the female offspring from obese sires were heavier, possessed larger renal fat deposits, higher plasma triglycerides and insulin resistance, both the diet or exercise interventions were effective at ameliorating the heritable metabolic consequences of paternal obesity[18] Therefore, paternal obesity was associated with

transmission of metabolic syndrome risk factors to the offspring and exercise was a successful intervention that prevented the harmful epigenetic inheritance of disease. There may be a vulnerability to other environment insults in those from a physically active ancestry who do not exercise. When challenged with a high fat diet for 12 weeks, the male offspring of exercised sires paired with non-litter mate control dams (C57B/6J mice) exhibited increased body weight and adiposity, glucose intolerance and insulin resistance due to a reduced basal metabolic rate.[17] The exercised sires showed a decreased skeletal muscle pyruvate dehydrogenase kinase 4 (*Pdk4*) mRNA expression, an important regulator of glucose metabolism, also reduced in skeletal muscle of the exercised sires' offspring[17] These data are consistent with human evidence suggesting *PDK4* mRNA is epigenetically controlled and decreased following exercise training.[8] Exercise-induced sperm epigenetic reprogramming could have contributed to the altered phenotypic and transcriptional changes to the skeletal muscle genes in the offspring from exercised sires, as differential miRNA expression (miR-21, miR-221, miR-431, miR-483-3p) and promoter DNA methylation was observed in metabolic genes (O-linked N-acetylglucosamine transferase [OGT], meningioma expressed antigen 5 [OGA], H19, imprinted maternally transcript [H19], protein tyrosine phosphatase non-receptor type 1 [Ptpn1]) in sperm from trained and untrained sires.[17] Notably, circulating miR-21 and miR-221 are controlled by exercise in humans.[63, 69, 70, 101] Thus, it is possible that preconception paternal exercise influences embryogenesis and the developing zygote through DNA methylation and miRNA regulation and this, in turn, increases offspring susceptibility to the metabolic consequences of high fat diet in the absence of exercise training in mice. The human implications of these data are troubling, given the widespread consumption of calorie-dense food and rising popularity of sedentarism throughout modern society. It will be critical to confirm these findings and identify whether exercise training could reverse the epigenetic programming and health consequences in F1

pups from a pre-conception paternal exercise ancestry. Despite the questionable translatability of rodent data to human, exercise-induced sperm epigenetic modifications are beginning to be explored in men.

In the only published exercise study analysing the human methylome in spermatozoa, three months of intense aerobic exercise training was associated with global DNA demethylation and specific differential DNA methylation in genes responsible for regulating the signalling milieus for health and performance adaptations (e.g. MAPK signalling, PI3K-Akt signalling and insulin secretion).[16] Of the paternally imprinted genes differentially methylated after intense exercise training, many were implicated in debilitating diseases, including cancers, Parkinson's disease and schizophrenia.[16] Regarding cognitive function, paternal exercise is associated with superior spatial learning and memory capacity in male pups with increased hippocampal *Bdnf* and reelin mRNA and protein expression.[102] The data supporting the potential for paternal exercise to confer intergenerational heritable changes through epigenetic reprogramming is limited and more work is needed to confirm the phenotypes transmittable by paternal exercise, impacts of different exercise modes and doses, epigenetic mechanisms and the risk associated with other environmental insults (e.g. high fat diet, psychological stress, etc) in context with exercise training interventions in the offspring.

Maternal exercise for inheritance of health and disease risk

Compared to the paternal effects, the maternal influences of exercise on the inheritance of health and disease risk are more established. There are critical time-points when maternal exercise reprograms the epigenetic landscape of the F0, F1 and F2 (Figure 3). The focus has been on F1 metabolic and psychological disorders following maternal gestational exercise,

with or without preconceptional exercise. The impacts of maternal exercise, at least on the first generation and in rodents, seem to elicit many of the known adaptations conferred by exercise training.

Maternal exercise during pregnancy has improved cognition (memory and learning) and reduced stressful behaviours (fear and anxiety), in first generation rodent offspring.[103-107] These positive cognitive and behavioural adaptations observed in pups following maternal gestational exercise are associated with increased brain Bdnf expression and neurogenesis,[103, 104, 107, 108] consistent with the elevated circulating BDNF observed in humans after acute and long term exercise training.[109, 110] These positive adaptations observed in pups exposed to maternal gestational exercise also extend to adulthood.[107] The behavioural and cognitive adaptations observed in offspring from a maternal prenatal (gestational) exercise – both forced swimming and voluntary wheel running – ancestries involved the adrenergic, serotonergic and N-methyl-D-aspartate pathways, as young offspring learning and memory ability is markedly reduced when the aforementioned pathways are pharmacologically inhibited.[106] Maternal exercise could be a powerful therapeutic strategy used to prevent harmful psychological impacts of substance abuse. For instance, pups from morphine-dependent, but maternally exercised rats displayed decreased anxiety and voluntary consumption of morphine compared to their peers from morphine-dependent, sedentary mothers.[111] Exercise elicits favourable outcomes on drug-addiction, such that it can suppress drug-seeking behaviour, reduce cravings and prevent relapse in humans.[112, 113] Similar findings suggest exercise as a maternal therapeutic strategy to buffer the harmful effects of psychological stress on the offspring,[114] though exercise performed by the offspring from stressed mothers may be the most effective treatment of prenatal stress exposure.[115] Supporting findings in rodents, it was revealed that maternal exercise before and during gestation was associated with improved academic performance in youth (6–18 y).[116]

Therefore, maternal exercise programs neurobiological pathways to promote resilience and cognitive performance, and the benefits of exercise for combatting substance abuse may extend to the progeny to prevent serious psychiatric disorders.

The ability of exercise to restore or abolish the harmful impacts of maternal environmental insults experienced by the first generation, such as intrauterine growth restriction[117, 118] and high-fat diet[119-122] were also recently revealed. Human studies indicate maternal gestational environment is linked to metabolic disease risk in the newborn.[123, 124] While maternal gestational weight gain was associated with an increased risk of offspring obesity at 8 years of age (n=5,125), maternal self-reported physical activity levels reduced the risk of infant obesity, assessed by body mass index.[123] Prenatal maternal exercise is also associated with lower birth weight in newborns and occurs with DNA methylation modifications. For instance, infants from mothers who self-reported the highest amounts of prenatal non-sedentary activities, exhibited lower body weights and less methylation at the pleiomorphic adenoma gene-like 1 (*PLAGL1*) gene differentially methylated region (DMR) compared to infants from less-active mothers.[125] Conversely, maternal gestational obesity is associated with increased DNA methylation (3.9%) of the *PLAGL1* gene was observed those newborns' cord blood (buffy coat), compared to newborns of mothers in a healthy weight range,[124] indicating maternal obesity could aberrantly program the offspring's epigenome to increase their susceptibility to obesity. A 3.9% does not seem a large magnitude of change, but it could be enough to elicit change of large biological significance. Remarkably, this hypothesis is supported by rodent experiments, as offspring from exercised (before and during pregnancy) mothers possessed less adiposity and circulating insulin, and improved glucose tolerance at 8 and 52 weeks of age.[120] Offspring of dams fed a high fat diet showed increased insulin and adiposity, though maternal exercise abolished the harmful effects of gestational maternal obesity.[120] Corroborating these findings, voluntary preconception and gestational

maternal exercise prevented the high-fat diet-induced glucose intolerance and DNA hypermethylation of the *Pgc1a* promoter region observed in 9-month old female offspring.[119] Thus, it is possible that both paternal and maternal obesity contribute to inheritance of metabolic syndrome in offspring and that preconceptional and gestational maternal exercise is an effective therapeutic strategy to prevent the dysfunctional epigenetic programming. Regarding maternal gestational exercise, it has been posited that whilst moderate doses could benefit the offspring, higher doses may lead to detrimental health consequences in later life.[126] It will be important to confirm this hypothesis and determine the potential affects of preconception paternal exercise has on epigenetic inheritance of health and disease risk in human.

Sex-differences are reported in some studies, such that maternal exercise with a high-fat diet[122] or exercise (before, during pregnancy and two weeks of lactation) alone[127] selectively prevents metabolic dysfunction in the male offspring, with much smaller or no effects in female offspring in rodents. Increased fat mass has been observed in male offspring from a maternal exercise ancestry, but not in female rats.[128] Differences were also noted at the molecular level, such that maternal exercise capable of preventing the harmful metabolic dysfunction in offspring in conjunction with restored gastrocnemius *Glut4* and *Myod1* mRNA expression in males, but not female rodent offspring.[122] An explanation for the observed sex-differences is the sexual dimorphic transcriptional profiles that occur in the placenta during gestational control and high-fat diet feeding in genes enriched for exercise signalling pathways (Akt, Erk1/2, Mapk, Vegf),[129] likely governed by genetic variants. Such dimorphic transcriptional profiles occur in placentas from dams fed high-fat or control diets and leads to modified risk of non-communicable diseases in the offspring, whereas preconceptional and gestational maternal exercise alone may not influence offspring metabolic disease risk factors (body composition and weight, and blood lipids), nor their propensity for voluntary exercise

or food consumption.[119, 130, 131] These are important findings for the consideration of future human studies. Conversely, maternal perinatal exercise was effective at decreasing offspring body weight, fat mass and glucose intolerance compared to their peers from sedentary dams[120, 122] and increasing skeletal muscle *Pgc1a* expression at post natal day 19.[122] Although mammary tumorigenesis[132] and the risk of age-related congenital heart disease[133] is reduced in the offspring from exercised dams, epigenetic modifications and regulating enzymes were not measured in these studies. Interestingly, the human foetus's heart may be responsive to maternal exercise, as maternal physical activity is positively correlated to foetal heart rate and duration of activity was negatively correlated with foetal heart rate variability.[134] Given the marked impact of exercise on the heart transcriptome and miRNome,[135-137] it is possible that epigenetic reprogramming is responsible for the reduced risk of offspring congenital heart disease risk[133] and other cardiovascular outcomes.

