Fit mothers for a healthy future

Breaking the intergenerational cycle of NAFLD with maternal exercise

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CONFLICT OF INTEREST

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Abstract

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Background: Non-alcoholic fatty liver disease (NAFLD) emerges as significant health burden worldwide. Lifestyles changes - unhealthy dietary habits and physical inactivity, can trigger NAFLD development. Persisting on these habits during pregnancy affects *in utero* environment and prompts a specific metabolic response in fetus resulting in offspring metabolic maladjustments potentially critical for developing NAFLD later in life. The increasing prevalence of NAFLD, particularly in children, has shifted the research focus towards preventive and therapeutic strategies. Yet, designing effective approaches that can break the NAFLD intergenerational cycle becomes even more complicated. Regular physical exercise (PE) is a powerful non-pharmacological strategy known to counteract deleterious metabolic outcomes. In this narrative review, we aimed to briefly describe NAFLD pathogenesis focusing on maternal nutritional challenge and fetal programming, and to provide potential mechanisms behind the putative intergenerational effect of PE against metabolic diseases, including liver diseases.

Methods: Following detailed electronic database search, recent existing evidence about NAFLD development, intergenerational programming, and gestational exercise effects were critically analysed and discussed.

Results: PE during pregnancy could have a great potential to counteract intergenerational transmission of metabolic burden. The interplay between different PE roles – metabolic, endocrine, and epigenetic - could offer a more stable *in utero* environment to the fetus, thus rescuing offspring vulnerability to metabolic disturbances.

Conclusions: The better understanding of maternal PE beneficial consequences on offspring metabolism could reinforce the importance of PE during pregnancy as an indispensable strategy in improving offspring health.

Keywords

NAFLD; Physical exercise; Maternal exercise; Fetal programming; Offspring health

Abbreviations

GDM – Gestational diabetes mellitus; HFD – High-fat diet; IR – Insulin resistance; NAFLD – Non-alcoholic fatty liver disease; PE – Physical exercise; PGC-1α – PPARγ coactivator-1α; PPAR – Peroxisome proliferator-activated receptor; SCD – Standard chow diet; SM – Skeletal muscle; TFAM – Mitochondrial transcription factor A

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is considered the most common worldwide cause of chronic liver diseases.[1] Described as the hepatic manifestation of metabolic syndrome, it includes a range of conditions characterized by excessive hepatic lipid accumulation. Hepatic steatosis reflects "simple" fat accumulation in the liver; however, if additionally pressured by the activation of inflammatory and fibrotic signalling pathways activation, steatosis can progress to non-alcoholic steatohepatitis, or even cirrhosis and hepatocellular carcinoma. As a complex disease, NAFLD results from the interaction of genetic and environmental factors, particularly unhealthy dietary patterns and physical inactivity.[2,3]

About 25% of the world's population has NAFLD, with very high prevalence even in children – 3-17%,[1] and up to 70-80% among obese children.[4] NAFLD in children may occur as a consequence of obesity and/or intrauterine environment.[5,6] Environmental factors to which mothers are exposed during pregnancy (diet, stress, smoking, alcohol consumption, and/or gestational diabetes mellitus (GDM)) are important determinants of fetal programming.[6] Given that maternal lifestyle can seriously affect the metabolic status of future generations[7-11], it is urgent to "design" efficient preventive tools against NAFLD. Since there are no approved medications for NAFLD treatment, lifestyle changes, mainly the engagement in physical exercise (PE) programs, are recommended.[12-14] Being already evidenced as effective in the prevention and treatment of numerous metabolic, neurological, oncological, cardiovascular, musculoskeletal, pulmonary, and psychiatric diseases,[14] PE has a great potential to prevent intergenerational transmission of metabolic impairments via metabolic, endocrine, and/or epigenetic mechanisms if implemented during critical moments of pregnancy. The promotion of PE during pregnancy should be emphasized to break the intergenerational cycle of NAFLD and provide healthy early-life environment. Thus, in this narrative review, we aim to critically analyse both the physiopathology of NAFLD and countermeasures to mitigate this disease, as well as how PE acts through distinct pathways and at several levels of systemic and cellular organization in order to counteract NAFLD. Insights into how skeletal muscle (SM) behaves as an endocrine organ, the metabolic action of PE through mitochondrial-related adaptations, and PE-induced epigenetic adaptations against NAFLD are provided. Also, recommendations regarding maternal PE and its beneficial consequences on offspring metabolism are discussed.

2. NAFLD as a multiorgan disease

Although NAFLD is characterised by excessive hepatic lipid accumulation, these complications are not exclusive to the liver tissue.[15,16] NAFLD is closely related to other metabolic disorders, like insulin

resistance (IR), diabetes mellitus, obesity, cardiovascular diseases, and GDM.[16-18] Extra-hepatic complications also involve chronic kidney disease, colorectal cancer, atherosclerosis, osteoporosis, thyroid dysfunction, obstructive sleep apnea, and polycystic ovarian syndrome.[16,18] Indeed, cardiovascular diseases are the most common cause of death in NAFLD patients.[18] Thus, NAFLD can be considered a multiorgan disease, manifesting in several organs, with a very complex interplay between different tissues.[15] Yet, NAFLD can occur even in non-obese subjects, where genetic and lifestyle factors play a part in the development of the so-called lean NAFLD.[19] The imbalance between energy intake and expenditure, due to high-caloric intake and low levels of physical activity, may result in ectopic lipid accumulation. In such conditions, lipid storage capacity of the adipose tissue gets saturated, and excess lipids are forwarded to liver, muscle and pancreas. Once these organs also become saturated, excess lipids can trigger the production of toxic reactive lipid species and lipotoxicity.[20] A significant role in the pathogenesis of metabolic diseases belongs to IR in SM[21,22] since SM acts as a sensor of low expenditure of stored energy and signal for events that will ultimately result in IR.[21] The subsequent hyperinsulinemia can trigger a sequence of metabolic pathways that favour the development of metabolic disorders, among which hepatic steatosis is of high concern.[21]

