Building-up fit muscles for the future

Transgenerational programming of skeletal muscle through physical exercise

Jorge Beleza[1*], Jelena Stevanović Silva[2], Pedro Coxito[2], Rui Carlos Costa[3], António Ascensão[2], Joan Ramon Torrella[1], José Magalhães[2]

Affiliations:

[1] Department of Cell Biology, Physiology & Immunology, Faculty of Biology, University of Barcelona, Barcelona, Spain;
[2] Laboratory of Metabolism and Exercise (LaMetEx), Research Centre in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sport, University of Porto, Porto, Portugal;
[3]Department of Communication and Art, Research Institute for Design, Media and Culture (ID+), Aveiro University, Aveiro, Portugal;

Running title: Building-up fit muscles for the future

***Address correspondence to:**
Jorge Beleza
Secció de Fisiologia, Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain

E-mail: jmsbeleza@gmail.com

Disclosure: The authors declared no conflict of interest.
Word count: 4036

Abstract

“Special issue - In Utero and Early Life Programming of Aging and Disease”

Background: Skeletal muscle (SM) adaptations to physical exercise (PE) has been extensively studied due, not only to the relevance of its *in situ* plasticity, but also to the SM endocrine-like effects in non-contractile tissues, such as brain, liver or adipocytes. Regular PE has been considered a pleiotropic nonpharmacological strategy to prevent and counteract the deleterious consequences of several metabolic, cardiovascular, oncological and neurodegenerative disorders. Additionally, PE performed by parents seems to have a direct impact in the offspring through the transgenerational programming of different tissues, such as SM. In fact, SM offspring programming mechanisms seems to be orchestrated, at least in part, by epigenetic machinery conditioning transcriptional or post-transcriptional processes. Ultimately, PE performed in the early in life is also a critical window of opportunity to positively modulate the juvenile and adult phenotype.

Methods: Following detailed electronic database search, recent existing evidences about the effects of parental and early-life PE on SM programming were reviewed.

Results: Parental PE have a positive impact in several health-related offspring outcomes, such as SM metabolism, differentiation, morphology and ultimately in offspring exercise volition and endurance. Also, early-life PE counteracts conceptional-related adverse effects and induces long-lasting healthy benefits throughout adulthood. Additionally, epigenetics mechanisms seem to play a key role in the PE-induced SM adaptations.

Conclusions: Despite the undoubtedly positive role of parental and early-life PE on SM phenotype, a strong research effort is still needed to better understand the mechanisms that positively regulate PE-induced SM programming.

Abbreviations:

FRW	Free running wheel
HDAC	Total histone deacetylases
HFD	High fat diet
ME	Maternal exercise
miRNA	MicroRNA
MYOD1	Factor myogenic differentiation 1
PE	Physical exercise
PGC-1α	Peroxisome proliferator-activated receptor-γ coactivator-1α
SM	Skeletal muscle
TFAM	Mitochondrial transcription factor A

Keywords: Epigenetics; Offspring; Maternal exercise; Paternal exercise, Early-life exercise**.**

1. Introduction

The skeletal muscle (SM) is a tissue primarily responsible for the force production and movement in the human body representing approximately 40% of total body weight[1]. Nowadays, SM is referred as a very specialized tissue with remarkable plasticity[2], therefore, one of the most studied tissue regarding physical exercise (PE) adaptations.[3]

PE imposes muscle stress that can disrupt homoeostasis during the exercise and recovery periods.[4] To cope with this physiological challenge, acute and chronic muscle structural, functional and metabolic adaptations occur.[2] Ultimately, alterations in key molecular pathways impact tissue phenotype preparing muscle towards similar future events and improving performance.[1] The impact of PE on muscle plasticity may be expressed as changes in muscle endurance and strength.[2] However, muscle adaptations are related to PE dose-response control such as frequency, intensity, time, type, volume and progression (the so-called FITT-VP principle).[5] Considering the crucial mitochondrial role in several cellular metabolic processes, a central point in the improvement of SM "fitness" is the remodeling of mitochondrial network.[3] These adaptations allow the muscle cells to be more functionally efficient with positive repercussions on SM phenotype and performance.[6] In addition, PE-induced SM contractile activity has pleiotropic repercussions on non-contractile tissues[6], being this SM endocrine-like effect mediated by several proteins secreted to the bloodstream, the so-called myokines.[6,7] Therefore, PE is consensually recognized as an important preventive and/or therapeutic non-pharmacological intervention able to mitigate the development or the deleterious effects of a wide range of metabolic, cardiovascular, oncological and neurodegenerative diseases.[8]

Recently, parental PE has also been proposed as having a positive role in the modulation of the offspring phenotype, such as in SM, with consequences in their fitness levels. In fact, besides maternal exercise (ME) performed during pregnancy[9-13], paternal exercise before conception has also been recently associated to healthy-related outcomes[14-17] highlighting the role of PE as a relevant strategy in transgenerational programming of the future generations. Furthermore, PE performed early in life also seems to induce mitigate adverse constrains inherited from gestational environment and prompt the juvenile with

long-lasting healthy benefits throughout adulthood. Apart from other mechanistic insights, these PE-related adaptations seem to be orchestrated by epigenetic signaling that fine-tune gene expression or silencing under the control of transient or stable transcriptional or post-transcriptional mechanisms.[18,19]

The aim of this review is to highlight the updated knowledge regarding the role of transgenerational programming and early-life healthy behaviors mediated by PE in the modulation of SM phenotype.

2. Maternal exercise impact in transgenerational programming of skeletal muscle offspring

Discoveries in the field of the next generation's health and early life environmental exposure shed light on the concept of developmental origins of health and disease. This concept helps to understand the sensitivity of an organism to certain environmental insults during critical periods of development, such as fetal and perinatal period, that may affect the offspring risk for chronic diseases in a short- or long-term manner.[20-22] An adverse *in utero* environment (i.e., maternal malnutrition, infections, or metabolic and hormonal disorders) might trigger fetal and/or perinatal adaptive responses, leading to persistent physiological and metabolic alterations.[23] In fact, different investigations using either maternal nutrient restriction[22,24,25] or increased high-fat diet (HFD) consumption[26-28] have shown impaired glucose regulation and altered insulin sensitivity in the adult offspring. Moreover, there is evidence suggesting that adverse maternal nutrition during the sensitive periods of pregnancy and lactation can also influence the offspring predisposition to develop distinct pathological conditions, including diabetes[29], non-alcoholic fatty liver disease[13,30] and obesity later in life.[21] Thus, ME during pregnancy may represent a cue by which fetus will cope with a hostile *in utero* environment (Figure 1). In fact, the benefits of active lifestyle during pregnancy, particularly ME, have been reported not only in mothers, but also in their children.[31,32] Data from human and animal model studies, demonstrated that ME induced maternal benefits including reduced body weight, improved cardiovascular function and reduced risk for gestational hypertension and hyperglycemia, preeclampsia, and cesarean section[12,33], whereas the offspring of exercised mothers have reduced risk of excessive body weight and fat mass,

as well as improved long-term glucose homeostasis.[11,12,33,34] Moreover, ME can also improve brain metabolism in the offspring and prevent neurodegenerative-related neurotoxicity and cognitive impairment.[35] Interestingly, as will be discussed below, ME can also prevent female offspring insulin resistance in the SM imposed by paternal obesity.[10] These studies highpoint the beneficial role of exercise before and/or during pregnancy in offspring short- or long-term health.