Some equivocal findings are potentially explained by the use of different rodent species and strains, timing of maternal exercise intervention (before and/or throughout gestation), potential instances of type 1 and 2 error.[138, 139] The inclusion of biological and technical replicates and validation experiments are warranted in future studies. Few studies have included an analysis of epigenetic modifications that will be necessary in order to ascertain epigenetic heritable effects. Addressing these considerations in future investigations will help isolate and provide insight on the epigenetic influence, and limit the genetic effects. Human studies will be required to verify the maternal and paternal exercise contributions to the inheritance of health and disease risk elicited by exercise and underpinning epigenetic mechanisms. Regardless, most findings support both maternal and paternal exercise as a therapeutic strategy to combat epigenetic inheritance of disease in the subsequent generation.

Epigenetic mechanisms for exercise-induced inheritance of health and disease risk

There are many molecular mechanisms and time-points (Figure 4) at which exercise could reprogram the epigenetic landscape of germ cells, primordial germ cells and the embryo to initiate exercise-induced intergenerational and transgenerational inheritance of health and disease risk. To data, there is no published evidence of transgenerational epigenetic inheritance elicited by exercise training. Whilst speculative, preconceptional and gestational exercise training could elicit transgenerational epigenetic adaptations in the offspring that enhance the capacity to respond to exercise training (e.g. $\dot{V}O_{2max}$, blood pressure and insulin sensitivity). This, in turn, could prevent disease by attenuating the harmful effects of environmental insults including high-caloric intake or psychological distress, and ultimately improve health span across multiple generations. The next section discusses possible mechanisms of inheritance of health and disease risk with consideration on critical time-points: preconception, embryogenesis and primordial germ cells maturation.

Germ line DNA methylation

Although mature germ cells (spermatozoa and oocyte) epigenomes are determined prenatally, they are not completely inert. DNMT, HDAC/HAT, TET enzymes are highly expressed in mammalian sperm[140-142] and oocytes,[143-145] which raises the question of whether or not exercise regulates the activity of epigenetic modifying enzymes of germ cells. Whilst mammalian oocytes are halted at the germinal vesicles until ovulation,[143] spermatogonia undergo mitosis to produce primary spermatocytes, which mature into spermatids through meiosis and then mature sperm (spermatozoa). DNMT1 is involved in DNA methylation maintenance and preferentially targets hemimethylated CpG sites.[146] As such, a decrease in DNMT1 activity could result in passive sperm DNA demethylation during replication. Progressive Dnmt3a and Dnmt3l-dependent methylation of mammalian oocytes occurs

during maturation.[147] Both sperm and oocytes methylomes could be demethylated actively by TET enzyme-mediated oxidation (hydroxymethylation, carboxylation, etc.). The production of reactive oxygen species, generated from the mitochondrial electron transport chain, is significantly increased during exercise[148] and could promote TET-mediated hydroxymethylation of cells, including somatic cells, gametes and primordial germ cells. The latter two could influence fertilisation, germ cell epigenetic modifications and underpin inheritance of health and disease risk via exercise. Conversely, DNMT3A or 3B enzymes cause *de novo* methylation changes and are regulated by exercise,[11, 58, 81] through energy sensing proteins (AMPK, HIF1A and NAD+) and metabolites (2-oxoglutarate, 2-hydroxyglutarate, succinate, fumarate, β-hydroxybutyrate) altered by exercise.[149]

While germ cell epigenome reprogramming through exercise-induced regulation of epigenetic enzymes seems plausible, an issue is that during early embryogenesis, the DNA methylomes of gametes, the zygote and primordial germ cells undergo widespread DNA methylation erasure. This is achieved by TET-mediated oxidation of 5-methylcytosine to 5-hydroxymethylcytosine[150] or 5-formylcytosine[151] before subsequent removal by base excision repair, through DNMT enzyme impairment.[152, 153] The purpose of the global DNA erasure is to promote a totipotent epigenome for the developing embryo. Between 59 and 137 days of development, the human prenatal primordial germ cells undergo *de novo* methylation modifications or undergo progressive demethylation in males and females, respectively.[154] At this stage any paternal epigenetic programming on the developing embryo are prevented, though physical interactions could cause indirect *in utero* consequences. Maternal exercise, however, during embryogenesis could program the epigenomes of the offspring and their primordial germ cells to promote intergenerational and transgenerational epigenetic inheritance of health and disease risk. However, some developmental, somatic and imprinted genes escape the global DNA erasure.[19] Of the human primordial germ cell CpG sites that

escape DNA methylation erasure at week 7 to 9 of development, many are within promoters, bodies and enhancers of genes involved in neural development and debilitating diseases (e.g. obesity, multiple sclerosis and schizophrenia).[155] For a specific discussion on exercise epigenetics and genomic imprinting, the reader is directed elsewhere.[5] Therefore, global DNA methylation erasure gene escapees offer candidate loci involved in inheritance of health and disease risk through environmental factors, including exercise.

Germ cell histone and protamine modifications

Histone modifications should also be considered as heritable programmers of health and disease risk. Oocyte histone modifications (methylation and acetylation) are crucial for their maturation and allow for the proper segregation of chromosomes during meiosis.[156, 157] Sperm histones are replaced with protamines upon maturation and again with oocyte nucleosomes during gamete fusion.[158] During the first phase of embryogenesis, approximately 31% and 8% of genes are expressed and possess H3K4me2 and H3K27me3, respectively.[158] Of note, oocytes at the germinal vesicle and MII phases are vulnerable to harmful effects of environmental factors, particularly ageing. For example, aberrant histone modifications in old oocytes that arise at the germinal vesicle phase, such that a large percentage lack H3K9me3, H3K36me2, H3K79me2 and H4K20me2.[159] Oocytes and pre-implantation embryos exhibit lower global DNA methylation at MII phase, 2-, 4- and 8-cell, morula and blastocyst stages in aged 35–40 week old compared to 6–8 week old mice, which is associated with a greater frequency of stillbirths and foetal malformations.[160] A gene microarray analysis revealed 530 differentially expressed genes between old and young oocytes including reduced expression of Dnmt enzymes (*Dnmt1o*, *Dnmt1s*, *Dnmt3a*, *Dnmt3l*), *Hdac2* and genes enriched for oxidative stress and mitochondrial function.[161] A single bout of exercise and long-term training regulates a host of metabolic genes[162-164] and

these are additional candidates for epigenetic inheritance and should be assessed in germ cells. Maternal obesity is not only linked to lower oocytes but DNA methylation is altered in metabolic genes (*Lep* and *Ppara*).[165] The aberrant DNA methylation profiles are transmitted to the oocytes of female offspring and caused liver mRNA expression changes.[165] Consistent with rodent data, human epidemiology studies support the concept that maternal and paternal obesity as a harmful environmental insult that are associated with modified cord blood methylation levels in *PLAGL1*, *MEG4* (maternal obesity) and *MEST*, *PEG3* and *NNAT* (paternal obesity).[124]

Spermatids house five histone proteins – H2AX, H2A, H2B (testes specific), H3, H4 and a linker protein, H1 – that collectively form a nucleosome vulnerable to post-translational epigenetic modifications (e.g. methylation, acetylation, ubiquitination and phosphorylation). Unlike oocytes, in sperm these histone proteins are disassembled and replaced by two protamine variants, PRM1 and PRM2, when the nucleus is elongated and compacted during the maturation of spermatids to spermatozoa.[166] Despite this, approximately 4% of the sperm genome retains nucleosomes with histone H3K9me and H3K27me3 and lie in developmental promoters (*HOX* genes), imprinted genes (*IGF2*, *MEST*, *HOTAIR*) and non-coding RNA (*XIST*, *TSIX*, *LET7E*, *MIR10a*, *MIR15a*, *MIR17*, *MIR96*, *MIR135b*, *MIR153*, *MIR488*, *MIR760*) loci.[167] The retained nucleosomes possess H3K27me3 around transcription start sites in sperm and decreased gene expression in the early embryo [158]. Moreover, sperm protamines are capable of epigenetic modifications, such as lysine acetylation and methylation.[168] Given the pronounced effects of exercise on some of the mature forms of the aforementioned miRNAs (miR-15a,[169] miR-17,[170] let-7e[169, 171] and miR-96[170]) and the correlation between histone modifications at retained sperm nucleosomes and embryonic gene expression, post-translational modifications are also potential mechanisms for epigenetic inheritance of health and disease risk through exercise.

Germ cell non-coding RNAs

Non-coding RNAs orchestrate the inheritance of health and disease risk. Both psychological stress and high-fat diet (obesity) perturb sperm non-coding RNAs that results in disorders in the subsequent progeny. When nine sperm-linked miRNAs – miR-29c, -30a, -30c, -32, -193-5p, -204, -375, 532-3p and -698 – that were previously up-regulated by in psychological stressed sires were micro-injected into a developing zygote, a reduction in the abundance of maternal mRNAs was observed.[172] Likewise, when total sperm RNA from high-fat diet fed mice with metabolic syndrome were injected into zygotes from healthy weight dams, the male offspring presented with symptoms of metabolic dysfunction.[173] Sperm RNAs 30–40 nucleotides long (predominantly transfer RNA-derived small RNAs [tsRNA]) caused metabolic syndrome in the male offspring of high fat diet-fed sires and regulated the expression of 62 genes enriched for oxidative stress, glucose input and apoptosis pathways in the eight-cell embryo.[173] Remarkably, an obesity legacy is transmitted F0 through to F1 and F2 via sperm non-coding RNAs, but the metabolic dysfunction is largely attenuated in the F3s, indicating a transgenerational effect that is diluted after the F2.[174] These are the most compelling evidence linking sperm non-coding RNAs to environmental inheritance of disorders. In line with rodent data, human spermatozoa small non-coding RNAs and DNA methylation patterns are deregulated by obesity and are significantly affected after eight weeks of gastric-bypass surgery,[100] suggesting the paternal gametes are vulnerable to epigenetic programming caused by environmental insults and that they may be heritable in humans. Additionally, those already studied, mainly miRNAs and other non-coding RNAs – long, piRNAs, snRNA, snoRNA, tRNA, rRNA, etc. – should be analysed in context with transmission of health and performance adaptations conferred by exercise. Whether sperm miRNAs involved in epigenetic inheritance of traits are transcribed from the sperm genome, sperm mitochondrial genome or are exogenously expressed and imported from semen,

oocytes or other organs via the circulation, or a combination, is relatively unknown. Sperm non-coding RNAs, as opposed to those found in semen, promote inheritance of metabolic disease,[173] yet those isolated from semen could also likely influence embryogenesis and deserves attention in future investigations.