2.1. The role of mitochondria in NAFLD

As a high-metabolic rate tissue with numerous metabolic functions, the liver is highly susceptible to disturbances due to excessive lipid accumulation that may lead to several metabolic impairments. In this context, the role of mitochondria in cellular energy production through oxidative phosphorylation seems to be crucial.[23] Additionally, liver mitochondrial dysfunction has been related to cellular death and inflammation, and thus, it is proposed as one of the first events during steatohepatitis development.[12]

Due to the imbalance between caloric intake and energy expenditure, perturbed regulation of glucose and lipid metabolism results in an excessive hepatic accumulation of fatty acids and triglycerides and consequently in steatosis. To recover from this lipid burden, hepatic β-oxidation rate increases in the beginning, as well as the electron flux through the electron transport chain. However, enhanced reductive supply to the electron transport chain, when coupled with membrane permeabilization and consequent cytochrome c release, through permeability transition pore opening, leads to an electron transport chain over-reductive state. In these circumstances, increased electron leakage promotes inefficiency of mitochondrial ATP synthesis, alongside augmented reactive oxygen species production and oxidative stress.[3,12,24] Moreover, inflammatory and fibrogenic responses can aggravate steatosis and “push” its progression towards steatohepatitis.[12,25]

The regulation and promotion of β-oxidation in liver mitochondria are dependent on key molecular players, such as peroxisome proliferator-activated receptors (PPARs) and their coactivator PPARγ-coactivator-1α (PGC-1α).[26] Yet, PGC-1α and its downstream target mitochondrial transcription factor A (TFAM) are reported to be decreased in hepatic steatosis.[27] The consequent change in mitochondrial homeostasis and efficiency, together with structural alterations in mitochondrial network morphology can be dangerous to cell viability. Therefore, the dynamic and inter-related adaptive processes involved in mitochondrial plasticity and turnover occur through an interplay of biogenesis, fusion, fission, and auto(mito)phagy activation, contributing to the maintenance of mitochondrial network. However, in NAFLD conditions, the function, structure, and dynamic processes of liver mitochondria are considerably altered. Disturbance in mitochondrial quality control contributes to hepatic lipid accumulation and subsequent pathogenesis of NAFLD.[23]

2.2. Intergenerational cycle of NAFLD

The attempts to describe pathogenesis of such a complex disorder can be summarized in a 'multiple hit hypothesis' considering that multiple insults (IR, release of adipokines and proinflammatory cytokines, over-nutrition, altered gut flora, and (epi)genetic challenges) work together to prompt NAFLD development.[2] Among these intertwined environmental and genetic factors in NAFLD pathogenesis, *in utero* environment emerges as the new 'first' hit.[28] In fact, children of steatosis-diagnosed parents are at increased risk for NAFLD development even if they do not have other metabolic-related risk factors.[28] Likewise, numerous animal studies stressed that maternal obesity or improper dietary habits during pregnancy and/or lactation are important factors that promote metabolic and mitochondrial dysfunction and eventually hepatic steatosis.[7-11,29] Intrauterine exposure to hyperglycaemia, typical of GDM, may increase the risk of later-life metabolic disorders, being *Pgc-1α* epigenetic alteration one of the potential mechanisms triggering this metabolic malprogramming.[30]

Accordingly, maternal nutrient overload and lipids excess may result in fetal lipotoxicity. In early gestation, *de novo* lipogenesis in fetal liver is limited and β-oxidation exists only at basal levels, making the liver inefficient when dealing with lipid surplus. Therefore, excessive lipid influx to fetal liver during early-to-mid gestation, before fetal adipose tissue development, results in ectopic lipid accumulation in liver making it susceptible to later-in-life NAFLD development (Fig.1).[28] This altered *in utero* environment that fetus is exposed to, specifically maternal high-fat diet (HFD), may provoke offspring metabolic disturbances, which extent depends on postnatal offspring diet. Actually, offspring from HFD-fed mice were vulnerable to develop steatosis even if fed with standard chow diet (SCD) in their early-life, whereas early-life HFD-feeding aggravated their liver metabolism to progressive

steatohepatitis.[8] Similarly, despite 23-week-long post-weaning SCD consumption, re-exposure to HFD in adult mice resulted in steatosis and inflammation, and higher susceptibility to steatohepatitis development.[31] In rats, even the prolonged SCD consumption by the offspring of HFD-fed mothers was not effective in reducing hepatic steatosis, suggesting that the metabolic intrauterine deleterious consequences might be irreversible at the post-weaning period.[7] Instead, HFD-fed offspring still developed steatosis, despite maternal SCD-consumption during pregnancy and lactation, whereas offspring vulnerability to develop steatohepatitis increases when mothers are fed with HFD during pregnancy.[7,31] Furthermore, HFD-feeding for three consecutive generations resulted in increasingly severe NAFLD manifestations at each generation, which can be explained, at least partly, as a consequence of transgenerational accumulation of epigenetic alterations.[32] In contrast, changing maternal diet from HFD to a low-fat diet in the subsequent pregnancy improved triglyceride levels in fetal livers,[33] suggesting that pregnancy can be proposed as a critical window for the implementation of intervention strategies. Taken together, these studies highlight that pregnancy is a critical period for programming offspring predisposition to NAFLD development and progression.

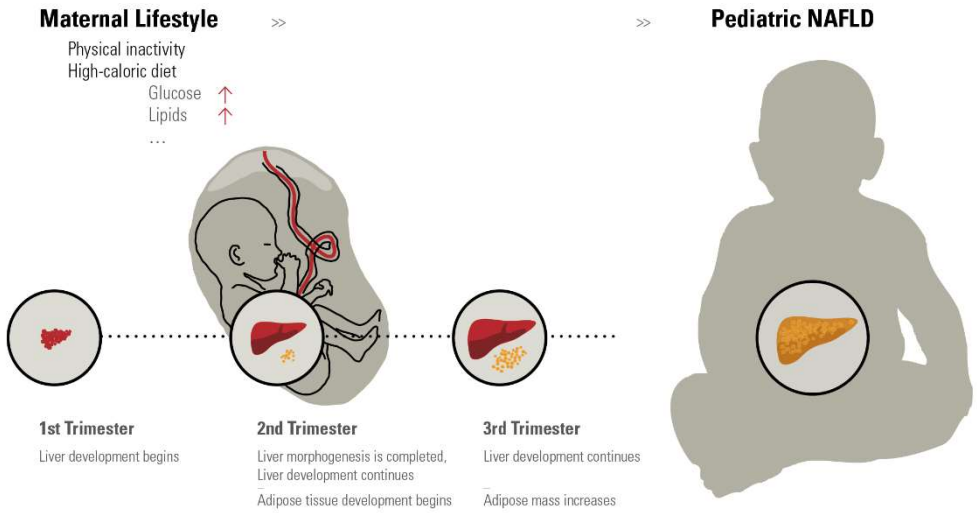

FIGURE 1. Potential mechanisms behind the development of non-alcoholic fatty liver disease (NAFLD) in children