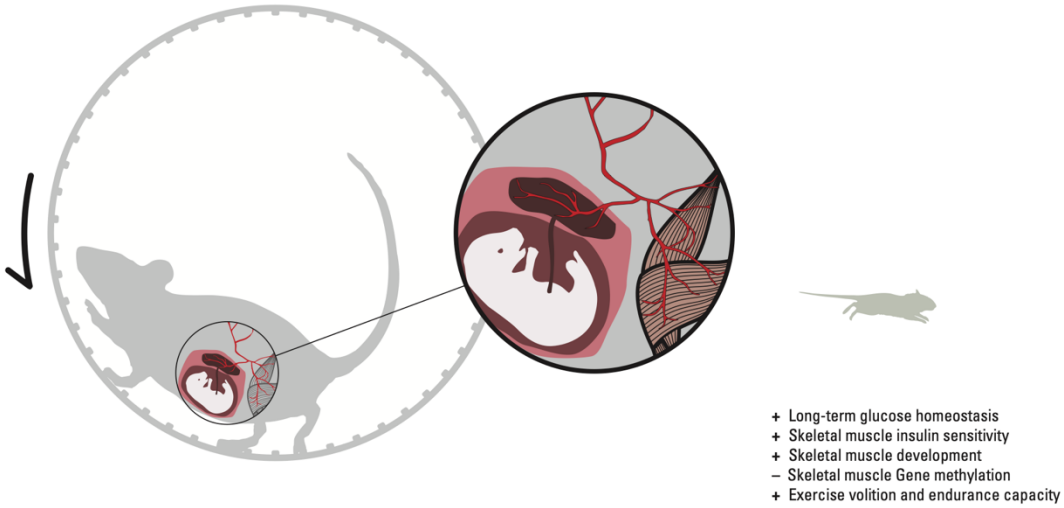

Fig. 1 Maternal exercise and active lifestyle during pregnancy induces numerous positive outcomes in the offspring generation. Skeletal muscle endocrine-like effect through the release of several muscle-derived molecules (myokines) into the blood stream and consequent effect on fetus throughout placental supply may represent one of the mechanisms associated to the positive healthy *status* in the offspring.

The SM has a significant relative "weight" in whole-body mass, an incontestable structural and physiological relevance. It also plays a central role as an endocrine-type stimulator of distinct signaling pathways in several other tissues preventing chronic non-communicable diseases, namely during the exercise and post-exercise recovery periods.[1,4,7] Therefore, conditions that impose constrains to SM normal development or induce degeneration may have a significant negative impact on the organism throughout life, and even in the healthy *status* of the next generations.

Accordingly, maternal HFD consumption seems to have a deleterious consequence on offspring SM development[36-38], being associated with reduced

gene expression of the specific transcription factor myogenic differentiation 1 (MYOD1).[39] Downregulation of MYOD1, the master regulator of SM differentiation, leads to a reduced number of myogenic cells and may be related to impaired SM development. In line, Bayol et al showed that offspring sired by HFD-fed mothers exhibited a 25% reduction in muscle cross-sectional area and approximately 20% fewer muscle fibers.[26] Moreover, decreases in capillary density, fiber cross-sectional area and type-IIa muscle fibers mRNA expression has been reported upon maternal HFD-consumption[38] with negative consequences on offspring muscle force production.[38,40] Furthermore, maternal HFD consumption induces alterations in SM mitochondrial physiology through a decrease in mRNA expression of several biomarkers of mitochondrial biogenesis and dynamics (peroxisome proliferator-activated receptor-γ coactivator-1α, PGC-1α; nuclear respiratory factor 1 and mitochondrial transcription factor A, TFAM)[38,41], associated to a downregulation of nuclear-encoded proteins linked to oxidative phosphorylation.[42]

As SM also play a key role in systemic insulin-sensitivity and glucose uptake[4], SM offspring-related metabolic impairments have been associated with deregulated parental nutritional regimens. In fact, maternal HFD-feeding lead to impairments in SM insulin-signaling[26,40], glucose and lipid metabolism[36], associated to reduced mRNA expression and content of glucose transporter type 1 and 4, fatty acid synthase and PGC-1α.[36,39,43,44] Furthermore, maternal HFD-consumption seems to impair exercise performance in offspring as well as training efficiency early in life.[44]

Bearing in mind the consensual health-related role of PE[7], ME has been proposed as a non-pharmacological strategy to counteract or attenuate morphology, function or metabolism downregulation in offspring induced by parental malnutrition.[33,45] However, as suggested before, even though considerable amount of data from several tissues, particularly liver[13], adipose tissue[46], brain[35] and pancreas[47] has been published, recent research has also been devoted to the impact of ME on offspring's SM[38,39]. Regarding the development of SM in the offspring of HFD-fed mothers, Raipuria et al reported that ME reverted HFD-related MYOD1 downregulation in male offspring.[39] Moreover, SM weight, fiber cross-sectional area, type-IIa muscle fibers mRNA expression and concomitant maximal strength increased in offspring sired from

exercised mothers.[38] The metabolic mechanisms underlying ME effects on offspring glucose tolerance and insulin-sensitivity in SM have also been studied in different rodent models. Albeit there are discrepancies among these studies due to the use of different strains of mice[28,34] and rats[9,48,49], and different durations and modalities[10,28,34] of ME, in general data suggest that ME before and during pregnancy improves glucose tolerance and insulin sensitivity in the offspring SM.[10,34,48-50] Regarding the different exercise models, although free-running wheel (FRW) has been associated to clear SM-related benefits in health animals[51], the absence of intensity control may be one the reasons associated to the variability and distinct outcomes described in studies involving metabolic constrains.

Alternatively, treadmill running is a well-established model used during pregnancy to control PE load. In fact, the intensity of exercise performed before and during gestation seems to play a key role in the offspring phenotypic adaptations. Low-intensity treadmill running exercise (55% of maximal aerobic speed) for 4-weeks before and during gestation improved SM glucose tolerance and insulin sensitivity in young male offspring.[33,45,52] Additionally, Falcão-Tebas et al showed that maternal treadmill exercise before (60-75% of VO_{2max}) and during pregnancy (65-50% of VO_{2max}) increased glucose transporter type 4 protein expression in the offspring[10], which is in line with some previous studies even when FRW was used[39], and may explain, at least in part, the attenuated lower insulin-stimulated glucose uptake in offspring sired by obese fathers. Moreover, citrate synthase activity, a marker of SM mitochondrial content, increased and reactive oxygen species production was normalized in female offspring, despite the lack of changes in the expression of mitochondrial biogenesis markers, such as PGC1α and TFAM, which contrasts with data reporting increased mRNA expression of PGC1α using a FRW model.[39]

Taking into account the positive impact of ME in the offspring SM metabolic phenotype, improvement in exercise volition and performance would also be expected. In contrast with data reporting that maternal HFD reduced fatty acid synthase and glucose transporter type 1 gene expression, together with offspring impairment of exercise performance[44], to our knowledge, only one study reported that ME performed before and during pregnancy positively modulated the propensity of the offspring for PE[53]. In fact, female offspring volition for exercise

in the FRW was higher during different time points throughout life when compared with offspring from sedentary mothers. Considering the positive health-related outcomes of the fitness levels throughout life[54], additional studies exploring offspring exercise ability, particularly maximal aerobic performance, could be of high interest to support the effectiveness of SM-related transgenerational programming of fitness in offspring sired from exercised mothers. In fact, only a very recent study by Son et al reported that gestational exercise improved endurance capacity in the offspring.[38]

Taken together, data suggest that ME may have a vital role in offspring's SM programming. However, more studies are necessary to identify the optimal ME training regimens and to fully elucidate the mechanisms responsible for the improvement in offspring SM responses to ME.