Conclusion

Exercise is a lifestyle factor capable of reprograming the epigenetic landscape of numerous tissues and these modifications may be responsible for orchestrating many of the well-established health and performance adaptations (e.g. improved cardiorespiratory fitness, mitochondrial content, insulin sensitivity and LVH). Current data indicates both maternal and paternal exercise may serve as effective therapeutic strategies to counteract the harmful, transmittable epigenetic impacts of cardio-metabolic diseases and could lead to improved metabolic health – reduced body weight, adiposity and glucose control – and cognitive functioning in the subsequent generation.[18, 103, 107, 120, 122] There are, however, inconsistencies regarding when offspring experience the aforementioned adaptations and sex-differences, which will need addressing in future work. Data on transgenerational inheritance is lacking and the underlying molecular mechanisms of exercise-induced intergenerational inheritance of health and disease risk are sparse. The translatability of rodent epigenetic inheritance of health and disease risk by exercise should be examined in future work. If exercise regulates the epigenome of human germ cells (DNA methylation, histone modifications and non-coding RNAs) the premise of epigenetic inheritance of health and disease risk by exercise training cannot be ignored. To establish the link between exercise and epigenetic inheritance for health and disease prevention, it will be essential to elucidate the impact of different modes and doses – intensity, frequency and duration – of exercise, relative paternal and maternal contributions of heritable traits, analysed at different time-points (Figure 4), and to

isolate epigenetic from genetic heritable effects. Such studies could establish an extraordinary role for maternal and paternal exercise training to promote exercise-induced adaptations and encourage the prevention age-related chronic disease in future generations. They may also emphasise the need for tailored maternal and paternal preconceptional and gestational exercise programs to combat the expanding burden of lifestyle-related chronic diseases plaguing our current society.

Conflict of interest

None.

Author contribution

JD wrote and revised the manuscript.

References

1. Egan, B, Zierath, JR: Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab, 17**:** 162-184, 2013.

2. Loos, RJ, Hagberg, JM, Perusse, L, Roth, SM, Sarzynski, MA, Wolfarth, B, Rankinen, T, Bouchard, C: Advances in exercise, fitness, and performance genomics in 2014. Med Sci Sports Exerc, 47**:** 1105-1112, 2015.

3. Haig, D: Commentary: The epidemiology of epigenetics. Int J Epidemiol, 41**:** 13-16, 2012.

4. Denham, J, Marques, FZ, O'Brien, BJ, Charchar, FJ: Exercise: putting action into our epigenome. Sports Med, 44**:** 189-209, 2014.

5. Brown, WM: Exercise-associated DNA methylation change in skeletal muscle and the importance of imprinted genes: a bioinformatics meta-analysis. Br J Sports Med, 49**:** 1567-1578, 2015.

6. Zawadzka, M, Jagodzinski, P, P.: Exercise-induced epigenetic regulations in inflammatory related cells. J Appl Biomed, 15**:** 63-70, 2016.

7. Nitert, MD, Dayeh, T, Volkov, P, Elgzyri, T, Hall, E, Nilsson, E, Yang, BT, Lang, S, Parikh, H, Wessman, Y, Weishaupt, H, Attema, J, Abels, M, Wierup, N, Almgren, P, Jansson, PA, Ronn, T, Hansson, O, Eriksson, KF, Groop, L, Ling, C: Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. Diabetes, 61**:** 3322-3332, 2012.

8. Barres, R, Yan, J, Egan, B, Treebak, JT, Rasmussen, M, Fritz, T, Caidahl, K, Krook, A, O'Gorman, DJ, Zierath, JR: Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab, 15**:** 405-411, 2012.

9. Rowlands, DS, Page, RA, Sukala, WR, Giri, M, Ghimbovschi, SD, Hayat, I, Cheema, BS, Lys, I, Leikis, M, Sheard, PW, Wakefield, SJ, Breier, B, Hathout, Y, Brown, K, Marathi, R, Orkunoglu-Suer, FE, Devaney, JM, Leiken, B, Many, G, Krebs, J, Hopkins, WG, Hoffman, EP: Multi-omic integrated networks connect DNA methylation and miRNA with skeletal muscle plasticity to chronic exercise in Type 2 diabetic obesity. Physiol Genomics, 46**:** 747-765, 2014.

10. Gomez-Pinilla, F, Zhuang, Y, Feng, J, Ying, Z, Fan, G: Exercise impacts brain-derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. Eur J Neurosci, 33**:** 383-390, 2011.

11. Abel, JL, Rissman, EF: Running-induced epigenetic and gene expression changes in the adolescent brain. Int J Dev Neurosci, 31**:** 382-390, 2013.

12. Portela, A, Esteller, M: Epigenetic modifications and human disease. Nature Biotechnology, 28**:** 1057-1068, 2010.

13. Gibney, ER, Nolan, CM: Epigenetics and gene expression. Heredity, 105**:** 4-13, 2010.

14. Ivanov, M, Barragan, I, Ingelman-Sundberg, M: Epigenetic mechanisms of importance for drug treatment. Trends Pharmacol Sci, 35**:** 384-396, 2014.

15. Bhutani, N, Burns, DM, Blau, HM: DNA Demethylation Dynamics. Cell, 146**:** 866-872, 2011.

16. Denham, J, O'Brien, BJ, Harvey, JT, Charchar, FJ: Genome-wide sperm DNA methylation changes after 3 months of exercise training in humans. Epigenomics, 7**:** 717-731, 2015.

17. Murashov, AK, Pak, ES, Koury, M, Ajmera, A, Jeyakumar, M, Parker, M, Williams, O, Ding, J, Walters, D, Neufer, PD: Paternal long-term exercise programs offspring for low energy expenditure and increased risk for obesity in mice. FASEB J, 30**:** 775-784, 2016.

18. McPherson, NO, Owens, JA, Fullston, T, Lane, M: Preconception diet or exercise intervention in obese fathers normalizes sperm microRNA profile and metabolic syndrome in female offspring. Am J Physiol Endocrinol Metab, 308**:** E805-821, 2015.

19. Borgel, J, Guibert, S, Li, Y, Chiba, H, Schubeler, D, Sasaki, H, Forne, T, Weber, M: Targets and dynamics of promoter DNA methylation during early mouse development. Nat Genet, 42**:** 1093-1100, 2010.

20. Guerrero-Bosagna, C, Settles, M, Lucker, B, Skinner, MK: Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. PLoS One, 5**:** e13100, 2010.

21. Skinner, MK, Guerrero-Bosagna, C: Role of CpG deserts in the epigenetic transgenerational inheritance of differential DNA methylation regions. BMC Genomics, 15**:** 692, 2014.

22. Hawley, JA, Hargreaves, M, Joyner, MJ, Zierath, JR: Integrative biology of exercise. Cell, 159**:** 738-749, 2014.

23. Yan, Z, Okutsu, M, Akhtar, YN, Lira, VA: Regulation of exercise-induced fiber type transformation, mitochondrial biogenesis, and angiogenesis in skeletal muscle. J Appl Physiol (1985), 110**:** 264-274, 2011.

24. Lochmann, TL, Thomas, RR, Bennett, JP, Jr., Taylor, SM: Epigenetic Modifications of the PGC-1alpha Promoter during Exercise Induced Expression in Mice. PLoS One, 10**:** e0129647, 2015.

25. Smith, JA, Kohn, TA, Chetty, AK, Ojuka, EO: CaMK activation during exercise is required for histone hyperacetylation and MEF2A binding at the MEF2 site on the Glut4 gene. Am J Physiol Endocrinol Metab, 295**:** E698-704, 2008.

26. McGee, SL, Fairlie, E, Garnham, AP, Hargreaves, M: Exercise-induced histone modifications in human skeletal muscle. J Physiol, 587**:** 5951-5958, 2009.

27. McLean, CS, Mielke, C, Cordova, JM, Langlais, PR, Bowen, B, Miranda, D, Coletta, DK, Mandarino, LJ: Gene and MicroRNA Expression Responses to Exercise; Relationship with Insulin Sensitivity. PLoS One, 10**:** e0127089, 2015.

28. Yamamoto, H, Morino, K, Nishio, Y, Ugi, S, Yoshizaki, T, Kashiwagi, A, Maegawa, H: MicroRNA-494 regulates mitochondrial biogenesis in skeletal muscle through mitochondrial transcription factor A and Forkhead box j3. Am J Physiol Endocrinol Metab, 303**:** E1419-1427, 2012.

29. Russell, AP, Lamon, S, Boon, H, Wada, S, Guller, I, Brown, EL, Chibalin, AV, Zierath, JR, Snow, RJ, Stepto, N, Wadley, GD, Akimoto, T: Regulation of miRNAs in human skeletal muscle following acute endurance exercise and short-term endurance training. J Physiol, 591**:** 4637-4653, 2013.

30. Nielsen, S, Scheele, C, Yfanti, C, Akerstrom, T, Nielsen, AR, Pedersen, BK, Laye, MJ: Muscle specific microRNAs are regulated by endurance exercise in human skeletal muscle. J Physiol, 588**:** 4029-4037, 2010.