In utero environment has been recognized as a critical factor in fetal development as it can affect a fetus' risk of developing metabolic disorders, such as NAFLD, later in life. In uncomplicated pregnancies, placental glucose and lipid transport is well regulated and matches with the development of fetal liver and adipose tissue. However, inappropriate maternal lifestyle, namely physical inactivity and high-caloric nutrition, results in the maternal nutrient overload that may cause adverse alterations to the *in utero* environment in early pregnancy. In such circumstances, the fetus is exposed to lipid excess that needs to be accumulated in not yet competent or developed organs. As the adipose tissue begins to develop during mid-pregnancy, a need for other lipid accumulation sites such as liver emerges. Fetal liver, still not completely developed, but already pressured by lipid excess, will become susceptible to metabolic disturbances, not only throughout intrauterine developmental

period but also later in life, especially if additionally "stressed" by an inadequate lifestyle during childhood. The likely end result is a health frailty childhood condition characterized by features of pediatric NAFLD.

2.3. Prevention and treatment of NAFLD

Given the social, economic, and healthcare burden of NAFLD, a need for effective preventive and/or therapeutic approaches is emerging. However, clinical management against such a complex multiorgan disease is a complicated process. As there are still no approved medications for NAFLD treatment, recommended medications usually target associated metabolic disorders - obesity, IR, diabetes, or lipid disorders. Some of the pharmacological treatments include antioxidant vitamin supplementation, PPAR agonists, or anti-diabetic, anti-obesity, anti-apoptotic, anti-inflammatory, anti-fibrotic agents.[34] As NAFLD prevalence is remarkably high in (morbidly) obese individuals,[17] bariatric surgery and surgery-related weight loss are proposed as another strategy against NAFLD. Still, this approach cannot be implemented in patients who are not either obese or have a high risk for surgical complications due to other metabolic disorders.[35] Thus, lifestyle interventions (diet and PE) appear as the first-line approach in the management of NAFLD, a disease that is, in fact, mainly triggered by lifestyle modifications. Numerous studies have proven that PE is efficient at reducing hepatic lipids even without dietary changes or weight loss.[13,36-39] A meta-analysis study also recommended PE as a therapeutic strategy against NAFLD, despite differences in PE type, duration, and/or intensity.[13] Accordingly, data from animal NAFLD models also showed that PE is able to ameliorate NAFLD-related metabolic and mitochondria-related impairments.[40,41]

With intergenerational transmission of metabolic impairments coming to the fore, efficient therapeutic strategies against developmental programming of NAFLD become a prime concern. Early-life intervention strategies, such as early and prolonged breastfeeding and dietary supplements with anti-inflammatory and antioxidant properties, are suggested as beneficial.[5,42] Moreover, some animal studies highlight the importance of offspring PE to ameliorate the detrimental metabolic influence of maternal obesity.[43-45] Yet, these approaches are focused on therapeutic levels, and not on breaking the intergenerational inheritance of NAFLD. Therefore, the critical timeframe of *in utero* development could be the right moment to implement potential preventive programs in pregnant mothers and avert children's metabolic impairment. Nevertheless, choosing the precise pharmacological therapy is not an easy task as pharmacokinetics and pharmacodynamics may vary even in normal pregnancies due to complex changes in maternal physiology, which can eventually affect fetus safety.[46] Thus, there is a need to encourage healthy lifestyle during pregnancy for the benefit of future generations health[47]. Since PE is proposed as the cornerstone in NAFLD prevention, PE during pregnancy may also have benefits in preventing the intergenerational inheritance of NAFLD.

3. "Exercise is medicine"

Considering the role of PE as a stimulus to mitochondrial biogenesis and function, PE can be an effective preventive or therapeutic approach to metabolic disorders in which mitochondrial failure is an essential cornerstone, such as NAFLD. Despite PE characteristics, including type, duration, intensity, volume, and/or frequency can differently contribute to the eventual beneficial short- or long-term adaptations in muscle and other tissues, it is unanimously considered a powerful non-pharmacological tool against deleterious effects of modern lifestyles. However, the underlying mechanisms associated with these PE-related beneficial effects in the intergenerational context are still not clearly understood. Thus, in the following paragraphs, we aimed to briefly review putative mechanisms of PE that could counteract deleterious metabolic outcomes.

3.1. Metabolic action of exercise

Physical inactivity has been associated with development of numerous chronic metabolic disorders. As discussed before, IR in SM due to physical inactivity can contribute to the development of hepatic steatosis. A decrease in energy storages turnover is one of the primary events associated to physical inactivity, eventually leading to decreased glycogen and lipid turnover in SM.[21] Actually, whereas SM insulin sensitive individuals store most of ingested energy as glycogen in both liver and muscle, in SM insulin resistant individuals, the storage of ingested energy is shifted towards liver over SM. This can initiate hepatic *de novo* lipogenesis and consequent increase in hepatic triglyceride synthesis and plasma hypertriglyceridemia.[22] Therefore, targeting SM-related IR might prevent the development of NAFLD and associated diseases. Indeed, resistance and aerobic training can improve insulin sensitivity in obese[48], diabetes[37], or NAFLD[36,39] patients. Interestingly, PE improves insulin action more strongly in the offspring of diabetic patients than in individuals with no family history of diabetes.[49,50]