3. Epigenetic mechanisms associated to skeletal muscle adaptation to physical exercise

The regulation of gene expression is a fundamental process that establishes and impacts the phenotype induced by different environmental stimuli, including PE, in the different tissues.[55,56] Nevertheless, although cells from an organism share identical DNA, its expression may be driven by distinct epigenetic-related influences, such as DNA methylation, histone modifications and microRNA (miRNA) regulation (Figure 2). Whilst DNA methylation and histone modifications involve a direct influence at chromatin level, but without changes in the genome sequence, miRNAs regulate the amount of proteins expressed in cells by modulating mRNA stability and the efficiency of translation.[57] In this context, PE has the ability to modulate the epigenetic signature mapping of different cells, thus mediating the subsequent modifications at transcription and post-transcription levels that characterizes distinct acute and chronic adaptations.[56,58,59]

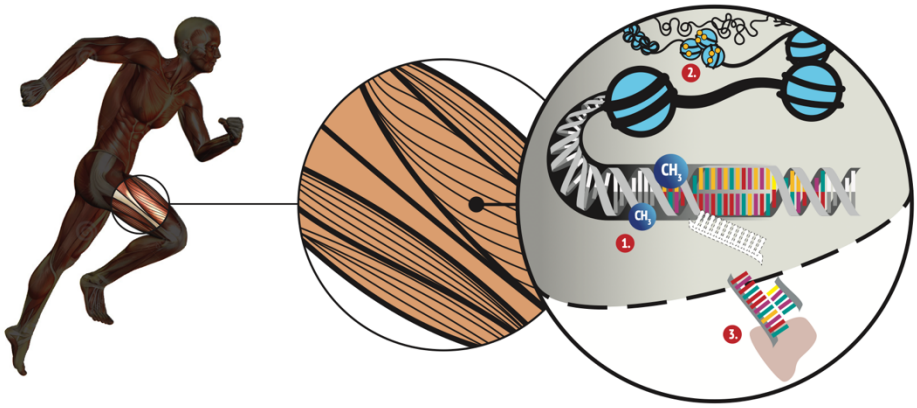

Fig. 2 Proposed epigenetic mechanisms that mediate the skeletal muscle gene expression in response to physical exercise, such as 1 – DNA methylation; 2 – histone modifications; 3 – microRNA post-transcriptional regulation.

3.1. **Variability of skeletal muscle epigenetic landscape mediated by physical exercise**

As illustrated in Figure 2, SM epigenetic landscape modulated by PE fine-tune the delicate balance between gene expression or silencing under the control of transient or stable transcriptional or post-transcriptional mechanisms.[18,19]

So far, an almost consensual idea supports that different types and regimens of PE, with distinct cohort of healthy and non-healthy active subjects[60-63] revert or attenuate the methylation profile of genes involved in crucial metabolic or structural remodeling of SM pathways, such as *Pgc-1α* and *Tfam*, in a dose-dependent manner.[60] Moreover, the SM genome-wide DNA hypomethylation pattern (measured as the number of hypomethylated CpG-sites) obtained after a 7-week resistance training program was retained and even exacerbated in the detraining and the retraining period, respectively.[63] Ultimately, a 9-day period of bed-rest in healthy active subjects induced an increased methylation of the *Pgc-1α* gene that was attenuated following 4-weeks of training.[64] This could be particularly interesting since SM is a programmable tissue and can 'remember' early-life mechanical and metabolic stimuli through epigenetic mechanisms that underly SM memory, affecting its function throughout life.[65]

Accordingly, histone modifications associated to phosphorylation, acetylation, and mono-, di-, and tri-methylation mechanisms seem to determine distinct spatial relationships between DNA and the histone core, conditioning SM transcriptional activation in response to exercise.[66] McGee et al showed that although no difference in total histone deacetylases (HDAC) activity was found following exercise in human SM, alterations in class IIa HDAC function occurred through changes in subcellular localization, as HDAC 4 and 5 nuclear abundance immediately after PE were significantly decreased.[66] This HDAC reallocation out of the nucleus[67] seems to be orchestrated, at least in part, by phosphorylation kinase cascades, namely AMP-activated protein kinase and the calcium–calmodulin-dependent protein kinase II.[66] In addition, endurance-trained subjects respond to intense cycling exercise through mitogen-activated protein kinase signaling. Extracellular regulated kinase 1/2 and p38 pathways are associated with activation of mitogen and stress-activated kinase 1 and histone H3 phosphorylation in human *vastus lateralis* from highly trained cyclists, which provides a potential cellular mechanism that facilitates exercise-related gene transcription.[68] Accordingly, it has been recently showed that increased acetylation levels in H3 were closely related to up-regulation of gene expression involved in resistance exercise-related SM adaptations.[69]

Ultimately, mounting data highlight the epigenetic-related role of miRNAs in the post-translational regulation of gene expression in SM response to exercise.[70-72] Although an impressive array of miRNAs is presently under accurate scientific surveillance regarding its impact in exercise-related adaptations, the inter-individual variability of miRNAs expression in SM is still a matter of debate. Studies using resistance training reported muscle mass gain responses that are positively correlated with the expression levels of miR-378 in a high or low responsive manner.[73] Moreover, data from endurance exercise participants emphasize the SM mRNA regulation of components of the miRNA biogenesis pathway (Drosha, Dicer and Exportin-5)[74], as well as the role of miRNAs in the stimulation of microvascular remodeling[62] and metabolic signaling pathways, such as mitochondrial biogenesis.[74-76] While miRNAs molecules are essential cellular mediators of processes related with PE adaptation, secreted miRNAs can also act as signaling molecules mediating intercellular communication via paracrine or endocrine signaling. Cells can release miRNAs to the blood stream

associated to lipid and protein carriers, namely extracellular microvesicles, exosomes and lipoproteins[77-79] and deliver them in recipient cells thus regulating target gene expression and cell function.[80] Nevertheless, data are still scarce regarding the intrinsic and accurate mechanisms associated to this circulating miRNA-mediated intercellular communication. In fact, further studies are needed to establish the biological cause-and-effect relationships between circulating and different tissue adaptations during exercise or post-exercise recovery.[70]

3.2. **Epigenome-mediated programming of offspring skeletal muscle through parental physical exercise**

Besides their fundamental role as modulators of the gene expression in several organs, epigenetic influence during prenatal and neonatal development has also been proposed to affect offspring metabolic health. Mounting evidence suggest that environmental factors, such as xenobiotics, diets and PE contribute to transgenerational epigenetic inheritance that alters cell/tissue function. Consequently, they may predispose to an increased disease risk or, in contrast, to putative beneficial health-related outcomes later in life.[57] In this regard, some studies suggest that epigenetic-related programming associated to ME contributes, at least in part, to mitigate disturbances associated with several pathophysiological conditions at distinct levels of cellular organization in the offspring.[30]