31. Keller, P, Vollaard, NB, Gustafsson, T, Gallagher, IJ, Sundberg, CJ, Rankinen, T, Britton, SL, Bouchard, C, Koch, LG, Timmons, JA: A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. J Appl Physiol (1985), 110**:** 46-59, 2011.

32. Nie, Y, Sato, Y, Wang, C, Yue, F, Kuang, S, Gavin, TP: Impaired exercise tolerance, mitochondrial biogenesis, and muscle fiber maintenance in miR-133a–deficient mice. FASEB J, 30**:** 3745-3758, 2016.

33. Lindholm, ME, Marabita, F, Gomez-Cabrero, D, Rundqvist, H, Ekstrom, TJ, Tegner, J, Sundberg, CJ: An integrative analysis reveals coordinated reprogramming of the epigenome and the transcriptome in human skeletal muscle after training. Epigenetics, 9**:** 1557-1569, 2014.

34. Alibegovic, AC, Sonne, MP, Hojbjerre, L, Bork-Jensen, J, Jacobsen, S, Nilsson, E, Faerch, K, Hiscock, N, Mortensen, B, Friedrichsen, M, Stallknecht, B, Dela, F, Vaag, A: Insulin resistance induced by physical inactivity is associated with multiple transcriptional changes in skeletal muscle in young men. Am J Physiol Endocrinol Metab, 299**:** E752-763, 2010.

35. Ringholm, S, Bienso, RS, Kiilerich, K, Guadalupe-Grau, A, Aachmann-Andersen, NJ, Saltin, B, Plomgaard, P, Lundby, C, Wojtaszewski, JF, Calbet, JA, Pilegaard, H: Bed rest reduces metabolic protein content and abolishes exercise-induced mRNA responses in human skeletal muscle. Am J Physiol Endocrinol Metab, 301**:** E649-658, 2011.

36. Aoi, W, Naito, Y, Mizushima, K, Takanami, Y, Kawai, Y, Ichikawa, H, Yoshikawa, T: The microRNA miR-696 regulates PGC-1{alpha} in mouse skeletal muscle in response to physical activity. Am J Physiol Endocrinol Metab, 298**:** E799-806, 2010.

37. Pluim, BM, Zwinderman, AH, van der Laarse, A, van der Wall, EE: The athlete's heart. A meta-analysis of cardiac structure and function. Circulation, 101**:** 336-344, 2000.

38. Prior, DL, La Gerche, A: The athlete's heart. Heart, 98**:** 947-955, 2012.

39. Fernandes, T, Hashimoto, NY, Magalhaes, FC, Fernandes, FB, Casarini, DE, Carmona, AK, Krieger, JE, Phillips, MI, Oliveira, EM: Aerobic exercise training-induced left ventricular hypertrophy involves regulatory MicroRNAs, decreased angiotensin-converting enzyme-angiotensin ii, and synergistic regulation of angiotensin-converting enzyme 2-angiotensin (1-7). Hypertension, 58**:** 182-189, 2011.

40. Soci, UPR, Fernandes, T, Barauna, VG, Hashimoto, NY, de Fatima Alves Mota, G, Rosa, KT, Irigoyen, MC, Philips, MI, de Oliveira, EM: Epigenetic control of exercise training-induced cardiac hypertrophy by miR-208. Clinical Science, 2016.

41. Ma, Z, Qi, J, Meng, S, Wen, B, Zhang, J: Swimming exercise training-induced left ventricular hypertrophy involves microRNAs and synergistic regulation of the PI3K/AKT/mTOR signaling pathway. Eur J Appl Physiol, 113**:** 2473-2486, 2013.

42. D. A. Silva ND, J, Fernandes, T, Soci, UP, Monteiro, AW, Phillips, MI, EM, DEO: Swimming training in rats increases cardiac MicroRNA-126 expression and angiogenesis. Med Sci Sports Exerc, 44**:** 1453-1462, 2012.

43. Liu, X, Xiao, J, Zhu, H, Wei, X, Platt, C, Damilano, F, Xiao, C, Bezzerides, V, Bostrom, P, Che, L, Zhang, C, Spiegelman, BM, Rosenzweig, A: miR-222 is necessary for exercise-induced cardiac growth and protects against pathological cardiac remodeling. Cell Metab, 21**:** 584-595, 2015.

44. Melo, SF, Barauna, VG, Neves, VJ, Fernandes, T, Lara Lda, S, Mazzotti, DR, Oliveira, EM: Exercise training restores the cardiac microRNA-1 and -214 levels regulating Ca(2+) handling after myocardial infarction. BMC Cardiovasc Disord, 15**:** 166, 2015.

45. Melo, SF, Fernandes, T, Barauna, VG, Matos, KC, Santos, AA, Tucci, PJ, Oliveira, EM: Expression of MicroRNA-29 and Collagen in Cardiac Muscle after Swimming Training in Myocardial-Infarcted Rats. Cell Physiol Biochem, 33**:** 657-669, 2014.

46. Galimov, A, Merry, TL, Luca, E, Rushing, EJ, Mizbani, A, Turcekova, K, Hartung, A, Croce, CM, Ristow, M, Krutzfeldt, J: MicroRNA-29a in Adult Muscle Stem Cells Controls Skeletal Muscle Regeneration During Injury and Exercise Downstream of Fibroblast Growth Factor-2. Stem Cells, 34**:** 768-780, 2016.

47. Riedel, S, Radzanowski, S, Bowen, TS, Werner, S, Erbs, S, Schuler, G, Adams, V: Exercise training improves high-density lipoprotein-mediated transcription of proangiogenic microRNA in endothelial cells. Eur J Prev Cardiol, 22**:** 899-903, 2015.

48. Chaturvedi, P, Kalani, A, Medina, I, Familtseva, A, Tyagi, SC: Cardiosome mediated regulation of MMP9 in diabetic heart: role of mir29b and mir455 in exercise. J Cell Mol Med, 19**:** 2153-2161, 2015.

49. White, AJ, Sandler, DP, Bolick, SC, Xu, Z, Taylor, JA, DeRoo, LA: Recreational and household physical activity at different time points and DNA global methylation. Eur J Cancer, 49**:** 2199-2206, 2013.

50. Zhang, FF, Cardarelli, R, Carroll, J, Zhang, S, Fulda, KG, Gonzalez, K, Vishwanatha, JK, Morabia, A, Santella, RM: Physical activity and global genomic DNA methylation in a cancer-free population. Epigenetics, 6**:** 293-299, 2011.

51. Luttropp, K, Nordfors, L, Ekstrom, TJ, Lind, L: Physical activity is associated with decreased global DNA methylation in Swedish older individuals. Scand J Clin Lab Invest, 73**:** 184-185, 2013.

52. Zhang, FF, Santella, RM, Wolff, M, Kappil, MA, Markowitz, SB, Morabia, A: White blood cell global methylation and IL-6 promoter methylation in association with diet and lifestyle risk factors in a cancer-free population. Epigenetics, 7**:** 606-614, 2012.

53. Houseman, EA, Accomando, WP, Koestler, DC, Christensen, BC, Marsit, CJ, Nelson, HH, Wiencke, JK, Kelsey, KT: DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics, 13**:** 86, 2012.

54. Koestler, DC, Christensen, B, Karagas, MR, Marsit, CJ, Langevin, SM, Kelsey, KT, Wiencke, JK, Houseman, EA: Blood-based profiles of DNA methylation predict the underlying distribution of cell types: a validation analysis. Epigenetics, 8**:** 816-826, 2013.

55. Denham, J, O'Brien, BJ, Marques, FZ, Charchar, FJ: Changes in the leukocyte methylome and its effect on cardiovascular-related genes after exercise. J Appl Physiol (1985), 118**:** 475-488, 2015.

56. Moleres, A, Campion, J, Milagro, FI, Marcos, A, Campoy, C, Garagorri, JM, Gomez-Martinez, S, Martinez, JA, Azcona-Sanjulian, MC, Marti, A, Group, ES: Differential DNA methylation patterns between high and low responders to a weight loss intervention in overweight or obese adolescents: the EVASYON study. FASEB J, 27**:** 2504-2512, 2013.

57. Nakajima, K, Takeoka, M, Mori, M, Hashimoto, S, Sakurai, A, Nose, H, Higuchi, K, Itano, N, Shiohara, M, Oh, T, Taniguchi, S: Exercise effects on methylation of ASC gene. Int J Sports Med, 31**:** 671-675, 2010.

58. Horsburgh, S, Todryk, S, Toms, C, Moran, CN, Ansley, L: Exercise-conditioned plasma attenuates nuclear concentrations of DNA methyltransferase 3B in human peripheral blood mononuclear cells. Physiol Rep, 3**:** e12621, 2015.

59. Pritchard, CC, Kroh, E, Wood, B, Arroyo, JD, Dougherty, KJ, Miyaji, MM, Tait, JF, Tewari, M: Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. Cancer Prev Res (Phila), 5**:** 492-497, 2012.

60. Ma, R, Jiang, T, Kang, X: Circulating microRNAs in cancer: origin, function and application. J Exp Clin Cancer Res, 31**:** 38, 2012.

61. Polakovicova, M, Musil, P, Laczo, E, Hamar, D, Kyselovic, J: Circulating MicroRNAs as Potential Biomarkers of Exercise Response. Int J Mol Sci, 17, 2016.

62. Xu, T, Liu, Q, Yao, J, Dai, Y, Wang, H, Xiao, J: Circulating microRNAs in response to exercise. Scand J Med Sci Sports, 25**:** e149-154, 2015.

63. Bye, A, Rosjo, H, Aspenes, ST, Condorelli, G, Omland, T, Wisloff, U: Circulating microRNAs and aerobic fitness--the HUNT-Study. PLoS One, 8**:** e57496, 2013.