Besides IR regulation, PE modulates multiple processes that may protect liver from NAFLD. Aerobic exercise can mitigate NAFLD through regulation of hepatic lipid metabolism by increasing β-oxidation-related cellular machinery and reducing lipogenesis, as well as through amelioration of redox-, inflammation- and apoptosis-driven status. Moreover, PE also activates hepatic protective pathways through autophagy-related mechanisms.[51] Among PE-induced adaptations, regulation of mitochondrial remodelling and quality control is of great importance in preventing pathological outcomes. PE can stimulate mitochondrial biogenesis, dynamics and auto(mito)phagy to promote the generation of new mitochondria, replacing, at the same time, the old and less-functional network fragments, thus maintaining the pool of healthy mitochondria and improving metabolic function. At

molecular level, particular relevance has been attributed to PGC-1α and its regulatory role in mitochondrial biogenesis in the context of PE.[52] In fact, data suggest that PE can improve mitochondrial function and bioenergetics which are compromised in various liver diseases, including NAFLD.[37,39-41,53]

3.2. Skeletal muscle as an endocrine organ

Besides its metabolic effects, SM also functions as an endocrine organ. Metabolic and mechanical stimuli promoted by PE's contractile activity can express pleiotropic effects on non-contractile tissues. During the exercise and post-exercise recovery periods, muscle cells release molecules with paracrine or endocrine effects, known as myokines.[54,55] Once released into the bloodstream and recognized by specific receptors on different target organs, myokines activate various signalling pathways and modulate metabolic processes. The first identified myokine – interleukin-6, is stimulated by PE in an intensity- and duration-dependent manner. It promotes glucose uptake by SM, thus contributing to the maintenance of SM glucose homeostasis and lipolysis .[55] PE-dependent interleukin-6 stimulation might be important in the management of NAFLD, considering that interleukin-6 can protect liver from steatosis and ischemia/reperfusion injury.[56] Interleukin-6 and fibronectin type-III domain-containing protein-5, cleaved as irisin, are recognized by specific receptors on liver and other tissues, initiating signalling pathways and ultimately activating mitochondrial master regulator PGC-1α.[54,57] PGC-1α activation further stimulates mitochondrial biogenesis, dynamics and quality control processes, as well as, electron transport chain complex activities,[54,55,57,58] which is an important countermeasure to NAFLD pathology. Considering that SM contributes to insulin-mediated glucose uptake and β-oxidation, myokines are likely to affect the whole-body metabolism in a beneficial manner. The myokine-induced positive modulation of liver mitochondrial remodelling can prepare liver to resist to adverse stimuli that are involved in development and pathogenesis of metabolic diseases, including NAFLD.

3.3. Epigenetic adaptations to exercise

Among numerous lifestyle factors that modulate human health via epigenetic alterations in a harmful or beneficial fashion, PE represents a beneficial epigenetic influence. It is known that PE-induced adaptive response also involves alterations in epigenetic landscape that includes DNA methylation, histone modifications, and microRNA regulation.[59] Barrès and co-workers showed that acute PE caused hypomethylation of metabolic genes, such as *Pgc-1α, TFAM, PPARβ*, and consequent increase of gene expression in SM of sedentary people, in a PE intensity-dependent manner.[60] Moreover, PE

prevented liver DNA hypermethylation and epigenome susceptibility to HFD-induced metabolic disturbance in mice.[61] Such PE effect could have clinical implications. In fact, hepatic *Pgc-1α* methylation seems to be highly correlated with peripheral IR and reduced mitochondrial biogenesis in NAFLD patients.[62]

Similarly, microRNA-related signalling networks can be regulated by PE, also supporting its therapeutic potential against the development of distinct metabolic diseases at epigenetic level.[63] The expression of circulating microRNAs involved in various functions (inflammation, muscle contractility, mitochondrial metabolism, hypoxia/ischemia adaptations, or lipid metabolism) - miR-21, miR-146a, miR-210, can be modulated by PE.[64] Accordingly, high-intensity PE induced increased expression of hepatic miR-122 in diabetic rats, which correlates with a rescuing-like phenotype of some NAFLD features.[65] This PE-induced miR-122 upregulation is important in the management of metabolic disorders as miR-122 regulates mitochondrial metabolism and its loss can cause liver dysfunction.[66] Interestingly, circulatory miR-122 levels are found to be reduced in GDM rats and their fetuses.[67] Acute endurance exercise decreased miR-23, a negative regulator of PGC-1α, thus promoting an increased expression of PGC-1α mRNA and its targets.[63] Also, while low levels of PE resulted in increased SM levels of miR-696 in mice, which further reduced its target PGC-1α, β-oxidation, and mitochondrial DNA content, endurance training downregulated miR-696 levels, thus rescuing inhibited mitochondrial biogenesis.[68] However, voluntary PE reduced miR-696 levels in mice SM, which seemed not to be correlated to PGC-1α.[69] This may suggest that PE type and intensity can differently modulate microRNAs roles in mitochondrial biogenesis.

As a master regulator of mitochondrial biogenesis and activity of transcription factors involved in β-oxidation, gluconeogenesis, and lipogenesis[70], PGC-1α is an attractive gene candidate for therapeutic interventions. Hence, it drags a lot of attention in PE studies, particularly when it comes to epigenetic regulation. Stimulation of PGC-1α and subsequently mitochondrial biogenesis through epigenetic modulation, could explain the positive modulation of PE in mitochondria-related diseases, such as NAFLD. Still, most studies are focused on PE-related epigenetic adaptations in SM rather than in liver. Further research on liver alterations and muscle-liver axis could provide a thorough explanation on PE modulation of liver diseases via epigenetic mechanisms (Fig.2).