Nowadays it's clear that parental environmental exposure has an influence on programming SM metabolism and phenotype. In fact, epigenetic alterations have been proposed as having a key role in the transgenerational transmission of metabolic impairments.[29] Therefore, understanding whether and how epigenetic modifications can manifest downstream implications for offspring in SM may be of extreme importance. Accordingly, Prats-Puig et al reported gestational obesity-related alterations in methylation levels of *MYL6, MYH11, TNNT3, TPM2, CXCL2* and *NCAM1* genes in the umbilical cord, with implications in infant's fasting glucose levels at 6 years of age, due to hypothetical in utero inappropriate epigenetic programming of SM.[81] Moreover, increased SM miR-15a and miR-15b expression in offspring of mothers with gestational diabetes seems to be

associated with fetal programming and consequent predisposition to develop type-2 diabetes and associated cardiometabolic disease later in life.[82]

In contrast, ME is a promising non-pharmacological strategy capable to reduce the susceptibility of offspring to developing metabolic disease in adulthood, and therefore contributes to break the vicious cycle of transgenerational transmission of maternal-associated malnutrition diseases. Although epigenetic-related mechanisms are the main players proposed so far[59,65,83], studies exploring the role of ME in the programming of offspring's landscape are still scarce. Laker et al reported that ME attenuated *Pgc-1α* promoter hypermethylation in the SM of newborns from HFD-mothers, which is of crucial physiological and functional relevance considering the role of PGC-1α as a master regulator of mitochondrial biogenesis and oxidative metabolism.[48] Moreover, other PGC-1α-related downstream targets such as glucose transporter type 4, cytochrome c oxidase subunit-4 and cytochrome c were also hypermethylated by the HFD and epigenetically prompted into a more positive phenotype by ME until 12-months of age.[45] This highlights the life-long protection provided by PE regarding epigenetic-transgenerational programming. In addition, ME induced DNA demethylation of the *Pgc-1α* with consequent overexpression in offspring muscle PGC-1α 1/4 isoforms content, mitochondrial biogenesis and improved muscle function.[38]

Until present, evidences are based on the relevance of ME on offspring health outcomes; however, recent research has shown that fathers' environmental exposure may also play an important role in offspring programming.[14-17] Sperm-mediated programming through epigenetic transmission of heritable alterations in chromatin structure may be one potential mechanism by which paternal physiology can impact offspring development. Father's environmental exposure leading to an obese phenotype may impair sperm number and motility[84,85] comprising fertility-related outcomes. In addition, the effects of paternal diet are likely to be transmitted to offspring through the sperm.[84,86] Data from rodent studies showed that paternal nutritional behavior can also influence both the sperm and offspring DNA methylation profile. In fact, the HFD-fed fathers and their male offspring-related spermatozoa epigenome showed overlapping DNA methylation that compromises distinct signaling metabolic pathways.[87] Moreover,

paternal HFD decreased expression of dimethyl H3-K9 protein in sperm with further consequences on placental and fetus weight.[88]

In contrast, paternal exercise is positively associated with progressive and total sperm motility[16,17] (Figure 3). In fact, endurance, resistance and combined training might improve sperm parameters, including viability and motility of sperms through increasing the serum testosterone and luteinizing hormone levels.[16,17] Recent data showed that reduced DNA methylation induced by exercise is present in germ cells suggesting that these positive outcomes can be transmitted to future generations.[89] In an elegant study, Guth et al demonstrated that parental exercise resulted in an increased SM mass and improved glucose tolerance in female offspring. This parental exercise program altered the expression of several genes involved in lipid metabolism and specifically intramuscular lipogenesis in *gastrocnemius* (*Adipoq, Cidec and Scd1*) throughout two generations of sedentary male offspring[14]. Genes involved in insulin and leptin signaling, as well as in the control of glucose metabolism (*H19*, *Ptpn1*, *Ogt* and *Oga*), were differently methylated in the sperm of paternal trained mice, which was associated with altered expression of these genes in the offspring SM and with an improvement in the metabolic efficiency.[15] Although controversial[15,88], the expression of several paternal sperm miRNAs levels may be positively modulated by exercise training.[90] The MiR-503 seems to be positively regulated by PE and have a role in target genes of cyclin family that are involved in cell cycle regulation.[90]

Taken together, data published so far highlight the relevant role that parental exercise may also have in the epigenetic-related transgenerational programming of SM offspring. However, a strong research effort should be done to explore the truly effects of epigenetic-related mechanisms that mediate the impact of paternal exercise on offspring phenotype.

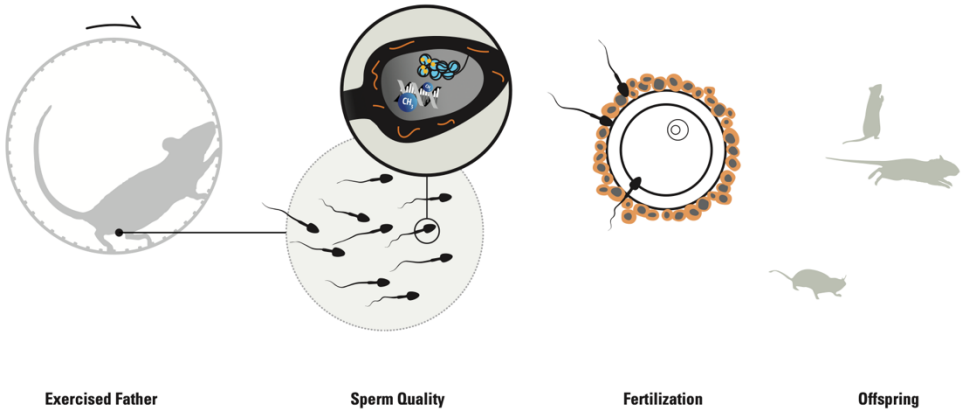

Fig. 3 Paternal exercise and active lifestyle before conception have a positive effect on sperm viability and motility. Additionally, paternal exercise also has an impact in sperm chromatin structure through alterations in DNA methylation and in histone protein expression as well as in the modulation and expression of distinct miRNAs. Some of the paternal-induced epigenetic alterations are likely to be transmitted to offspring through the germ cells. In fact, paternal exercise, altered the skeletal muscle expression of several genes involved in the offspring glucose and lipid metabolism.

4. Early-life exercise counteracts negative impact of parents' lifestyle

Besides the relevant role of PE before conception and during the gestational period, there is increasing evidence that early-life PE may also play a significant impact in the modulation of the juvenile offspring[20,22,91-93] and adult phenotype[94], as developmental programming is not restricted to the gestational period. In fact, the postnatal period is also a critical window of opportunity[95,96], being PE performed during childhood negatively correlated to constrains inherited from an adverse gestational environment and induces lower prevalence of chronic diseases in adulthood.[94,97] In fact, early-life exercise seems to mitigate the deleterious consequences of undernutrition during pregnancy and subsequent intrauterine growth restriction.[22,91] Huber et al reported that in offspring exposed to maternal undernutrition, long-term daily moderate exercise reverted the decrease in fatty acid synthase protein expression and consequently improved overall energy expenditure and enhanced SM oxidative capacity.[91] Therefore, the exercise-induced improvement in muscle metabolism contributed to the

prevention of the paradoxical obesity development reported in undergrown offspring at birth.[40] Furthermore, exercise engagement from early-life throughout adulthood in this model of undernourished mothers had the same effect in the prevention of obesity, when a moderate level of daily exercise was controlled by a locked FRW model only allowing 56m/day of running distance, which suggests that even moderate daily exercise prevents prenatally-induced obesity.[97]