64. Mooren, FC, Viereck, J, Kruger, K, Thum, T: Circulating microRNAs as potential biomarkers of aerobic exercise capacity. Am J Physiol Heart Circ Physiol, 306**:** H557-563, 2014.

65. Denham, J, Prestes, P, R.: Muscle-Enriched MicroRNAs Isolated From Whole Blood Are Regulated By Exercise And Are Potential Biomarkers of Cardiorespiratory Fitness. Front Genet, 7**:** 196, 2016.

66. Uhlemann, M, Mobius-Winkler, S, Fikenzer, S, Adam, J, Redlich, M, Mohlenkamp, S, Hilberg, T, Schuler, GC, Adams, V: Circulating microRNA-126 increases after different forms of endurance exercise in healthy adults. Eur J Prev Cardiol, 21**:** 484-491, 2014.

67. Aoi, W, Ichikawa, H, Mune, K, Tanimura, Y, Mizushima, K, Naito, Y, Yoshikawa, T: Muscle-enriched microRNA miR-486 decreases in circulation in response to exercise in young men. Front Physiol, 4**:** 80, 2013.

68. Baggish, AL, Hale, A, Weiner, RB, Lewis, GD, Systrom, D, Wang, F, Wang, TJ, Chan, SY: Dynamic regulation of circulating microRNA during acute exhaustive exercise and sustained aerobic exercise training. J Physiol, 589**:** 3983-3994, 2011.

69. Nielsen, S, Akerstrom, T, Rinnov, A, Yfanti, C, Scheele, C, Pedersen, BK, Laye, MJ: The miRNA plasma signature in response to acute aerobic exercise and endurance training. PLoS One, 9**:** e87308, 2014.

70. Wardle, SL, Bailey, ME, Kilikevicius, A, Malkova, D, Wilson, RH, Venckunas, T, Moran, CN: Plasma microRNA levels differ between endurance and strength athletes. PLoS One, 10**:** e0122107, 2015.

71. Hecksteden, A, Leidinger, P, Backes, C, Rheinheimer, S, Pfeiffer, M, Ferrauti, A, Kellmann, M, Sedaghat, F, Meder, B, Meese, E, Meyer, T, Keller, A: miRNAs and sports: tracking training status and potentially confounding diagnoses. J Transl Med, 14**:** 219, 2016.

72. Lu, YL, Jing, W, Feng, LS, Zhang, L, Xu, JF, You, TJ, Zhao, J: Effects of hypoxic exercise training on microRNA expression and lipid metabolism in obese rat livers. J Zhejiang Univ Sci B, 15**:** 820-829, 2014.

73. Oghbaei, H, Ahmadi Asl, N, Sheikhzadeh, F, Alipour, MR, Khamaneh, AM: The Effect of Regular Moderate Exercise on miRNA-192 Expression Changes in Kidney of Streptozotocin-Induced Diabetic Male Rats. Adv Pharm Bull, 5**:** 127-132, 2015.

74. Liu, G, Detloff, MR, Miller, KN, Santi, L, Houle, JD: Exercise modulates microRNAs that affect the PTEN/mTOR pathway in rats after spinal cord injury. Exp Neurol, 233**:** 447-456, 2012.

75. Liu, G, Keeler, BE, Zhukareva, V, Houle, JD: Cycling exercise affects the expression of apoptosis-associated microRNAs after spinal cord injury in rats. Exp Neurol, 226**:** 200-206, 2010.

76. Miao, W, Bao, TH, Han, JH, Yin, M, Yan, Y, Wang, WW, Zhu, YH: Voluntary exercise prior to traumatic brain injury alters miRNA expression in the injured mouse cerebral cortex. Braz J Med Biol Res, 48**:** 433-439, 2015.

77. Pan-Vazquez, A, Rye, N, Ameri, M, McSparron, B, Smallwood, G, Bickerdyke, J, Rathbone, A, Dajas-Bailador, F, Toledo-Rodriguez, M: Impact of voluntary exercise and housing conditions on hippocampal glucocorticoid receptor, miR-124 and anxiety. Mol Brain, 8**:** 40, 2015.

78. Dawes, M, Kochan, KJ, Riggs, PK, Timothy Lightfoot, J: Differential miRNA expression in inherently high- and low-active inbred mice. Physiol Rep, 3: e12469, 2015.

79. Rodrigues, GM, Jr., Toffoli, LV, Manfredo, MH, Francis-Oliveira, J, Silva, AS, Raquel, HA, Martins-Pinge, MC, Moreira, EG, Fernandes, KB, Pelosi, GG, Gomes, MV: Acute stress affects the global DNA methylation profile in rat brain: modulation by physical exercise. Behav Brain Res, 279**:** 123-128, 2015.

80. Kashimoto, RK, Toffoli, LV, Manfredo, MH, Volpini, VL, Martins-Pinge, MC, Pelosi, GG, Gomes, MV: Physical exercise affects the epigenetic programming of rat brain and modulates the adaptive response evoked by repeated restraint stress. Behav Brain Res, 296**:** 286-289, 2016.

81. Elsner, VR, Lovatel, GA, Moyses, F, Bertoldi, K, Spindler, C, Cechinel, LR, Muotri, AR, Siqueira, IR: Exercise induces age-dependent changes on epigenetic parameters in rat hippocampus: a preliminary study. Exp Gerontol, 48**:** 136-139, 2013.

82. Chandramohan, Y, Droste, SK, Arthur, JS, Reul, JM: The forced swimming-induced behavioural immobility response involves histone H3 phospho-acetylation and c-Fos induction in dentate gyrus granule neurons via activation of the N-methyl-D-aspartate/extracellular signal-regulated kinase/mitogen- and stress-activated kinase signalling pathway. Eur J Neurosci, 27**:** 2701-2713, 2008.

83. Collins, A, Hill, LE, Chandramohan, Y, Whitcomb, D, Droste, SK, Reul, JM: Exercise improves cognitive responses to psychological stress through enhancement of

epigenetic mechanisms and gene expression in the dentate gyrus. PLoS One, 4**:** e4330, 2009.

84. Elsner, VR, Lovatel, GA, Bertoldi, K, Vanzella, C, Santos, FM, Spindler, C, de Almeida, EF, Nardin, P, Siqueira, IR: Effect of different exercise protocols on histone acetyltransferases and histone deacetylases activities in rat hippocampus. Neuroscience, 192**:** 580-587, 2011.

85. Anway, MD, Cupp, AS, Uzumcu, M, Skinner, MK: Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science, 308**:** 1466-1469, 2005.

86. Hanson, MA, Skinner, MK: Developmental origins of epigenetic transgenerational inheritance. Environ Epigenet, 2**:** dvw002, 2016.

87. Nilsson, EE, Skinner, MK: Environmentally induced epigenetic transgenerational inheritance of disease susceptibility. Transl Res, 165**:** 12-17, 2015.

88. Brown-Séquard, C-E: Hereditary transmission of an epileptiform affection accidentally produced. Proc R Soc London, 10**:** 297-298, 1859.

89. Radford, EJ, Ito, M, Shi, H, Corish, JA, Yamazawa, K, Isganaitis, E, Seisenberger, S, Hore, TA, Reik, W, Erkek, S, Peters, AH, Patti, ME, Ferguson-Smith, AC: In utero effects. In utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism. Science, 345**:** 1255903, 2014.

90. Martinez, D, Pentinat, T, Ribo, S, Daviaud, C, Bloks, VW, Cebria, J, Villalmanzo, N, Kalko, SG, Ramon-Krauel, M, Diaz, R, Plosch, T, Tost, J, Jimenez-Chillaron, JC: In utero undernutrition in male mice programs liver lipid metabolism in the second-generation offspring involving altered Lxra DNA methylation. Cell Metab, 19**:** 941-951, 2014.

91. Lee, HS: Impact of Maternal Diet on the Epigenome during In Utero Life and the Developmental Programming of Diseases in Childhood and Adulthood. Nutrients, 7**:** 9492-9507, 2015.

92. Schuster, A, Skinner, MK, Yan, W: Ancestral vinclozolin exposure alters the epigenetic transgenerational inheritance of sperm small noncoding RNAs. Environ Epigenet, 2: dvw001, 2016.

93. Wu, L, Lu, Y, Jiao, Y, Liu, B, Li, S, Li, Y, Xing, F, Chen, D, Liu, X, Zhao, J, Xiong, X, Gu, Y, Lu, J, Chen, X, Li, X: Paternal Psychological Stress Reprograms Hepatic Gluconeogenesis in Offspring. Cell Metab, 23**:** 735-743, 2016.

94. Gapp, K, Jawaid, A, Sarkies, P, Bohacek, J, Pelczar, P, Prados, J, Farinelli, L, Miska, E, Mansuy, IM: Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. Nat Neurosci, 17**:** 667-669, 2014.

95. Rodgers, AB, Morgan, CP, Bronson, SL, Revello, S, Bale, TL: Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation. J Neurosci, 33**:** 9003-9012, 2013.

96. Carone, BR, Fauquier, L, Habib, N, Shea, JM, Hart, CE, Li, R, Bock, C, Li, C, Gu, H, Zamore, PD, Meissner, A, Weng, Z, Hofmann, HA, Friedman, N, Rando, OJ: Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell, 143**:** 1084-1096, 2010.

97. Vassoler, FM, White, SL, Schmidt, HD, Sadri-Vakili, G, Pierce, RC: Epigenetic inheritance of a cocaine-resistance phenotype. Nat Neurosci, 16**:** 42-47, 2013.

98. Atsem, S, Reichenbach, J, Potabattula, R, Dittrich, M, Nava, C, Depienne, C, Bohm, L, Rost, S, Hahn, T, Schorsch, M, Haaf, T, El Hajj, N: Paternal age effects on sperm FOXK1 and KCNA7 methylation and transmission into the next generation. Hum Mol Genet, 25**:** 4996-5005, 2016.

