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Perspectives

PERSPECTIVES

POHaD: why we should study future fathers

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Abstract

The growing field of 'Developmental Origin of Health and Disease' (DOHaD) generally reflects environmental influences from mother to child. The importance of maternal lifestyle, diet and other environmental exposures before and during gestation period is well recognized. However, few epidemiological designs explore potential influences from the paternal environment on offspring health. This is surprising given that numerous animal models have provided evidence that the paternal environment plays a role in a non-genetic inheritance of pre-conceptional exposures through the male germ line. Recent findings in humans suggest that the epigenome of sperm cells can indeed be affected by paternal exposures. Defects in epigenetic sperm mechanisms may result in persistent modifications, affecting male fertility or offspring health status. We addressed this issue at the LATSIS Symposium 'Transgenerational Epigenetic Inheritance: Impact for Biology and Society', in Zürich, 28–30 August 2017, and here provide important arguments why environmental and lifestyle-related exposures in young men should be studied. The *Paternal Origins of Health and Disease* (POHaD) paradigm was introduced to stress the need for more research on the role of the father in the transmission of acquired environmental messages from his environment to his offspring. A better understanding of pre-conceptional origins of disease through the *paternal exposome* will be informative to the field of transgenerational epigenetics and will ultimately help instruct and guide public health policies in the future.

Key words: men; obesity; EDCs; sperm; DOHaD; epigenetic epidemiology

Introduction

Parents contribute in many ways to development and health of their children. A mother's lifestyle is well recognized to have a positive or negative impact on her offspring's health. However, the paternal contribution of early exposures is often overlooked in human studies. In recent years, a substantial number of animal studies have reported that fat-induced obesity, as well as several pre-conceptional environmental exposures (e.g. to certain food items, bisphenol A, heavy metals, stress, etc.), affect developing male germ cells. Acquired epimutations at this pre-conceptional stage of development may influence offspring's future health [1]. Although not completely understood, comparative epigenetic mechanisms have been suggested in humans; discussed by others and us [2–7]. However, to date, empirical evidence on paternally transmitted effects in humans is still limited [8].

We here discuss two lifestyle exposures with a major public health burden: obesity and exposures to environmental pollutants, such as endocrine disruptors (EDCs) and other toxins from lifestyle habits, including smoking, alcohol intake and indoor pollution. Although excessive body fat is primarily due to a

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combination of unhealthy dietary patterns and a sedentary lifestyle, exposure to a mix of as yet less well understood environmental factors, including endocrine-disrupting chemicals [9], may also affect metabolic homeostasis in the human body. These factors together contribute to what is currently termed the 'obesogenic' environment [10], which is omnipresent in a typical Western-lifestyle environment and part of the paternal exposome [8]. On the basis of animal data and a limited number of studies in humans, we recently introduced Paternal Origins of Health and Disease (or POHaD) as an extension of the DOHaD paradigm. We here explain why there is a need to study paternal influences on offspring and to inform young men who are planning to have a child. We provide a possible biological explanation and future research avenues. This will ultimately be beneficial to human health, especially in the context of the growing obesity burden and increasing environmental exposures to EDCs in the general population worldwide.

Why Focus on Obese Fathers-to-Be?

Obesity in Men

Adult overweight and obesity are major public health issues. Worldwide, reported prevalence has been increasing steadily in many populations [11] (http://www.who.int/mediacentre/factsheets/fs311/en/). While associations between obesity in women and offspring health are currently being extensively documented, paternal obesity is seldom included in epidemiological designs to investigate influences from parental lifestyles. Given the high frequency of obesity in adult men, this line of research should not be ignored. For instance, most recent national data of the US population show that overweight or obesity (BMI $\geq$ 25) is 10% higher in men than in women (72.1% in men *versus* 61.2% in women) [11]. It is known that high BMI in men has deleterious effects on fertility parameters [12–14]. However, long-term effects, such as in subsequent generations, are unclear. We earlier showed a link between paternal obesity and offspring DNA methylation differences in humans [15, 16]. Furthermore, DNA methylation alterations in sperm have been associated with male obesity [17]. Yet, most evidence for an epigenetic link in the transmission of environmental conditions through the paternal germ line originates from animal data.