In a model of impaired uterine blood flow-induced low birth weight in offspring, Laker et al [92] showed that early-life PE until 9-weeks of age elicited SM mitochondrial biogenesis and oxidative capacity, through increased protein expression of PGC-1α, elevated β-Hydroxyacyl-CoA dehydrogenase and citrate synthase enzyme activities.[92] However, no sustained effects of early exercise on the expression and activity of these proteins were still evident after the cessation of exercise (at 24-weeks of age), compromising the long-term SM beneficial programing induced by early-life exercise.[47]

As previously discussed, similarly to the impact of maternal undernutrition during pregnancy, an excessive caloric intake during gestation is also associated with health-related complications in offspring.[29,38] Recent works have demonstrated beneficial effects of early-life voluntary exercise on the metabolic profile of offspring from obese mothers with exercise reversing maternal obesity-induced adiposity.[93] On the other hand, Falcão-Tebas et al focused on the offspring early-life PE reprograming effect against paternal HFD consumption.[20] Data revealed that only 4-weeks of exercise early in life normalized whole-body insulin sensitivity and reduced body weight in offspring later in adulthood (at 25-weeks of age), which seems to be influenced by SM adaptations, namely the increased levels of SM glucose transporter type 1 protein content in adulthood.[20] Moreover, there have been reported improvements in mitochondrial respiration were reported[20] suggesting that mitochondrial function 4-months *post-partum* in offspring from HFD parents may have contributed, at least in part, to the normalization of insulin sensitivity in SM. In addition, a work from Patterson et al showed that a 3-week FRW programme in rats, performed immediately after weaning, prevented the onset of diet-induced obesity for at least 10weeks after the cessation of exercise training, despite the continued HFD-consumption.[98]

Besides the relevance of prenatal exercise on offspring health, recent striking findings show that maternal perinatal exercise (i.e., during lactation) can also

impose metabolic reprogramming in the offspring, through changes in the composition breastmilk. Ribeiro et al reported that elevated insulin levels in the milk of exercising mothers could, at least partly, contribute to lessening the effects of early-life overfeeding on obesity development of their offspring.[99] The better insight into exercise effects on breastmilk composition was given by a cross-fostering study by Harris et al. Findings showed that offspring from sedentary mothers when cross-fostered by exercising dams improved glucose and insulin tolerance, in addition to a decrease in adiposity and body mass, compared to the offspring of exercising mothers nurtured by sedentary dams.[100] It seems that ME expresses some of its beneficial metabolic effects through altering breast milk composition, particularly an oligosaccharide 3'-sialyllactose, which levels correlate positively with exercise.[100] These remarkable cross-fostering data actually demonstrate the role that ME may have on altering breast milk composition and thus, it's possible involvement in the offspring metabolic status.[100] Even though studies on this new aspect of ME mechanisms are in its infancy, it undoubtedly offers a new perspective on the benefits that ME during lactation may also have on the offspring metabolic programming.

In summary, although the beneficial the role of parental and early-life PE seems to be sufficiently robust in rodent model studies, a strong effort is still needed to highlight the potential translation power of the benefits and related mechanisms into humans. Nevertheless, the current knowledge seems to be appropriated to establish clinical guidelines and scientifically supported evidences for the promotion of parental PE before and during gestation, as well as offspring PE early in life. In this sense, behavior' changes regarding the practice of PE during pregnancy should be adopted as part of the huge parental responsibility with their offspring and with the society.

Acknowledgments:
This work was supported by the EU's Horizon 2020 Research and Innovation program under the Marie Skłodowska-Curie Actions (No.722619, FOIE GRAS; No.734719, mtFOIE GRAS) and by the Portuguese Foundation for Science and Technology (FCT) (UIDB/00617/2020-base; POCI-01-0145-FEDER-016690-PTDC/DTP-DES/7087/2014; POCI-01-0145-FEDER-016657-PTDC/DTP-DES/1082/2014), to JB PhD scholarship (SFRH/BD/129645/2017).

Contributions:
Conceptualization: JB, JSS, JRT, JM
Literature survey: JB, JSS, PC
Original Draft: JB, JSS, JM
Final editing and approval: JB, JSS, PC, AA, RCC, JRT, JM
Information visualization: RCC
Funding acquisition: JM, JB

ORCID:
JB - https://orcid.org/0000-0002-7573-3757
JSS - https://orcid.org/0000-0003-4641-1078
PC - https://orcid.org/0000-0003-0573-7418
RCC - https://orcid.org/0000-0002-8881-3174
AA - https://orcid.org/0000-0001-5269-0857
JRT - https://orcid.org/0000-0001-6314-6523
JM - https://orcid.org/0000-0003-4808-837

References

1. Mukund K, Subramaniam S. Skeletal muscle: A review of molecular structure and function, in health and disease. *Wiley Interdiscip Rev Syst Biol Med*. Jan 2020;12(1):e1462. doi:10.1002/wsbm.1462
2. Hoppeler H. Molecular networks in skeletal muscle plasticity. *J Exp Biol*. Jan 2016;219(Pt 2):205-13. doi:10.1242/jeb.128207
3. Lundby C, Jacobs RA. Adaptations of skeletal muscle mitochondria to exercise training. *Exp Physiol*. Jan 2016;101(1):17-22. doi:10.1113/ep085319
4. Hargreaves M, Spriet LL. Skeletal muscle energy metabolism during exercise. *Nat Metab*. Sep 2020;2(9):817-828. doi:10.1038/s42255-020-0251-4
5. Pescatello LS, Riebe D, Thompson PD. *ACSM's guidelines for exercise testing and prescription*. Lippincott Williams & Wilkins; 2014.
6. Beleza J, Rizo-Roca D, Ascensão A, Magalhães J. Targeting Mitochondria with Sweat: Improving Mitochondrial Function with Physical Activity. *Mitochondrial Biology and Experimental Therapeutics*. Springer; 2018:379-406.
7. Murphy RM, Watt MJ, Febbraio MA. Metabolic communication during exercise. *Nat Metab*. Sep 2020;2(9):805-816. doi:10.1038/s42255-020-0258-x
8. Pedersen BK, Saltin B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scand J Med Sci Sports*. Dec 2015;25 Suppl 3:1-72. doi:10.1111/sms.12581
9. Carter LG, Qi NR, De Cabo R, Pearson KJ. Maternal exercise improves insulin sensitivity in mature rat offspring. *Med Sci Sports Exerc*. May 2013;45(5):832-40. doi:10.1249/MSS.0b013e31827de953
10. Falcão-Tebas F, Marin EC, Kuang J, Bishop DJ, McConell GK. Maternal exercise attenuates the lower skeletal muscle glucose uptake and insulin secretion caused by paternal obesity in female adult rat offspring. *The Journal of physiology*. Jun 15 2020;doi:10.1113/jp279582
11. Ferrari N, Bae-Gartz I, Bauer C, et al. Exercise during pregnancy and its impact on mothers and offspring in humans and mice. *J Dev Orig Health Dis*. Feb 2018;9(1):63-76. doi:10.1017/s2040174417000617
12. Mottola MF. Physical activity and maternal obesity: cardiovascular adaptations, exercise recommendations, and pregnancy outcomes. *Nutrition reviews*. 2013;71(suppl_1):S31-S36.
13. Sheldon RD, Nicole Blaize A, Fletcher JA, et al. Gestational exercise protects adult male offspring from high-fat diet-induced hepatic steatosis. *Journal of hepatology*. Jan 2016;64(1):171-8. doi:10.1016/j.jhep.2015.08.022
14. Guth LM, Ludlow AT, Witkowski S, et al. Sex-specific effects of exercise ancestry on metabolic, morphological and gene expression phenotypes in multiple generations of mouse offspring. *Exp Physiol*. Oct 2013;98(10):1469-84. doi:10.1113/expphysiol.2012.070300
15. Murashov AK, Pak ES, Koury M, et al. Paternal long-term exercise programs offspring for low energy expenditure and increased risk for obesity in mice. *Faseb j*. Feb 2016;30(2):775-84. doi:10.1096/fj.15-274274
16. Parastesh M, Heidarianpour A, Sadegh M. Investigating the effects of endurance, resistance and combined training on reproductive hormones and sperm parameters of streptozotocin-nicotinamide diabetic male rats. *J Diabetes Metab Disord*. Dec 2019;18(2):273-279. doi:10.1007/s40200-018-0380-4