99. Kobayashi, N, Okae, H, Hiura, H, Chiba, H, Shirakata, Y, Hara, K, Tanemura, K, Arima, T: Genome-Scale Assessment of Age-Related DNA Methylation Changes in Mouse Spermatozoa. PLoS One, 11**:** e0167127, 2016.

100. Donkin, I, Versteyhe, S, Ingerslev, LR, Qian, K, Mechta, M, Nordkap, L, Mortensen, B, Appel, EV, Jorgensen, N, Kristiansen, VB, Hansen, T, Workman, CT, Zierath, JR, Barres, R: Obesity and Bariatric Surgery Drive Epigenetic Variation of Spermatozoa in Humans. Cell Metab, 23**:** 369-378, 2016.

101. Xu, T, Zhou, Q, Che, L, Das, S, Wang, L, Jiang, J, Li, G, Xu, J, Yao, J, Wang, H, Dai, Y, Xiao, J: Circulating miR-21, miR-378, and miR-940 increase in response to an acute exhaustive exercise in chronic heart failure patients. Oncotarget, 7**:** 12414-12425, 2016.

102. Yin, MM, Wang, W, Sun, J, Liu, S, Liu, XL, Niu, YM, Yuan, HR, Yang, FY, Fu, L: Paternal treadmill exercise enhances spatial learning and memory related to hippocampus among male offspring. Behav Brain Res, 253**:** 297-304, 2013.

103. Gomes da Silva, S, de Almeida, AA, Fernandes, J, Lopim, GM, Cabral, FR, Scerni, DA, de Oliveira-Pinto, AV, Lent, R, Arida, RM: Maternal Exercise during Pregnancy Increases BDNF Levels and Cell Numbers in the Hippocampal Formation but Not in the Cerebral Cortex of Adult Rat Offspring. PLoS One, 11**:** e0147200, 2016.

104. Lee, HH, Kim, H, Lee, JW, Kim, YS, Yang, HY, Chang, HK, Lee, TH, Shin, MC, Lee, MH, Shin, MS, Park, S, Baek, S, Kim, CJ: Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups. Brain Dev, 28**:** 147-154, 2006.

105. Kim, H, Lee, SH, Kim, SS, Yoo, JH, Kim, CJ: The influence of maternal treadmill running during pregnancy on short-term memory and hippocampal cell survival in rat pups. Int J Dev Neurosci, 25**:** 243-249, 2007.

106. Akhavan, MM, Emami-Abarghoie, M, Safari, M, Sadighi-Moghaddam, B, Vafaei, AA, Bandegi, AR, Rashidy-Pour, A: Serotonergic and noradrenergic lesions suppress the enhancing effect of maternal exercise during pregnancy on learning and memory in rat pups. Neuroscience, 151**:** 1173-1183, 2008.

107. Aksu, I, Baykara, B, Ozbal, S, Cetin, F, Sisman, AR, Dayi, A, Gencoglu, C, Tas, A, Buyuk, E, Gonenc-Arda, S, Uysal, N: Maternal treadmill exercise during pregnancy decreases anxiety and increases prefrontal cortex VEGF and BDNF levels of rat pups in early and late periods of life. Neurosci Lett, 516**:** 221-225, 2012.

108. Robinson, AM, Bucci, DJ: Physical exercise during pregnancy improves object recognition memory in adult offspring. Neuroscience, 256**:** 53-60, 2014.

109. Griffin, EW, Mullally, S, Foley, C, Warmington, SA, O'Mara, SM, Kelly, AM: Aerobic exercise improves hippocampal function and increases BDNF in the serum of young adult males. Physiol Behav, 104**:** 934-941, 2011.

110. Seifert, T, Brassard, P, Wissenberg, M, Rasmussen, P, Nordby, P, Stallknecht, B, Adser, H, Jakobsen, AH, Pilegaard, H, Nielsen, HB, Secher, NH: Endurance training enhances BDNF release from the human brain. Am J Physiol Regul Integr Comp Physiol, 298**:** R372-377, 2010.

111. Haydari, S, Miladi-Gorji, H, Mokhtari, A, Safari, M: Effects of voluntary exercise on anxiety-like behavior and voluntary morphine consumption in rat pups borne from morphine-dependent mothers during pregnancy. Neurosci Lett, 578**:** 50-54, 2014.

112. Lynch, WJ, Peterson, AB, Sanchez, V, Abel, J, Smith, MA: Exercise as a novel treatment for drug addiction: a neurobiological and stage-dependent hypothesis. Neurosci Biobehav Rev, 37**:** 1622-1644, 2013.

113. Zhou, Y, Zhao, M, Zhou, C, Li, R: Sex differences in drug addiction and response to exercise intervention: From human to animal studies. Front Neuroendocrinol, 40**:** 24-41, 2016.

114. Bustamante, C, Henriquez, R, Medina, F, Reinoso, C, Vargas, R, Pascual, R: Maternal exercise during pregnancy ameliorates the postnatal neuronal impairments induced by prenatal restraint stress in mice. Int J Dev Neurosci, 31**:** 267-273, 2013.

115. Kim, TW, Ji, ES, Kim, TW, Lee, SW, Lee, CY, Lee, SJ: Postnatal treadmill exercise attenuates prenatal stress-induced apoptosis through enhancing serotonin expression in aged-offspring rats. J Exerc Rehabil, 11**:** 12-19, 2015.

116. Esteban-Cornejo, I, Martinez-Gomez, D, Tejero-Gonzalez, CM, Izquierdo-Gomez, R, Carbonell-Baeza, A, Castro-Pinero, J, Sallis, JF, Veiga, OL, Up, Group, DS: Maternal physical activity before and during the prenatal period and the offspring's academic performance in youth. The UP&DOWN study. J Matern Fetal Neonatal Med, 29**:** 1414-1420, 2016.

117. Corvino, SB, Volpato, GT, Rudge, MV, Damasceno, DC: Intrauterine Growth Restricted Rats Exercised before and during Pregnancy: Maternal and Perinatal Repercussions. Evid Based Complement Alternat Med, 2015**:** 294850, 2015.

118. Corvino, SB, Netto, AO, Sinzato, YK, Campos, KE, Calderon, IM, Rudge, MV, Volpato, GT, Zambrano, E, Damasceno, DC: Intrauterine Growth Restricted Rats Exercised at Pregnancy: Maternal-Fetal Repercussions. Reprod Sci, 22**:** 991-999, 2015.

119. Laker, RC, Lillard, TS, Okutsu, M, Zhang, M, Hoehn, KL, Connelly, JJ, Yan, Z: Exercise prevents maternal high-fat diet-induced hypermethylation of the Pgc-1alpha gene and age-dependent metabolic dysfunction in the offspring. Diabetes, 63**:** 1605-1611, 2014.

120. Stanford, KI, Lee, MY, Getchell, KM, So, K, Hirshman, MF, Goodyear, LJ: Exercise before and during pregnancy prevents the deleterious effects of maternal high-fat feeding on metabolic health of male offspring. Diabetes, 64**:** 427-433, 2015.

121. Sheldon, RD, Nicole Blaize, A, Fletcher, JA, Pearson, KJ, Donkin, SS, Newcomer, SC, Rector, RS: Gestational exercise protects adult male offspring from high-fat diet-induced hepatic steatosis. J Hepatol, 64**:** 171-178, 2016.

122. Raipuria, M, Bahari, H, Morris, MJ: Effects of maternal diet and exercise during pregnancy on glucose metabolism in skeletal muscle and fat of weanling rats. PLoS One, 10**:** e0120980, 2015.

123. Mourtakos, SP, Tambalis, KD, Panagiotakos, DB, Antonogeorgos, G, Arnaoutis, G, Karteroliotis, K, Sidossis, LS: Maternal lifestyle characteristics during pregnancy, and the risk of obesity in the offspring: a study of 5,125 children. BMC Pregnancy Childbirth, 15**:** 66, 2015.

124. Soubry, A, Murphy, SK, Wang, F, Huang, Z, Vidal, AC, Fuemmeler, BF, Kurtzberg, J, Murtha, A, Jirtle, RL, Schildkraut, JM, Hoyo, C: Newborns of obese parents have altered DNA methylation patterns at imprinted genes. Int J Obes (Lond), 39**:** 650-657, 2015.

125. McCullough, LE, Mendez, MA, Miller, EE, Murtha, AP, Murphy, SK, Hoyo, C: Associations between prenatal physical activity, birth weight, and DNA methylation at genomically imprinted domains in a multiethnic newborn cohort. Epigenetics, 10**:** 597-606, 2015.

126. Chalk, TE, Brown, WM: Exercise epigenetics and the fetal origins of disease. Epigenomics, 6**:** 469-472, 2014.

127. Carter, LG, Lewis, KN, Wilkerson, DC, Tobia, CM, Ngo Tenlep, SY, Shridas, P, Garcia-Cazarin, ML, Wolff, G, Andrade, FH, Charnigo, RJ, Esser, KA, Egan, JM, de

Cabo, R, Pearson, KJ: Perinatal exercise improves glucose homeostasis in adult offspring. Am J Physiol Endocrinol Metab, 303**:** E1061-1068, 2012.

128. Rosa, BV, Blair, HT, Vickers, MH, Dittmer, KE, Morel, PC, Knight, CG, Firth, EC: Moderate exercise during pregnancy in Wistar rats alters bone and body composition of the adult offspring in a sex-dependent manner. PLoS One, 8**:** e82378, 2013.

129. Gabory, A, Ferry, L, Fajardy, I, Jouneau, L, Gothie, JD, Vige, A, Fleur, C, Mayeur, S, Gallou-Kabani, C, Gross, MS, Attig, L, Vambergue, A, Lesage, J, Reusens, B, Vieau, D, Remacle, C, Jais, JP, Junien, C: Maternal diets trigger sex-specific divergent trajectories of gene expression and epigenetic systems in mouse placenta. PLoS One, 7**:** e47986, 2012.