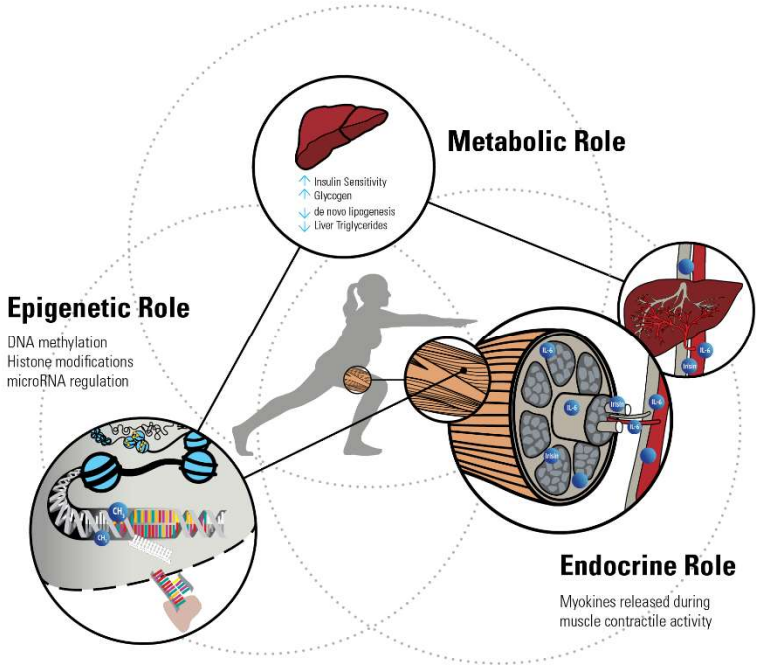

FIGURE 2. Liver-muscle crosstalk
The interaction between skeletal muscle and liver takes place on several intertwined and interconnected levels – metabolic, endocrine, and epigenetic. This crosstalk stimulated by physical exercise (PE) has effects on skeletal muscle (SM), but also on non-contractile tissues, such as liver. Generally, exercise promotes energy turnover in SM and improves insulin sensitivity in both SM and liver, and consequently decreases *de novo* lipogenesis and hepatic lipid accumulation. Moreover, SM contractile activity promotes myokines secretion to the bloodstream. After reaching the liver, myokines can activate signaling pathways and thus regulate metabolic processes. Metabolic processes in the liver can also be modulated by epigenetic-related mechanisms - DNA methylation, post-transcriptional regulation by microRNAs, and post-translational modification of histone proteins. These precisely orchestrated metabolic, endocrine, and epigenetic mechanisms induced by PE may impact SM and liver adaptive response, thus improving liver „fitness“ and promoting beneficial health-related adaptations.

4. Prenatal exercise

Although PE is consensually considered as a preventive and therapeutic strategy that mitigates lifestyle-associated metabolic pathologies, questions still arise on whether and how PE can prevent the transmission of maternal metabolic impairments to offspring and break the intergenerational cycle of NAFLD. Maternal PE during pregnancy is a promising, yet challenging approach in managing a possible transmission of maternal metabolic burden to the next generation (Fig.3).

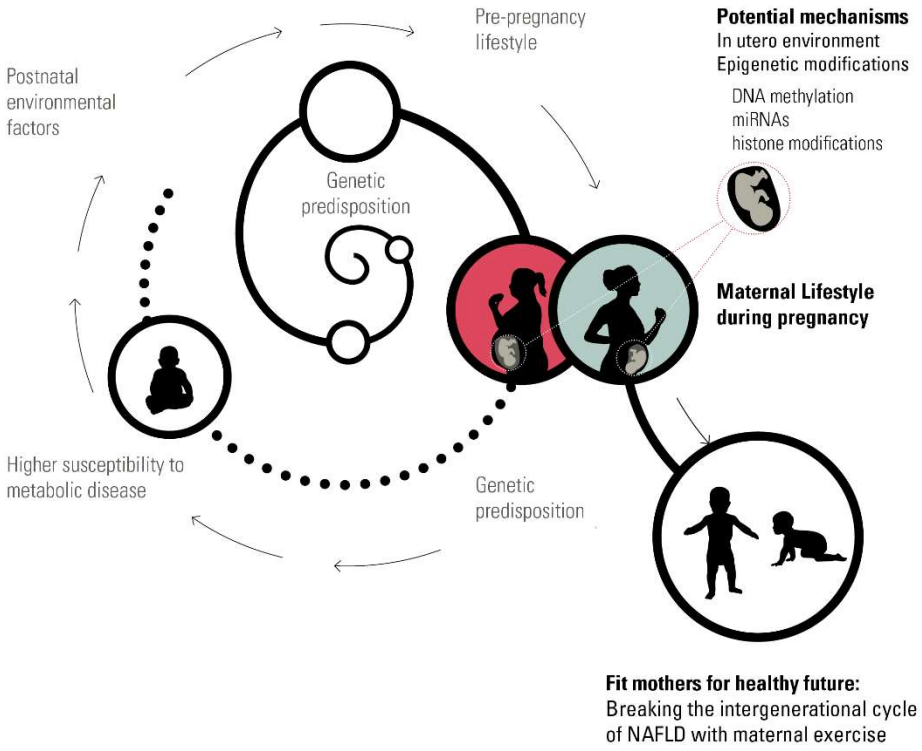

FIGURE 3. Breaking the intergenerational cycle of NAFLD with maternal exercise
Early fetal development is a critical timeframe that may decide the metabolic destiny of the new-growing human being. Genetic predisposition, as well as pre-pregnancy lifestyle, such as physical (in)activity and (im)proper dietary habits, remarkably affect *in utero* environment in a metabolic and/or epigenetic manner, influencing fetal development. A fetus developing in an adverse *in utero* environment has an increased susceptibility to NAFLD later in life, especially when further pressured by genetic predisposition and postnatal environmental factors, resulting in more severe NAFLD manifestations in the following generations. However, an active lifestyle during pregnancy provides health-beneficial outcomes in the offspring generation, potentially contributing to breaking the disease-related cycle. It is therefore critical to highlight that maternal exercise during pregnancy represents a promising approach in breaking the intergenerational cycle of NAFLD and preventing transmission of maternal metabolic burden to children.

4.1. Safety and recommendations

During pregnancy, a woman's body undergoes considerable anatomical and physiological alterations, which may result in lifestyle changes and eventual shift towards more sedentary behaviours. However, since the earliest recorded recommendations from the 18th century until nowadays, an active lifestyle during pregnancy has been considered favourable for mothers' and babies' health. Earlier, prenatal PE was only associated with easier labor and reduced fetal size; however, the awareness of the health-related benefits of prenatal PE changed throughout time, being nowadays extended to several dimensions of mothers´ and offspring´s wellness. Still, the concept of individualized moderation, regarding PE´s intensity and volume during pregnancy, remains important in this particular life period.[71]

Regular PE in pregnancy is recommended to promote health benefits and associated with minimal risks. Some of the benefits of PE during pregnancy include lower risk for GDM, preterm birth, excessive gestational weight gain, caesarean delivery, and macrosomia.[72] However, considering pregnancy-related physiological and anatomical changes and/or medical complications, some modifications and/or individualized PE programs are recommended.[72,73] Recent guidelines consider some activities safe to be performed during pregnancy, such as walking, swimming, stationary cycling, dancing, low-impact aerobic, resistance and stretching exercises,[72,73] even running/jogging and racquet sports if regularly practiced prior to pregnancy,[73] whereas contact sports and activities with high risk of falling should be avoided.[72] The American College of Obstetricians and Gynecologists and American College of Sports Medicine agree on moderate-intensity PE during pregnancy, recommending if not every day, then at least 20-30 minutes/day on most days of the week,[72] or at least 3 days/week with accumulated 150 minutes/week of PE[73], respectively. PE is considered safe and beneficial for health in uncomplicated pregnancies, it would be expected that prenatal PE might also mitigate maternal, fetal, and neonatal metabolic impairments that could trigger the development of NAFLD in both generations.