Obesity and Nutrition in Animal Models

A large number of experiments in animals showed that epigenetic marks can persist in a next generation, pre-disposing offspring with a different epigenetic pattern and altered phenotype which may often persist for many generations. In 2010, Ng et al. reported that high-fat diet consumption in male rats programmed β-cell dysfunction in F1 female offspring; combined with an early onset of impaired insulin secretion and glucose tolerance worsening over time [18]. DNA methylation changes were measured at a key pancreatic islet gene, *Il13ra2*. Ng et al. added that the transcriptome of retroperitoneal white adipose tissue of offspring rat was also concomitantly affected [19]. However, no analyses were performed on sperm. Alhashem et al. demonstrated in a rat experiment that obesity, induced by a high-fat diet, has deleterious effects on semen. It diminishes total sperm count and the number of motile spermatozoa, while increasing morphological abnormalities. Parameters reflecting the oxidative status were also negatively impacted [20]. Other rodent studies showed that paternal obesity or malnutrition not only decreased sperm quality, it also affected early stages of development, with a decrease in embryo numbers and quality [21]. Aberrant offspring outcomes included an increased risk for impaired insulin sensitivity and adiposity [22]. Underlying molecular mechanisms in the male germ line are most likely the reason for these observations. Obesity or high-fat diet in rodents has been linked with changes in the sperm epigenome at multiple levels (microRNA and/or DNA methylation) [22–24]. De Castro Barbosa et al. showed that a diet-induced epigenetic response was paralleled by transgenerational inheritance of metabolic dysfunction throughout two generations [23]. Interestingly, also paternal vitamin levels may influence sperm and offspring health. If male rats were administered a folate deficient diet before mating, a decrease in global DNA methylation in the liver of the offspring was detected [25].

Physical Exercise Interventions in Animal Models

Further indications for the importance of a role of the sperm epigenome as an intermediate player between paternal health and offspring phenotypes originate from exercise intervention studies. McPherson et al. showed that an exercise-only intervention in obese male mice reduced the susceptibility to metabolic syndrome in offspring. The abundance of X-linked sperm microRNA was normalized after exercising [22]. Similarly, Palmer et al. showed that moderate exercising in obese mice reduced Reactive Oxygen Species (ROS) and DNA damage, and improved sperm motility and morphology [26]. Sperm quality and oxidative stress parameters were restored in rodents that were engaged in daily swimming, while simultaneously being fed a high-fat diet [20].

Some studies tried to quantify the effect of forced swimming on rodent sperm quality [27–29]. In brief, they found that forced exercising decreased normal morphology in spermatozoa [29], negatively affected spermatid production [27] and impaired fertilization capacity [28]. The forced exercising in these rodents is comparable to a high-intensity workout in humans.

Translating Animal Data on Physical Exercising to Humans

The animal models described above offer epidemiologists tools to help understand cellular processes in humans. At the same time, these animal data are useful to prevent that certain 'Western' lifestyle conditions—and resulting epigenetic marks in sperm—would be handed down to offspring. A remarkable observation from animal experiments is the improvement measured in sperm and offspring health after physical interventions. Furthermore, animal data show that extreme exercising or related stress prohibit the potential for amelioration. Hence, it is worth to explore if and how exercise intervention programs in humans influence sperm and offspring characteristics, including the epigenome.

In humans, along with dietary patterns, physical activity plays a major role in weight control [30]. Globally, 23% of adults are not active enough [11]. The Youth Fitness Survey as being part of NHANES in 2012 confirmed that obesity and physically inactivity are closely interconnected. Moreover, this relation already starts in adolescence. Only 29.5% of normal-weight and overweight boys (aged 12–15 years old) met the daily recommended activity guidelines, meaning a moderate-to-vigorous activity for at least 60 min per day. Only 18% of the obese boys met this criterium [31]. Besides the negative health effects of obesity or a sedentary lifestyle, it is also well known that physical activity has positive effects on sperm quality in young men. However, the human body—and microenvironment of semen—responds differentially according to the sport's intensity (moderate *versus*

high) [32], chronicity (continuous *versus* intermittent) [33], type of sport (e.g. running *versus* cycling) [34, 35] or oxygen supply [36–38] (e.g. mountaineering). Interestingly, the positive effects of exercise interventions are often more pronounced in obese men, compared with those in normal-weight men [39].

To the best of our knowledge, only one intervention study explored epigenetic effects of exercising in humans. Denham et al. reported epigenetic changes in human sperm cells after a 3-month period of a physical interventional programme [40]. They found that changes in DNA methylation at CpG sites in genes associated with a wide range of diseases, such as schizophrenia, Parkinson's disease, cervical cancer and leukaemia [40].