130. Kelly, SA, Hua, K, Wallace, JN, Wells, SE, Nehrenberg, DL, Pomp, D: Maternal exercise before and during pregnancy does not impact offspring exercise or body composition in mice. J Negat Results Biomed, 14**:** 13, 2015.

131. Blaize, AN, Breslin, E, Donkin, SS, Cabot, R, Pearson, KJ, Newcomer, SC: Maternal Exercise Does Not Significantly Alter Adult Rat Offspring Vascular Function. Med Sci Sports Exerc, 47**:** 2340-2346, 2015.

132. Camarillo, IG, Clah, L, Zheng, W, Zhou, X, Larrick, B, Blaize, N, Breslin, E, Patel, N, Johnson, D, Teegarden, D, Donkin, SS, Gavin, TP, Newcomer, S: Maternal exercise during pregnancy reduces risk of mammary tumorigenesis in rat offspring. Eur J Cancer Prev, 23**:** 502-505, 2014.

133. Schulkey, CE, Regmi, SD, Magnan, RA, Danzo, MT, Luther, H, Hutchinson, AK, Panzer, AA, Grady, MM, Wilson, DB, Jay, PY: The maternal-age-associated risk of congenital heart disease is modifiable. Nature, 520**:** 230-233, 2015.

134. May, LE, Suminski, RR, Langaker, MD, Yeh, HW, Gustafson, KM: Regular maternal exercise dose and fetal heart outcome. Med Sci Sports Exerc, 44**:** 1252-1258, 2012.

135. Bronikowski, AM, Carter, PA, Morgan, TJ, Garland, T, Ung, N, Pugh, TD, Weindruch, R, Prolla, TA: Lifelong voluntary exercise in the mouse prevents age-related alterations in gene expression in the heart. Physiological Genomics, 12**:** 129-138, 2003.

136. Ellison, GM, Waring, CD, Vicinanza, C, Torella, D: Physiological cardiac remodelling in response to endurance exercise training: cellular and molecular mechanisms. Heart, 98**:** 5-10, 2012.

137. Martinelli, NC, Cohen, CR, Santos, KG, Castro, MA, Biolo, A, Frick, L, Silvello, D, Lopes, A, Schneider, S, Andrades, ME, Clausell, N, Matte, U, Rohde, LE: An analysis of the global expression of microRNAs in an experimental model of physiological left ventricular hypertrophy. PLoS One, 9**:** e93271, 2014.

138. Iqbal, K, Tran, DA, Li, AX, Warden, C, Bai, AY, Singh, P, Madaj, ZB, Winn, ME, Wu, X, Pfeifer, GP, Szabo, PE: High type I error and misrepresentations in search for transgenerational epigenetic inheritance: response to Guerrero-Bosagna. Genome Biol, 17**:** 154, 2016.

139. Guerrero-Bosagna, C: High type II error and interpretation inconsistencies when attempting to refute transgenerational epigenetic inheritance. Genome Biol, 17**:** 153, 2016.

140. Oakes, CC, La Salle, S, Smiraglia, DJ, Robaire, B, Trasler, JM: Developmental acquisition of genome-wide DNA methylation occurs prior to meiosis in male germ cells. Dev Biol, 307**:** 368-379, 2007.

141. Kim, JH, Jee, BC, Lee, JM, Suh, CS, Kim, SH: Histone acetylation level and histone acetyltransferase/deacetylase activity in ejaculated sperm from normozoospermic men. Yonsei Med J, 55**:** 1333-1340, 2014.

142. Yamaguchi, S, Shen, L, Liu, Y, Sendler, D, Zhang, Y: Role of Tet1 in erasure of genomic imprinting. Nature, 504**:** 460-464, 2013.

143. Gu, L, Wang, Q, Sun, QY: Histone modifications during mammalian oocyte maturation: dynamics, regulation and functions. Cell Cycle, 9**:** 1942-1950, 2010.

144. Yu, C, Zhang, YL, Pan, WW, Li, XM, Wang, ZW, Ge, ZJ, Zhou, JJ, Cang, Y, Tong, C, Sun, QY, Fan, HY: CRL4 complex regulates mammalian oocyte survival and reprogramming by activation of TET proteins. Science, 342**:** 1518-1521, 2013.

145. Lucifero, D, Mann, MR, Bartolomei, MS, Trasler, JM: Gene-specific timing and epigenetic memory in oocyte imprinting. Hum Mol Genet, 13**:** 839-849, 2004.

146. Wu, SC, Zhang, Y: Active DNA demethylation: many roads lead to Rome. Nat Rev Mol Cell Biol, 11**:** 607-620, 2010.

147. Smallwood, SA, Tomizawa, S, Krueger, F, Ruf, N, Carli, N, Segonds-Pichon, A, Sato, S, Hata, K, Andrews, SR, Kelsey, G: Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. Nat Genet, 43**:** 811-814, 2011.

148. Fisher-Wellman, K, Bloomer, RJ: Acute exercise and oxidative stress: a 30 year history. Dyn Med, 8**:** 1, 2009.

149. Pareja-Galeano, H, Sanchis-Gomar, F, Garcia-Gimenez, JL: Physical exercise and epigenetic modulation: elucidating intricate mechanisms. Sports Med, 44**:** 429-436, 2014.

150. Hackett, JA, Sengupta, R, Zylicz, JJ, Murakami, K, Lee, C, Down, TA, Surani, MA: Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. Science, 339**:** 448-452, 2013.

151. Wang, L, Zhang, J, Duan, J, Gao, X, Zhu, W, Lu, X, Yang, L, Zhang, J, Li, G, Ci, W, Li, W, Zhou, Q, Aluru, N, Tang, F, He, C, Huang, X, Liu, J: Programming and inheritance of parental DNA methylomes in mammals. Cell, 157**:** 979-991, 2014.

152. Seisenberger, S, Andrews, S, Krueger, F, Arand, J, Walter, J, Santos, F, Popp, C, Thienpont, B, Dean, W, Reik, W: The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. Mol Cell, 48**:** 849-862, 2012.

153. Arand, J, Wossidlo, M, Lepikhov, K, Peat, JR, Reik, W, Walter, J: Selective impairment of methylation maintenance is the major cause of DNA methylation reprogramming in the early embryo. Epigenetics Chromatin, 8**:** 1, 2015.

154. Gkountela, S, Zhang, KX, Shafiq, TA, Liao, WW, Hargan-Calvopina, J, Chen, PY, Clark, AT: DNA Demethylation Dynamics in the Human Prenatal Germline. Cell, 161**:** 1425-1436, 2015.

155. Tang, WW, Dietmann, S, Irie, N, Leitch, HG, Floros, VI, Bradshaw, CR, Hackett, JA, Chinnery, PF, Surani, MA: A Unique Gene Regulatory Network Resets the Human Germline Epigenome for Development. Cell, 161**:** 1453-1467, 2015.

156. Ma, P, Schultz, RM: Histone deacetylase 2 (HDAC2) regulates chromosome segregation and kinetochore function via H4K16 deacetylation during oocyte maturation in mouse. PLoS Genet, 9**:** e1003377, 2013.

157. Stewart, KR, Veselovska, L, Kim, J, Huang, J, Saadeh, H, Tomizawa, S, Smallwood, SA, Chen, T, Kelsey, G: Dynamic changes in histone modifications precede de novo DNA methylation in oocytes. Genes Dev, 29**:** 2449-2462, 2015.

158. Brykczynska, U, Hisano, M, Erkek, S, Ramos, L, Oakeley, EJ, Roloff, TC, Beisel, C, Schubeler, D, Stadler, MB, Peters, AH: Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. Nat Struct Mol Biol, 17**:** 679-687, 2010.

159. Manosalva, I, Gonzalez, A: Aging changes the chromatin configuration and histone methylation of mouse oocytes at germinal vesicle stage. Theriogenology, 74**:** 1539-1547, 2010.

160. Yue, MX, Fu, XW, Zhou, GB, Hou, YP, Du, M, Wang, L, Zhu, SE: Abnormal DNA methylation in oocytes could be associated with a decrease in reproductive potential in old mice. J Assist Reprod Genet, 29**:** 643-650, 2012.

161. Hamatani, T, Falco, G, Carter, MG, Akutsu, H, Stagg, CA, Sharov, AA, Dudekula, DB, VanBuren, V, Ko, MS: Age-associated alteration of gene expression patterns in mouse oocytes. Hum Mol Genet, 13**:** 2263-2278, 2004.

162. Pedersen, BK, Febbraio, MA: Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol, 8**:** 457-465, 2012.

163. Stepto, NK, Coffey, VG, Carey, AL, Ponnampalam, AP, Canny, BJ, Powell, D, Hawley, JA: Global gene expression in skeletal muscle from well-trained strength and endurance athletes. Med Sci Sports Exerc, 41**:** 546-565, 2009.

164. Camera, DM, Smiles, WJ, Hawley, JA: Exercise-induced skeletal muscle signaling pathways and human athletic performance. Free Radic Biol Med, 98**:** 131-143, 2016.

165. Ge, ZJ, Luo, SM, Lin, F, Liang, QX, Huang, L, Wei, YC, Hou, Y, Han, ZM, Schatten, H, Sun, QY: DNA methylation in oocytes and liver of female mice and their offspring: effects of high-fat-diet-induced obesity. Environ Health Perspect, 122**:** 159-164, 2014.

166. Govin, J, Caron, C, Lestrat, C, Rousseaux, S, Khochbin, S: The role of histones in chromatin remodelling during mammalian spermiogenesis. Eur J Biochem, 271**:** 3459-3469, 2004.

167. Hammoud, SS, Nix, DA, Zhang, H, Purwar, J, Carrell, DT, Cairns, BR: Distinctive chromatin in human sperm packages genes for embryo development. Nature, 460**:** 473-478, 2009.