4.2. Maternal exercise and offspring metabolism

Recent research regarding prenatal PE and offspring health provides sufficient evidence to support maternal engagement in PE programs during pregnancy. A recent meta-analysis corroborated the beneficial effects of maternal PE on newborns, as prenatal PE was not associated with any neonatal complications and harmful outcomes, and the risk for macrosomia was reduced.[74] Moreover, infants of mothers that exercised during pregnancy had improved neuro-motor development, suggesting that they may be more physically active, thus with potentially lower risk to develop obesity later in life.[75] In fact, moderate prenatal PE can reduce infant adiposity at 6 months of age[76] and the offspring risk to develop overweight or obesity in childhood and pre-adolescence.[77]

Considering human natural life-span, the study of inter- and transgenerational effects of maternal lifestyle on the next generations is rather complicated. Therefore, rodent models can provide valuable mechanistic information in an appropriate time period. Early animal studies of prenatal PE from three decades ago, demonstrated that offspring of exercised (diabetic) mothers have enhanced glucose tolerance compared to offspring of sedentary mothers.[78-81] These findings were corroborated by recent studies that include offspring of various ages, from fetuses and neonates to 72-week-old, and maternal PE at different time points: during pregnancy, before and during pregnancy, or during pregnancy and lactation[82-93] (for details see Table 1).

Table 1. Alterations in offspring hepatic metabolism in response to maternal exercise performed before and/or during pregnancy.

Reference	Species/Strain and maternal age	Maternal diet	Maternal exercise protocol	Offspring	Effects of maternal exercise on offspring hepatic metabolism
Carlson et al. 1986 [78]	Sprague-Dawley rats	SCD	• Acute PE at GD20.5 Treadmill: 60 min at 12m/min • Acute PE at GD21.5 Treadmill: 60 min at 16 m/min at 10% grade	Fetuses, - E20.5 - E21.5	~ hepatic glycogenolysis ~ hepatic glycogen content
Rodgers et al. 1991 [80]			• PE 4 wks before and during pregnancy Treadmill: 26.8 m/min, 1 hour/day, 5 days/week	28-days-old	~ hepatic glycogen content
Houghton et al. 1997 [79]	Sprague-Dawley rats; 50-60 days of age	SCD	• PE 3 wks before pregnancy; • PE 3 wks before and during pregnancy. Treadmill: 30m/min, 10^{o} inclination, (60-70% VO_2max), 1 hour/day, 5 days/week	Fetuses E20	↓ hepatic glycogen content (maternal PE before pregnancy); ~ hepatic glycogen content (maternal PE before and during pregnancy)
Carter et al. 2013 [84]	Sprague-Dawley rats; 12-wk-old	SCD	• PE 7-10 days before and during pregnancy, and 12 days after delivery Free-running wheel	Female offspring; 17-mo-old; 18% protein diet; Sedentary	↑ glucose tolerance ↑ glucose disposal ↑ glucose infusion rate ↑ glucose turnover rate ↓ hepatic glucose production
Laker et al. 2014 [86]	C57BL/6 mice; 8-wk-old	SCD or HFD	• PE 6 wks before and during pregnancy Free-running wheel	Offspring from neonatal until 12-mo-old; SCD; Sedentary	~ hepatic *Pgc-1α* methylation: neonatals
Songstad et al. 2015 [92]	Sprague-Dawley rats; 9-11-wk-old	SCD	• PE 3 wks before and during pregnancy Treadmill: HIIT protocol 5 days/wk – 5 minutes warm up at low speed (at <60%	Male and female fetuses (E20)	~ hepatic oxidative stress level ~ hepatic total antioxidant capacity ↓ hepatic GPx4.2 gene expression

			of V_{O2max}), 10 bouts of 4 minutes high intensity running on a treadmill at 25° inclination (at 85–90% of VO_2max) alternating with two minutes of active recovery (at 50–60% of V_{O2max})		↓ hepatic eNOS gene expression ↓ hepatic HIF1A gene expression ~ hepatic SOD1, SOD2, iNOS, CAT, HK2, GPx1, GPx2, GPx4.1 gene expression
Quiclet et al. 2016 [88]	Wistar rats; 15-wk-old	SCD	• PE 4 wks before and during pregnancy (until GD18) Treadmill: gradually increased to the speed of 25 m/min (55% of maximal aerobic speed), 1 hour/day	Male offspring; - 3/4-wk-old - 7-mo-old SCD; Sedentary	~ glucose tolerance: 3-wk-old offspring ↑ glucose tolerance: 7-mo-old offspring ~ insulin sensitivity ~ hepatic insulin signaling pathway activation (pPKB/PKB ratio): 3-wk-old and 7-mo-old offspring
Sheldon et al. 2016 [89]	Sprague-Dawley rats	SCD	• PE during pregnancy Free-running wheel	Male and female offspring; - 4-mo-old; - 8-mo-old; SCD or HFD; Sedentary	~ glucose tolerance ~ hepatic TG: 4-mo-old male offspring; ↓ hepatic TG: 8-mo-old male offspring ~ hepatic TG: female offspring ~ hepatic 1-[14C] palmitate oxidation to CO2 ~ hepatic β-HAD activity ~ hepatic CS activity ↑ enhanced mitochondrial biogenesis (PGC-1α, TFAM gene expression): 8-months old male offspring ↑ elevated basal autophagic potential (ATG12:5 conjugate, pAMPK/AMPK protein expression): 8-months old male offspring
Quicklet et al. 2017 [87]	Wistar rats; 15-wk-old	SCD	• PE 4 wks before and during pregnancy (until GD18) Treadmill: gradually increased to the speed of 25 m/min (55% of maximal aerobic speed), 1 hour/day	Male offspring; 3-mo-old; SCD or HFHS; Sedentary	~ glucose tolerance ~ insulin sensitivity ~ hepatic insulin signaling pathway activation (pPKB/PKB ratio) ↓ hepatic CS activity ~ hepatic HAD: SCD-fed offspring ↓ hepatic HAD: HFHS-fed offspring