17. Sun B, Messerlian C, Sun ZH, et al. Physical activity and sedentary time in relation to semen quality in healthy men screened as potential sperm donors. *Hum Reprod*. Dec 1 2019;34(12):2330-2339. doi:10.1093/humrep/dez226
18. Barrès R, Zierath JR. The role of diet and exercise in the transgenerational epigenetic landscape of T2DM. *Nat Rev Endocrinol*. Aug 2016;12(8):441-51. doi:10.1038/nrendo.2016.87
19. McGee SL, Walder KR. Exercise and the Skeletal Muscle Epigenome. *Cold Spring Harb Perspect Med*. Sep 1 2017;7(9)doi:10.1101/cshperspect.a029876
20. Falcão-Tebas F, Kuang J, Arceri C, et al. Four weeks of exercise early in life reprograms adult skeletal muscle insulin resistance caused by a paternal high-fat diet. *The Journal of physiology*. Jan 2019;597(1):121-136. doi:10.1113/jp276386
21. Armitage JA, Poston L, Taylor PD. Developmental origins of obesity and the metabolic syndrome: the role of maternal obesity. *Front Horm Res*. 2008;36:73-84. doi:10.1159/000115355
22. Falcão-Tebas F, Bento-Santos A, Fidalgo MA, et al. Maternal low-protein diet-induced delayed reflex ontogeny is attenuated by moderate physical training during gestation in rats. *Br J Nutr*. Feb 2012;107(3):372-7. doi:10.1017/s0007114511002947
23. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med*. Jul 3 2008;359(1):61-73. doi:10.1056/NEJMra0708473
24. Fidalgo M, Falcão-Tebas F, Bento-Santos A, et al. Programmed changes in the adult rat offspring caused by maternal protein restriction during gestation and lactation are attenuated by maternal moderate-low physical training. *Br J Nutr*. Feb 14 2013;109(3):449-56. doi:10.1017/s0007114512001316
25. Simmons RA, Templeton LJ, Gertz SJ. Intrauterine growth retardation leads to the development of type 2 diabetes in the rat. *Diabetes*. Oct 2001;50(10):2279-86. doi:10.2337/diabetes.50.10.2279
26. Bayol SA, Simbi BH, Stickland NC. A maternal cafeteria diet during gestation and lactation promotes adiposity and impairs skeletal muscle development and metabolism in rat offspring at weaning. *The Journal of physiology*. Sep 15 2005;567(Pt 3):951-61. doi:10.1113/jphysiol.2005.088989
27. Masuyama H, Hiramatsu Y. Effects of a high-fat diet exposure in utero on the metabolic syndrome-like phenomenon in mouse offspring through epigenetic changes in adipocytokine gene expression. *Endocrinology*. Jun 2012;153(6):2823-30. doi:10.1210/en.2011-2161
28. 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*. Feb 2015;64(2):427-33. doi:10.2337/db13-1848
29. Godfrey KM, Reynolds RM, Prescott SL, et al. Influence of maternal obesity on the long-term health of offspring. *Lancet Diabetes Endocrinol*. Jan 2017;5(1):53-64. doi:10.1016/s2213-8587(16)30107-3
30. Stevanović J, Beleza J, Coxito P, Ascensão A, Magalhães J. Physical exercise and liver "fitness": Role of mitochondrial function and epigenetics-related mechanisms in non-alcoholic fatty liver disease. *Mol Metab*. Feb 2020;32:1-14. doi:10.1016/j.molmet.2019.11.015
31. Gilbert JS, Banek CT, Bauer AJ, Gingery A, Dreyer HC. Placental and vascular adaptations to exercise training before and during pregnancy in the rat. *Am J Physiol Regul Integr Comp Physiol*. Sep 1 2012;303(5):R520-6. doi:10.1152/ajpregu.00253.2012

32. Mottola MF, Artal R. Fetal and maternal metabolic responses to exercise during pregnancy. *Early Hum Dev*. Mar 2016;94:33-41. doi:10.1016/j.earlhumdev.2016.01.008
33. Harris JE, Baer LA, Stanford KI. Maternal Exercise Improves the Metabolic Health of Adult Offspring. *Trends Endocrinol Metab*. Mar 2018;29(3):164-177. doi:10.1016/j.tem.2018.01.003
34. Carter LG, Lewis KN, Wilkerson DC, et al. Perinatal exercise improves glucose homeostasis in adult offspring. *Am J Physiol Endocrinol Metab*. Oct 15 2012;303(8):E1061-8. doi:10.1152/ajpendo.00213.2012
35. Klein CP, Hoppe JB, Saccomori AB, et al. Physical Exercise During Pregnancy Prevents Cognitive Impairment Induced by Amyloid-β in Adult Offspring Rats. *Mol Neurobiol*. Mar 2019;56(3):2022-2038. doi:10.1007/s12035-018-1210-x
36. Simar D, Chen H, Lambert K, Mercier J, Morris MJ. Interaction between maternal obesity and post-natal over-nutrition on skeletal muscle metabolism. *Nutrition, metabolism, and cardiovascular diseases : NMCD*. Mar 2012;22(3):269-76. doi:10.1016/j.numecd.2010.11.007
37. Pileggi CA, Segovia SA, Markworth JF, et al. Maternal conjugated linoleic acid supplementation reverses high-fat diet-induced skeletal muscle atrophy and inflammation in adult male rat offspring. *Am J Physiol Regul Integr Comp Physiol*. Mar 1 2016;310(5):R432-9. doi:10.1152/ajpregu.00351.2015
38. Son JS, Chae SA, Wang H, et al. Maternal Inactivity Programs Skeletal Muscle Dysfunction in Offspring Mice by Attenuating Apelin Signaling and Mitochondrial Biogenesis. *Cell Rep*. Dec 1 2020;33(9):108461. doi:10.1016/j.celrep.2020.108461
39. 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*. 2015;10(4):e0120980. doi:10.1371/journal.pone.0120980
40. Bayol SA, Macharia R, Farrington SJ, Simbi BH, Stickland NC. Evidence that a maternal "junk food" diet during pregnancy and lactation can reduce muscle force in offspring. *Eur J Nutr*. Feb 2009;48(1):62-5. doi:10.1007/s00394-008-0760-5
41. Pileggi CA, Hedges CP, Segovia SA, et al. Maternal High Fat Diet Alters Skeletal Muscle Mitochondrial Catalytic Activity in Adult Male Rat Offspring. *Front Physiol*. 2016;7:546. doi:10.3389/fphys.2016.00546
42. Latouche C, Heywood SE, Henry SL, et al. Maternal overnutrition programs changes in the expression of skeletal muscle genes that are associated with insulin resistance and defects of oxidative phosphorylation in adult male rat offspring. *J Nutr*. Mar 2014;144(3):237-44. doi:10.3945/jn.113.186775
43. Chen H, Simar D, Lambert K, Mercier J, Morris MJ. Maternal and postnatal overnutrition differentially impact appetite regulators and fuel metabolism. *Endocrinology*. Nov 2008;149(11):5348-56. doi:10.1210/en.2008-0582
44. Walter I, Klaus S. Maternal high-fat diet consumption impairs exercise performance in offspring. *J Nutr Sci*. 2014;3:e61. doi:10.1017/jns.2014.55
45. Kusuyama J, Alves-Wagner AB, Makarewicz NS, Goodyear LJ. Effects of maternal and paternal exercise on offspring metabolism. *Nat Metab*. Sep 2020;2(9):858-872. doi:10.1038/s42255-020-00274-7
46. Son JS, Zhao L, Chen Y, et al. Maternal exercise via exerkine apelin enhances brown adipogenesis and prevents metabolic dysfunction in offspring mice. *Sci Adv*. Apr 2020;6(16):eaaz0359. doi:10.1126/sciadv.aaz0359
47. Zheng J, Alves-Wagner AB, Stanford KI, et al. Maternal and paternal exercise regulate offspring metabolic health and beta cell phenotype. *BMJ Open Diabetes Res Care*. Feb 2020;8(1)doi:10.1136/bmjdrc-2019-000890