168. Brunner, AM, Nanni, P, Mansuy, IM: Epigenetic marking of sperm by post-translational modification of histones and protamines. Epigenetics Chromatin, 7**:** 2, 2014.

169. Radom-Aizik, S, Zaldivar, F, Jr., Leu, SY, Adams, GR, Oliver, S, Cooper, DM: Effects of exercise on microRNA expression in young males peripheral blood mononuclear cells. Clin Transl Sci, 5**:** 32-38, 2012.

170. Radom-Aizik, S, Zaldivar, F, Jr., Oliver, S, Galassetti, P, Cooper, DM: Evidence for microRNA involvement in exercise-associated neutrophil gene expression changes. J Appl Physiol (1985), 109**:** 252-261, 2010.

171. Radom-Aizik, S, Zaldivar, F, Haddad, F, Cooper, DM: Impact of brief exercise on peripheral blood NK cell gene and microRNA expression in young adults. J Appl Physiol (1985), 114**:** 628-636, 2013.

172. Rodgers, AB, Morgan, CP, Leu, NA, Bale, TL: Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress. Proc Natl Acad Sci U S A, 112**:** 13699-13704, 2015.

173. Chen, Q, Yan, M, Cao, Z, Li, X, Zhang, Y, Shi, J, Feng, GH, Peng, H, Zhang, X, Zhang, Y, Qian, J, Duan, E, Zhai, Q, Zhou, Q: Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. Science, 351**:** 397-400, 2016.

174. Cropley, JE, Eaton, SA, Aiken, A, Young, PE, Giannoulatou, E, Ho, JWK, Buckland, ME, Keam, SP, Hutvagner, G, Humphreys, DT, Langley, KG, Henstridge, DC, Martin, DIK, Febbraio, MA, Suter, CM: Male-lineage transmission of an acquired metabolic phenotype induced by grand-paternal obesity. Mol Metab, 5**:** 699–708, 2016.

Table

Table 1. Epigenetic modifications and regulating enzymes controlled by exercise training.

Epigenetic modifications/enzymes	**Description**
DNA methylation	The addition of a methyl (CH_3) group to the 5^{th} carbon of cytosine (C) nucleobase neighbouring a guanine nucleobase. This produces 5-methylcytosine and is typically referred to as CpG – cytosine-phosphate-guanine dinucleotide – methylation. Whilst methylation typically occurs at CpGs, non-CpG methylation is also present.
DNA hydroxymethylation	The conversion of 5-methylcytosine to 5-hydroxymethylcytosine by replacing the methyl ($-CH_3$) group with a hydroxymethyl (CH_2OH) group.
DNA methyltransferase	Enzymes involved in DNA methylation. DNMT1 is responsible for maintaining DNA methylation during mitosis. DNMT3A and DNMT3B control *de novo* methylation during development and new methylation patterns. DNMT3L does not actively methylate DNA, rather it stimulates other DNMTs.
Tet methylcytosine dioxygenases	Three enzymes (TET1, TET2 and TET3) that actively convert 5-methylcytosine to 5-hydroxymethylcytosine through an oxidative process.
S-adenosyl methionine	A methyl donor used for active DNA methylation.

Histone acetylation	The acquisition of an acetyl (CH_3CO) group to lysine (K) residues on histone proteins (H2A, H2B, H2AX, H3).
Histone acetyltransferases	A class of enzymes responsible for actively acetylating histone proteins (e.g. CBP/p300).
Histone deacetylases	Different enzymes responsible for de-acetylating histone proteins (e.g. HDAC1, HDAC2 and HDAC4).
MicroRNAs	Small (~22 nt) non-coding RNAs (e.g. miR-1, miR-21 and miR-133a) that combine with argonaute proteins to from the RNA-induced silencing complex (RISC) and subsequently post-transcriptionally regulate gene expression, through negative regulation or mRNA degradation.

Legend: C, carbon; H, hydrogen; OH, hydroxy; DNMT1, DNA methyltransferase 1; DNMT3A, DNA methyltransferase 3A; DNMT3B, DNA methyltransferase 3B; DNA methyltransferase 3L; SAM, S-adenosyl methionine; CH_3CO, acetyl; K, lysine; H2A, histone protein 2A; H2B, histone protein 2B; H2AX, histone protein 2AX; H3, histone protein 3; GNAT, Gcn5-related N-acetyltransferase; HDAC1, histone deacetylase 1; HDAC2, histone deacetylase 2; HDAC4, histone deacetylase 4; nt, nucleotides; miR, microRNA; RISC, RNA-induced silencing complex; mRNA, messenger RNA.

Figure legends

Figure 1. Epigenetic modifications linked to exercise-induced adaptations.

Chronic exercise training regulates epigenetic modifications, including DNA methylation, histone post-translational modifications (histone methylation and acetylation) and microRNAs. The epigenetic landscape of peripheral blood (A), brain (B), cardiac exosomes (C), heart (D), skeletal muscle (E) and sperm (F) cells are all response to chronic exercise training. The epigenetic modifications caused by exercise training are associated with health benefits, such as blood lipid and glucose control LVH, psychological resilience and neurogenesis, the prevention of premature ageing, and it is hypothesised that sperm and oocyte, and *in utero* epigenetic changes influences embryogenesis and affects the developmental origins of health and disease in the offspring (DOHaD).

Legend: ↑, increased; ↓, decreased; 5-mc, 5-methylcytosine; *SNCAIP*, synuclein alpha interacting protein; *ANGPT1*, angiopoietin 1; *GHRH*, growth hormone-releasing hormone; *IGF1R*, insulin like growth factor 1; *PLAG1*, pleomorphic adenoma gene 1; *ASC*, PYD and CARD domain containing; miR, microRNA; H3, histone 3; ac, acetylation; K9, lysine 9; me, methylation; LVH, left ventricular hypertrophy; K4, lysine 4; VO_{2max}, maximum oxygen uptake (cardiorespiratory fitness); me3, trimethylation; CpG, cytosine neighbouring a guanine dinucleotide; *IGF2*, insulin like growth factor 2; *GABRG3*, gamma-aminobutyric acid type A receptor gamma3 subunit; *PIK3CD*, phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit delta; DOHaD, developmental origins of health and disease. This figure was produced using Servier Medical Art, http://www.servier.com/.

Figure 2. Epigenetic modifications associated with health consequences caused by physical inactivity. Physical inactivity is associated with numerous health consequences and tissues – e.g. blood (A), brain (B), heart (C), skeletal muscle (D) and sperm (E) – that are responsive to

epigenetic modifications caused by exercise are also prone to aberrant epigenetic programming through lack of activity. Such inactivity-related epigenetic modifications are linked to detrimental health consequences such as cardiorespiratory deconditioning, cardiac dysfunction, insulin resistance, loss of lean muscle mass and it is hypothesised that physical inactivity in conjunction with excess caloric intake leads to embryological and developmental impacts that increases the risk of obesity and metabolic dysfunction in offspring.
Legend: ↑, increased; ↓, decreased; miR, microRNA; 5-mc, 5-methylcytosine; VO_{2max}, maximum oxygen uptake (cardiorespiratory fitness); piRNAs, Piwi-interacting RNA; *BDNF*, brain-derived neurotrophic factor; *CART*, cocaine and amphetamine regulated transcript; *FTO*, fat mass and obesity associated; *NPY*, neuropeptide Y. This figure was produced using Servier Medical Art, http://www.servier.com/.

Figure 3. A schematic of the different types of epigenetic inheritance caused by maternal and paternal environmental (lifestyle) factors. Transgenerational inheritance occurs when phenotypic changes are experienced by the offspring of the founder generation independent of exposure to the environmental exposure (or intervention). A transgenerational epigenetic heritable influence has occurred when the phenotype and epigenetic profile is altered in the third generation (F3), if a maternal environmental effect has occurred during gestation, or in the second, if a maternal or paternal environmental stimuli was experienced pre-conception (Figure 3 A, B and C, respectively). Conversely, intergenerational epigenetic inheritance occurs when an environmental stressor directly impacts the epigenetic landscape and phenotype of the offspring or offspring's germ line. The difference being that the environmental stressor directly impacts the individual and their gametes (spermatozoa and

oocytes – F1) in intergenerational inheritance, whereas in the case of transgenerational inheritance, the offspring have not been exposed to the environmental factor or intervention. Legend: F0, base (parent) generation, parent; F1, first generation; F2, second generation; F3, third generation.

Figure 4. Time-points environmental factors could reprogram epigenomes of maternal and paternal germ cells and lead to epigenetic inheritance of health and disease risk. There are critical time-points when exercise could reprogram germ cells influence the offspring. While preconceptional paternal (A) and maternal (B) exercise could modify the epigenetic landscape of spermatozoa and oocytes, respectively, paternal exercise could also influence the abundance non-coding RNAs and proteins in semen (C). These epigenetic modifications could facilitate transcriptional reprogramming *in utero* and affect fertilisation and/or embryogenesis (D). Upon fertilisation and embryogenesis the effects of paternal exercise on the developing offspring (F1) are limited and any additional epigenetic reprogramming are implemented *in utero* by maternal exercise (E). Finally, it is plausible that newborn (F1) offspring are vulnerable to epigenetic regulation by maternal exercise-induced by milk-bound non-coding RNAs or proteins, etc, that are consumed during suckling (F). These critical time-points, paternal and maternal impact of exercise-induced epigenetic reprogramming could ultimately lead to health adaptations and modified disease risk in the future generations in youth and adulthood. Exercise may be an effective therapeutic strategy to prevent the epigenetic inheritance of disease transmitted by the exposed parents to harmful environmental insults (psychological stress, age-associated diseases, metabolic syndrome). Nonetheless, the harmful, heritable effects of environmental insults (obesity, psychological stress, etc.) experienced by the offspring (F1) of those exposed (F0) are most likely effectively prevented or managed by lifestyle strategies, including exercise.