					↑ hepatic glycogen content ~ pGSK3/GSK3 ~ hepatic PEPCK ↓ hepatic FAS: SCD-fed offspring ↓↓ hepatic FAS: HFHS-fed offspring
Stanford et al. 2017 [93]	C57BL/6 mice; 6-wk-old	SCD or HFD	• PE 2 wks before and during pregnancy; • PE 2 wks before pregnancy; • PE during pregnancy Free-running wheel	Female offspring; - 8-wk-old; - 12-wk-old; - 24-wk-old; - 36-wk-old; - 52-wk old. Sedentary	↑ glucose tolerance: 8-wk-old (SCD-fed mothers), 36-wk-old (HFD-fed mothers), 52-wk-old (HFD-fed mothers) offspring ~ glucose tolerance: 12-wk-old and 24-wk-old. ↑ glucose tolerance: 36-wk-old and 52-wk-old offspring of mothers with PE before and during pregnancy ~ glucose tolerance: 36-wk-old and 52-wk-old offspring of mothers with PE before pregnancy or PE during pregnancy ↑ insulin tolerance: 52-wk-old offspring of mothers with PE before and during pregnancy or with PE during pregnancy ~ insulin tolerance: 52-wk-old offspring of mothers with PE before pregnancy ↓ basal rate of hepatic glucose production: 6-, 12-, 24-wk-old offspring ↓ insulin-mediated hepatic glucose production: 12-, 24-wk-old offspring ~ insulin-mediated hepatic glucose production: 6-wk-old offspring ↓ glucagon-mediated hepatic glucose production: 6-, 12-, 24-wk-old offspring ~ glucose infusion rate, rates of glucose appearance, basal rates of glucose disappearance, insulin-stimulated rates of glucose disappearance: 52-wk-old offspring ↓ hepatic TG content: 52-wk-old offspring ↑ hepatic expression of genes involved in pyruvate metabolism, Krebs cycle, and FA transport and oxidation

Cunningham et al. 2018 [85]	Wistar rats; 7-8-wk-old	SCD or WD	• PE 5 wks before and during pregnancy Free-running wheel	Male and female offspring; 18-wk-old; SCD; Sedentary	~ hepatic TG: offspring of exercised SCD-fed mothers ~ hepatic TG: offspring of exercised WD-fed mothers ~ hepatic CS activity ~ protein content of the OXPHOS subunits ↑ OXPHOS subunit CIII: male offspring of exercised SCD-fed mothers ↓ hepatic β-HAD activity: offspring of exercised WD mothers ~ hepatic mitochondrial biogenesis markers (~PPARγ, ↑TFAM, ~PGC-1α) ↑ hepatic markers of autophagy/mitophagy
Siti et al. 2019 [90]	Wistar rats; 12-wk-old	SCD	• PE 4 wks before and during pregnancy (until GD18) Treadmill: gradually increased to the speed of 25 m/min with a 10% slope, 1 hour/day	Male offspring; 3-wk-old	~ hepatic RCR (glutamate/malate) ↓ hepatic RCR (succinate; succinate + glutamate/malate) ~ hepatic OXPHOS subunit CI ↑ hepatic OXPHOS subunits CII, CIII, CIV ↓ hepatic mitochondrial H_2O_2 release (succinate or succinate + glutamate/malate) ~ hepatic mitochondrial H_2O_2 release (rotenone or antimycin A) ↑ hepatic cytochrome a+a3 ~ hepatic cytochrome b, c, c+c1 ↑ hepatic GPx activity ~ hepatic thiols ↑ hepatic % short-chain FA ↓ hepatic % long-chain FA ↓ hepatic unsaturated FA ↑ hepatic saturated FA ↑ hepatic (n-3) FA ↓ hepatic (n-6) FA ↑ hepatic (n-3)/(n-6) FA
Boonpattrawong et al. 2020 [83]	C57BL/6 mice;	SCD or WD	• PE 1 week before and during pregnancy and 3-wks after delivery Free-running wheel	Male offspring; 17-wk-old;	↑ hepatic Mat1a mRNA (one-carbon metabolism) ~ hepatic Mat1a mRNA (one-carbon metabolism) ~ hepatic Mthfr, Mtrr, an Cbs (one-carbon metabolism)

				SCD or WD; Sedentary	
Bae-Gartz et al. 2020 [82]	C57BL/6 mice; 3-wk old	SCD	• PE during pregnancy (until GD18) Free-running wheel	Male offspring; - 21-day-old; - 112-day-old. SCD or HFD (only after 70 days of age)	↓ steatosis score: 112-day-old HFD-fed offspring ↓ NAFLD activity score: 112-day-old HFD-fed offspring ↓ hepatic FAS: 21-day-old offspring ↑ hepatic PEPCK protein expression: 21-day-old offspring ↑ hepatic CYP7A1 and SREBP2 protein expression: 21-day-old offspring ↓ hepatic SREPB1 protein expression ↑ tendency for hepatic insulin signalling activation ↑ hepatic PPARα and PGC-1α signalling: 21-day-old offspring ~ hepatic TFAM mRNA levels: 21-day-old offspring ~ hepatic *PGC-1α* methylation: 21-day-old offspring ↑ hepatic β-oxidation: 21-day-old offspring
Son et al. 2020 [91]	C57BL/6J mice; 8-wk-old	SCD	• PE during pregnancy (until GD16.5 or GD20.5) Treadmill: 10-14 m/min, 1 hour/day, at 40% VO_2max (E1.5 to E7.5), then 65% VO_2max (E8.5 to E14.5), and 50% VO_2max (E15.5 to E20.5)	Male and female offspring; 3-mo-old; SCD or HFD; Sedentary	↑ glucose tolerance ↓ hepatic lipid accumulation
Stevanović-Silva et al. 2021 [53]	Sprague-Dawley rats; 7-wks-old;	SCD or HFHS	• PE during pregnancy Treadmill: gradually increased to the speed of 21 m/min, 1 hour/day, 6 days/wk Free-running wheel	Male offspring; 6-wks-old; SCD; Sedentary	~ glucose tolerance ↓ TG content ↓ NAFLD score ↓ long-chain acylcarnitines (C16, C18, C18:1) ↑ RCR (glutamate/malate) ~ RCR (succinate)