Translating Animal Data on Nutrition or Obesity to Humans

Unfortunately, while animal models show that diet and sperm quality are related, there is limited evidence in humans that paternal dietary factors influence male fertility or offspring health. Evidence in humans is even harder to find if searching for mechanistic processes, such as epimutations. Consumption of high-energy diets (HEDs) has been related to impaired sperm concentration, motility and morphology [41]. More specifically, a higher intake of saturated fat has been associated with lower sperm concentration and total sperm count [42], and a negative correlation was detected between dietary trans fatty acid intake and sperm concentration [43]. Rafiee et al. showed that vitamin C administration improves sperm concentration and motility [39]. A recent systematic review confirmed that nutritional supplements (including vitamin C, E, CoQ10 and alpha-tocopherol) can indeed improve semen parameters [44]. Restoration of cellular oxidative stress is accepted as the underlying biological mechanism (briefly explained below), but potential downstream epigenetic changes in the germ line that may be inherited to other generations have not been explored yet. To the best of our knowledge, one research group explored the epigenetic effects of a vitamin intervention in humans. Tunc et al. showed that if infertile men received a 3-month supplementation of folate and anti-oxidants sperm quality was improved and global sperm DNA methylation was increased [45]. Further research is needed to qualify and quantify the effect of obesity, nutrition or physical activity on sperm epigenetics and potential phenotypic effects in future offspring. If harmful consequences could be normalized through specific dietary supplements in young fathers-to-be, this would make research in this field even more important.

Although animal models may help to contribute unravelling the mechanisms of trans- or intergenerational epigenetic inheritance of lifestyle-related exposures, the multitude of exposures and the differences in genetic background—causing different responses to environmental traits—make it impossible to investigate these processes in a single animal model. Furthermore, extrapolation of results from an animal model to humans remains an artificial attempt to mimic the complex human responses and needs to be considered with caution [46]. Studying humans is challenging, because of ethical reasons, but there is the advantage that unexpected and new findings can be generated from observational studies. Of note, studies on domestic animals may also be beneficial in exploring epigenetic effects from 'their' (human-induced) obesogenic environment.

Oxidative Stress as a Mediator between Lifestyle and Epimutations in Sperm

One possible mechanistic view that could explain how obesity or related factors could influence the sperm epigenome involves unbalanced ROS. As mentioned above, the testicular microenvironment, such as the abundance of oxidative stress or ROS, can theoretically alter sperm characteristics. However, an intermediate role of the epigenome remains speculative within this dynamic or responsive molecular network. We earlier discussed the potential role of paternal diet and obesity in this process, and how in addition to DNA damage through direct attack of free radicals on DNA molecules, ROS signalling may trigger epigenetic responses [6]. In brief, obesity has been linked to ROS overproduction in the testes [47]. These toxic molecules cause direct damage to cell structures, including DNA. Low concentrations of ROS are a normal byproduct of cellular metabolism and are necessary in signal transduction and protection against pathogens [48]. ROS also play a role in sperm capacitation and acrosome reaction when spermatozoa and oocyts join [49, 50]. Production of ROS in sperm occurs mainly in mitochondria as a byproduct of the electron transfer chain. If unbalanced, excessive ROS can overrule its protective function and induce damage to mitochondrial DNA, which has an impact on energy balance of spermatozoa, sperm motility and capacity for fertilization [50]. Next to its damaging effects on DNA integrity, the spermatozoa membranes—rich in polyunsaturated fatty acids—are also potential targets [49]. Animal data showed that impaired spermatogenesis linked to a HED is attributed to unbalanced ROS generation [51]. Rodents fed with HED had lower expression of ROS-detoxifying enzymes. This contributed to a decrease in testicular ROS-defence and a decline in sperm quality [52]. Similarly in humans, an association was found between a high-fat paternal diet, ROS and sperm DNA damage [53, 54]. Oxidative stress or ROS overproduction has been shown to mediate epigenetic changes in several cell types [55]. Therefore, it is important to study both phenomena (epigenetic and oxidative stress/ROS status) in sperm of men in specific lifestyle conditions.

Why Study Exposure to Environmental Toxins in Future Fathers?

Humans are exposed to environmental chemicals on a daily basis, such as EDCs found in personal care products [56], plastics or food packaging [57] and the surrounding environment [58]. EDCs are known to disrupt the endocrine and metabolic homeostasis in the body, but other potential consequences include decreased reproductive function [57, 59, 60], neurodevelopmental delays in children [61] and increased risk of diabetes [62], or other chronic disorders via transgenerational inheritance of these exposures [63]. A worrisome observation is the fact that exposure to EDCs has increased >10-fold over the past ten years [64]. Taking into account findings from Skinner's research team, showing that environmental chemicals can have long-term transgenerational effects in animal models [65], the ubiquitous presence of chemicals may well tip the epigenetic balance and program an individual for developing a chronic condition later in life.