48. Laker RC, Lillard TS, Okutsu M, et al. Exercise prevents maternal high-fat diet-induced hypermethylation of the Pgc-1α gene and age-dependent metabolic dysfunction in the offspring. *Diabetes*. May 2014;63(5):1605-11. doi:10.2337/db13-1614
49. Quiclet C, Siti F, Dubouchaud H, et al. Short-term and long-term effects of submaximal maternal exercise on offspring glucose homeostasis and pancreatic function. *Am J Physiol Endocrinol Metab*. Aug 1 2016;311(2):E508-18. doi:10.1152/ajpendo.00126.2016
50. Stanford KI, Rasmussen M, Baer LA, et al. Paternal Exercise Improves Glucose Metabolism in Adult Offspring. *Diabetes*. Dec 2018;67(12):2530-2540. doi:10.2337/db18-0667
51. Beleza J, Albuquerque J, Santos-Alves E, et al. Self-Paced Free-Running Wheel Mimics High-Intensity Interval Training Impact on Rats' Functional, Physiological, Biochemical, and Morphological Features. *Front Physiol*. 2019;10:593. doi:10.3389/fphys.2019.00593
52. Quiclet C, Dubouchaud H, Berthon P, et al. Maternal exercise modifies body composition and energy substrates handling in male offspring fed a high-fat/high-sucrose diet. *The Journal of physiology*. Dec 1 2017;595(23):7049-7062. doi:10.1113/jp274739
53. Eclarinal JD, Zhu S, Baker MS, et al. Maternal exercise during pregnancy promotes physical activity in adult offspring. *Faseb j*. Jul 2016;30(7):2541-8. doi:10.1096/fj.201500018R
54. Organization WH. WHO guidelines on physical activity and sedentary behaviour: at a glance. 2020;
55. Gensous N, Bacalini MG, Franceschi C, Meskers CGM, Maier AB, Garagnani P. Age-Related DNA Methylation Changes: Potential Impact on Skeletal Muscle Aging in Humans. *Front Physiol*. 2019;10:996. doi:10.3389/fphys.2019.00996
56. Dimauro I, Paronetto MP, Caporossi D. Exercise, redox homeostasis and the epigenetic landscape. *Redox Biol*. Aug 2020;35:101477. doi:10.1016/j.redox.2020.101477
57. Jiménez-Chillarón JC, Nijland MJ, Ascensão AA, et al. Back to the future: transgenerational transmission of xenobiotic-induced epigenetic remodeling. *Epigenetics*. 2015;10(4):259-73. doi:10.1080/15592294.2015.1020267
58. Barrón-Cabrera E, Ramos-Lopez O, González-Becerra K, et al. Epigenetic Modifications as Outcomes of Exercise Interventions Related to Specific Metabolic Alterations: A Systematic Review. *Lifestyle Genom*. 2019;12(1-6):25-44. doi:10.1159/000503289
59. McGee SL, Hargreaves M. Epigenetics and Exercise. *Trends Endocrinol Metab*. Sep 2019;30(9):636-645. doi:10.1016/j.tem.2019.06.002
60. Barrès R, Yan J, Egan B, et al. Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metab*. Mar 7 2012;15(3):405-11. doi:10.1016/j.cmet.2012.01.001
61. Nitert MD, Dayeh T, Volkov P, et al. Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. *Diabetes*. Dec 2012;61(12):3322-32. doi:10.2337/db11-1653
62. Rowlands DS, Page RA, Sukala WR, et al. Multi-omic integrated networks connect DNA methylation and miRNA with skeletal muscle plasticity to chronic exercise in Type 2 diabetic obesity. *Physiol Genomics*. Oct 15 2014;46(20):747-65. doi:10.1152/physiolgenomics.00024.2014
63. Seaborne RA, Strauss J, Cocks M, et al. Methylome of human skeletal muscle after acute & chronic resistance exercise training, detraining & retraining. *Sci Data*. Oct 30 2018;5:180213. doi:10.1038/sdata.2018.213

64. Alibegovic AC, Sonne MP, Højbjerre L, et al. Insulin resistance induced by physical inactivity is associated with multiple transcriptional changes in skeletal muscle in young men. *Am J Physiol Endocrinol Metab*. Nov 2010;299(5):E752-63. doi:10.1152/ajpendo.00590.2009
65. Sharples AP, Stewart CE, Seaborne RA. Does skeletal muscle have an 'epi'-memory? The role of epigenetics in nutritional programming, metabolic disease, aging and exercise. *Aging Cell*. Aug 2016;15(4):603-16. doi:10.1111/acel.12486
66. McGee SL, Fairlie E, Garnham AP, Hargreaves M. Exercise-induced histone modifications in human skeletal muscle. *The Journal of physiology*. Dec 15 2009;587(Pt 24):5951-8. doi:10.1113/jphysiol.2009.181065
67. McGee SL, Hargreaves M. Exercise and myocyte enhancer factor 2 regulation in human skeletal muscle. *Diabetes*. May 2004;53(5):1208-14. doi:10.2337/diabetes.53.5.1208
68. Yu M, Stepto NK, Chibalin AV, et al. Metabolic and mitogenic signal transduction in human skeletal muscle after intense cycling exercise. *The Journal of physiology*. Jan 15 2003;546(Pt 2):327-35. doi:10.1113/jphysiol.2002.034223
69. Lim C, Shimizu J, Kawano F, Kim HJ, Kim CK. Adaptive responses of histone modifications to resistance exercise in human skeletal muscle. *PLoS One*. 2020;15(4):e0231321. doi:10.1371/journal.pone.0231321
70. Russell AP, Lamon S. Exercise, Skeletal Muscle and Circulating microRNAs. *Prog Mol Biol Transl Sci*. 2015;135:471-96. doi:10.1016/bs.pmbts.2015.07.018
71. Silva GJJ, Bye A, El Azzouzi H, Wisløff U. MicroRNAs as Important Regulators of Exercise Adaptation. *Prog Cardiovasc Dis*. Jun-Jul 2017;60(1):130-151. doi:10.1016/j.pcad.2017.06.003
72. Ultimo S, Zauli G, Martelli AM, et al. Influence of physical exercise on microRNAs in skeletal muscle regeneration, aging and diseases. *Oncotarget*. Mar 30 2018;9(24):17220-17237. doi:10.18632/oncotarget.24991
73. Davidsen PK, Gallagher IJ, Hartman JW, et al. High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. *J Appl Physiol (1985)*. Feb 2011;110(2):309-17. doi:10.1152/japplphysiol.00901.2010
74. Russell AP, Lamon S, Boon H, et al. Regulation of miRNAs in human skeletal muscle following acute endurance exercise and short-term endurance training. *The Journal of physiology*. Sep 15 2013;591(18):4637-53. doi:10.1113/jphysiol.2013.255695
75. Aoi W, Naito Y, Mizushima K, et al. The microRNA miR-696 regulates PGC-1{alpha} in mouse skeletal muscle in response to physical activity. *Am J Physiol Endocrinol Metab*. Apr 2010;298(4):E799-806. doi:10.1152/ajpendo.00448.2009
76. Xu Y, Zhao C, Sun X, Liu Z, Zhang J. MicroRNA-761 regulates mitochondrial biogenesis in mouse skeletal muscle in response to exercise. *Biochem Biophys Res Commun*. Nov 6 2015;467(1):103-8. doi:10.1016/j.bbrc.2015.09.113
77. Guescini M, Canonico B, Lucertini F, et al. Muscle Releases Alpha-Sarcoglycan Positive Extracellular Vesicles Carrying miRNAs in the Bloodstream. *PLoS One*. 2015;10(5):e0125094. doi:10.1371/journal.pone.0125094
78. Michell DL, Vickers KC. Lipoprotein carriers of microRNAs. *Biochim Biophys Acta*. Dec 2016;1861(12 Pt B):2069-2074. doi:10.1016/j.bbalip.2016.01.011
79. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. Jun 2007;9(6):654-9. doi:10.1038/ncb1596