					↓ respiratory rate with oligomycin ~ ADP/O ratio ↑ OXPHOS subunits CI and CIV protein expression ↓ UCP2 protein expression

Abbreviations: AMPK: Adenosine monophosphate-activated protein kinase; ATG: Autophagy-related gene; CAT: Catalase; Cbs: Cystathionine-β-synthase; CS: Citrate synthase; CYP7A1: Cholesterol 7 alpha-hydroxylase; E: Embryonic day; eNOS; Endothelial nitric oxide synthases; FA: Fatty acids; FAS: Fatty acid synthase; GD: Gestational day; GPx: Glutathione peroxidase; GSK3: Glycogen synthase kinase 3; HAD: hydroxyacyl-CoA dehydrogenase; HFD: High-fat diet; HFHS: High-fat high-sucrose diet; HIF1A: Hypoxia inducible factor 1 subunit alpha; HK2: Hexokinase 2; iNOS: Inducible nitric oxide synthases; Mat1a: Methionine adenosyltransferase; Mthfr: Methylenetetrahydrofolate reductase; Mtrr: Methionine synthase reductase; NAFLD: Non-alcoholic fatty liver disease; OXPHOS: Oxidative phosphorylation; pAMPK: Phosphorylated adenosine monophosphate-activated protein kinase; PE: Physical exercise; PEPCK: Phosphoenolpyruvate carboxykinase; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; pGSK3: Phosphorylated glycogen synthase kinase 3; PKB: Protein kinase B; PND: Postnatal day; PPAR: Peroxisome proliferator-activated receptor; pPKB: Phosphorylated protein kinase B; RCR: Respiratory control ratio; SCD: Standard chow diet; SOD1: Superoxide dismutase 1; SOD2: Superoxide dismutase 2; SREBP1: Sterol regulatory element-binding protein 1; SREPB2: Sterol regulatory element-binding protein 1; TFAM: Mitochondrial transcription factor A; TG: Triglycerides; UCP2: Uncoupling protein 2; WD: Western diet.

Maternal PE can be beneficial against metabolic disturbances when offspring are challenged by HFD. Adult offspring of SCD-fed mothers that exercised before and during pregnancy, were protected from offspring HFD-induced obesity.[94] In a similar study, maternal PE during pregnancy prevented offspring HFD-induced hepatic steatosis, as observed through reduction of hepatic TG accumulation, and increased hepatic PGC-1α and TFAM expression. However, this effect was noticed in male but not in female offspring.[89] Actually, maternal prenatal PE seems to modulate offspring metabolism in a gender-specific way. Adult male but not female offspring from exercised mothers had lower fat mass percentage and higher lean mass percentage than offspring of sedentary mothers.[95] Some of these gender-specific effects may be explained by synergistic effect of maternal PE and HFD feeding. Apparently, prenatal PE showed greater effects in male than in female offspring of HFD-fed obese mothers. Male weaning offspring had lower metabolic risk and improved glucose metabolism. While PE performed during pregnancy by lean mothers improved PGC-1α expression in offspring of both genders, this effect of PE performed by HFD-fed obese mothers was only observed in male offspring.[96] Some of these *in utero* and gender-specific effects could be explained by epigenetic mechanisms; however, the data on offspring liver epigenetic alterations are still lacking.

Prenatal PE is important to improve offspring metabolism even in the presence of maternal HFD, which adverse effects are mitigated by maternal PE.[93,96,97] Prenatal PE can also counteract the effects of maternal HFD-feeding on adult female offspring hepatic glucose metabolism and reduce adiposity and hepatic triglyceride content.[93] Surprisingly, maternal prenatal PE can rescue IR in SM of female offspring that were actually prompted by paternal obesity.[98] Besides the positive modulation of maternal diet-induced insulin sensitivity in both mothers and male offspring, maternal PE prevented an excessive lipid accumulation and hypoxia in placenta.[99] This PE effect is very important considering the remarkable function of placenta and the fact that possible alterations in intrauterine environment can be detrimental to fetus development. A recent study of our group also reported that PE programs during pregnancy improved maternal diet-induced GDM and related liver mitochondrial dysfunction and remarkably reduced hepatic triglyceride accumulation in male offspring.[53]

5. Conclusions

Many studies suggest that maternal PE can enhance offspring insulin sensitivity and glucose metabolism, which can be very important in glucose and insulin impairments-related disorders. However, studies analysing the effects of maternal PE as a preventive tool against offspring NAFLD are still lacking. Special focus in future research should also be given to mitochondrial alterations as one of the first key events in NAFLD development. Mechanistic explanation of how PE improves offspring

metabolic health is out of reach so far. Gene-environment *in utero* interactions that lead to epigenetic alterations may be among the mechanisms lowering disease risk. Exercise-induced epigenetic modifications can provide further mechanistic insights into the preventive and therapeutic role of PE against metabolic disorders. In mothers with altered metabolic status (hyperglycaemia, hyperinsulinemia, dyslipidemia), PE can rescue these changes, providing a stabilised *in utero* metabolic *milieu* that will positively affect fetal developmental programming and eventually reduce the offspring susceptibility to later-life metabolic diseases.[100] Nevertheless, it is hypothesised that maternal PE can have a dose-dependent effect on fetal epigenome, with lower doses considered beneficial, but higher doses detrimental.[59] Therefore, exercise programs applied during pregnancy should be carefully designed, with particular emphasis on both mother's and fetal welfare, and following the concept of general moderation, as suggested by recent guidelines. Better understanding of *in utero* environment and the consequences of its alterations for the offspring, could reinforce exercise programs as interventions to improve health and reduce disease risk in offspring.

Contributions:

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