In order to understand how environmentally induced effects can last for several generations, research at the level of human germ cells is necessary. Few studies have been performed on the impact of these contaminants on the sperm epigenome in humans. In a cross-sectional study in healthy volunteers living in North Carolina, we showed that the sperm epigenome may be responsible for transmission of environmental chemicals/factors (organophosphates, from exposures to flame-retardants) from father to child [66]. DNA methylation at imprinted genes

that were not supposed to be paternally methylated were methylated if men were exposed to specific environmental chemicals. Interestingly, the more metabolites of these OP chemicals were measured in their urine, the higher the risk for producing a sperm sample that was hypermethylated at these imprinting regions. This suggests that a 'cocktail' of chemicals from the environment—which is realistic in our current daily environment—increases the risk of aberrant methylation in male germ cells. Interestingly, exposures to EDCs have also been shown to alter fatty acid composition and decrease anti-oxidant enzyme level in the testis [67], inducing oxidative stress [68] and ultimately affecting spermatogenesis [69]. Occupational exposures to bisphenol A was also shown to impact global sperm DNA methylation in humans [70].

Lifestyle habits such as smoking and alcohol intake can also be classified among environmental toxins. Chronic consumption of alcoholic beverages has been associated with sperm epigenetic aberrancies, such as DNA methylation at the *IGF2* and *H19* DMRs [71]. Smoking habits have been correlated with changes in miRNA expression in spermatozoa [72]. Unfortunately, sample sizes were small in these studies. Noteworthy, the 'Avon Longitudinal Study of Parents and Children' (ALSPAC) provided evidence that sons of fathers who started smoking before puberty are at high risk of becoming obese [73]. Taken into account potential effects in children from smoking fathers it is worthwhile to proceed this line of research.

Conclusions

The environment of the father before conception not only predisposes him to obesity and related chronic disorders, but it can also affect his sperm quality, epigenetic profiles in spermatozoa, and increase the risk that his children will be obese or develop other chronic diseases [2].

At the LATSIS symposium we presented a number of epidemiological studies showing altered DNA methylation profiles in offspring at several differentially imprinting regions in children born from obese fathers [15, 16]. Some of these imprinted genes were also differentially methylated in sperm DNA of obese men (prospective fathers) [17]. Additional reports in healthy volunteers showed epigenetic aberrancies in sperm by increasing exposure to processed food and environmental toxins [74]. Although these findings in humans should be replicated in other populations, several experiments in animal models convincingly demonstrate that the sperm epigenome functions as a vector to transfer pre-conceptional environment messages from paternal environment to offspring.

Obesity and exposures to EDCs are serious global health challenges. The increases in magnitude of obesity rates and exposures to environmental toxins have paralleled reports of rising frequencies of poor sperm quality, male infertility and several adverse health conditions. Next to these relatively immediate effects, the possibility that epigenetic marks can be transmitted to offspring opens a new field: that of POHaD [8]. An intermediate factor between obesity or related conditions and epigenetic signatures in sperm is oxidative stress. For instance, increased ROS can unbalance the epigenetic state. Hence, understanding interactions between the environment, ROS, and the epigenome in sperm is critical to acquire a comprehensive view on typical 'Western' diseases, today and in the future.

We believe that public health would doubly benefit from studies in men on protective effects from dietary supplementation and moderate exercising before conception. Besides advantages for the individual's general and reproductive health, health of future offspring may also gain from this research. Health promotion and disease prevention is more challenging than curing diseases. However, without research in humans, we will continue to try and translate results from animal models to human beings, which may at best lead to speculation on the molecular processes in the human body.

As discussed by Rothstein et al. [75], evaluating the implications of personal lifestyles on epigenetics and offspring health becomes a social and ethical issue, even more so than research on the genetic origin of diseases. For instance, an unhealthy lifestyle could be used as evidence of lack of personal responsibility. The new and growing field of transgenerational epigenetics will thus generate the need for explicit attention to concerns about environmental justice in environmental regulations and health policies [75]. We here agree with the opinion of Rothstein et al. that additional scientific research (in humans) is needed before protective environmental policies can be implemented. Nevertheless, while our knowledge regarding the etiology of 'transgenerational phenotypes or diseases' is growing, it is advisable to inform and protect our most vulnerable populations, such as men with occupational exposures to EDCs, men with an unhealthy lifestyle, or those who are suffering from the consequences of subfertility.

In conclusion, understanding the mechanisms of action of EDCs or obesity-related exposures through germ cells will offer a unique opportunity to modulate risk for diseases in offspring via targeted recommendations of food supplements and a better lifestyle, or by introducing health policy recommendations requesting the industry to reduce or replace the use of certain chemicals. The current DOHaD concepts are already being used to guide public health policies that support mothers and children's health. It has been well established that advising future mothers not to drink alcohol, not to smoke and to eat healthy, has important benefits for their offspring. Unfortunately, there is currently a striking lack of awareness in the general population regarding the father's contribution to offspring health. We foresee a change in the historical concept of the mothers' only contribution, to recognize and bolster the father's role in success of having healthy children.

Declaration of interest

The author declares to have no competing financial, professional or personal interests.

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