80. Siracusa J, Koulmann N, Banzet S. Circulating myomiRs: a new class of biomarkers to monitor skeletal muscle in physiology and medicine. *J Cachexia Sarcopenia Muscle*. Feb 2018;9(1):20-27. doi:10.1002/jcsm.12227
81. Prats-Puig A, García-Retortillo S, Puig-Parnau M, et al. DNA Methylation Reorganization of Skeletal Muscle-Specific Genes in Response to Gestational Obesity. *Front Physiol*. 2020;11:938. doi:10.3389/fphys.2020.00938
82. Houshmand-Oeregaard A, Schrölkamp M, Kelstrup L, et al. Increased expression of microRNA-15a and microRNA-15b in skeletal muscle from adult offspring of women with diabetes in pregnancy. *Hum Mol Genet*. May 15 2018;27(10):1763-1771. doi:10.1093/hmg/ddy085
83. Laker RC, Wlodek ME, Connelly JJ, Yan Z. Epigenetic origins of metabolic disease: the impact of the maternal condition to the offspring epigenome and later health consequences. *Food Science and Human Wellness*. 2013;2(1):1-11.
84. Bodden C, Hannan AJ, Reichelt AC. Diet-Induced Modification of the Sperm Epigenome Programs Metabolism and Behavior. *Trends Endocrinol Metab*. Feb 2020;31(2):131-149. doi:10.1016/j.tem.2019.10.005
85. Kort HI, Massey JB, Elsner CW, et al. Impact of body mass index values on sperm quantity and quality. *J Androl*. May-Jun 2006;27(3):450-2. doi:10.2164/jandrol.05124
86. Su L, Patti ME. Paternal Nongenetic Intergenerational Transmission of Metabolic Disease Risk. *Curr Diab Rep*. May 24 2019;19(7):38. doi:10.1007/s11892-019-1163-0
87. de Castro Barbosa T, Ingerslev LR, Alm PS, et al. High-fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring. *Mol Metab*. Mar 2016;5(3):184-197. doi:10.1016/j.molmet.2015.12.002
88. Claycombe-Larson KG, Bundy AN, Roemmich JN. Paternal high-fat diet and exercise regulate sperm miRNA and histone methylation to modify placental inflammation, nutrient transporter mRNA expression and fetal weight in a sex-dependent manner. *J Nutr Biochem*. Jul 2020;81:108373. doi:10.1016/j.jnutbio.2020.108373
89. Denham J, O'Brien BJ, Harvey JT, Charchar FJ. Genome-wide sperm DNA methylation changes after 3 months of exercise training in humans. *Epigenomics*. Aug 2015;7(5):717-31. doi:10.2217/epi.15.29
90. 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*. May 1 2015;308(9):E805-21. doi:10.1152/ajpendo.00013.2015
91. Huber K, Miles JL, Norman AM, Thompson NM, Davison M, Breier BH. Prenatally induced changes in muscle structure and metabolic function facilitate exercise-induced obesity prevention. *Endocrinology*. Sep 2009;150(9):4135-44. doi:10.1210/en.2009-0125
92. Laker RC, Wlodek ME, Wadley GD, Gallo LA, Meikle PJ, McConell GK. Exercise early in life in rats born small does not normalize reductions in skeletal muscle PGC-1α in adulthood. *Am J Physiol Endocrinol Metab*. May 1 2012;302(10):E1221-30. doi:10.1152/ajpendo.00583.2011
93. Rajia S, Chen H, Morris MJ. Voluntary post weaning exercise restores metabolic homeostasis in offspring of obese rats. *Nutrition, metabolism, and cardiovascular diseases : NMCD*. Jun 2013;23(6):574-81. doi:10.1016/j.numecd.2011.12.009
94. Fernandes RA, Zanesco A. Early physical activity promotes lower prevalence of chronic diseases in adulthood. *Hypertens Res*. Sep 2010;33(9):926-31. doi:10.1038/hr.2010.106
95. Linnér A, Almgren M. Epigenetic programming-The important first 1000 days. *Acta Paediatr*. Mar 2020;109(3):443-452. doi:10.1111/apa.15050

96. Bahari H, Caruso V, Morris MJ. Late-onset exercise in female rat offspring ameliorates the detrimental metabolic impact of maternal obesity. *Endocrinology*. Oct 2013;154(10):3610-21. doi:10.1210/en.2013-1059
97. Miles JL, Huber K, Thompson NM, Davison M, Breier BH. Moderate daily exercise activates metabolic flexibility to prevent prenatally induced obesity. *Endocrinology*. Jan 2009;150(1):179-86. doi:10.1210/en.2008-1035
98. Patterson CM, Dunn-Meynell AA, Levin BE. Three weeks of early-onset exercise prolongs obesity resistance in DIO rats after exercise cessation. *Am J Physiol Regul Integr Comp Physiol*. Feb 2008;294(2):R290-301. doi:10.1152/ajpregu.00661.2007
99. Ribeiro TA, Tófolo LP, Martins IP, et al. Maternal low intensity physical exercise prevents obesity in offspring rats exposed to early overnutrition. *Scientific reports*. Aug 9 2017;7(1):7634. doi:10.1038/s41598-017-07395-2
100. Harris JE, Pinckard KM, Wright KR, et al. Exercise-induced 3'-sialyllactose in breast milk is a critical mediator to improve metabolic health and cardiac function in mouse offspring. *Nat Metab*. Aug 2020;2(8):678-687. doi:10.1038/s42255-020-0223-8