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Paternal dietary programming of metabolic health

Effects and mechanisms of paternal nutrition during preconception on the offspring's risk of developing metabolic syndrome

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Content

Abstract

Metabolic syndrome increases the risk of type 2 diabetes and cardiovascular diseases and is one of the greatest public health concerns. Nutrition of parents can have a large impact on offspring's development and its adult health. This concept is called 'programming'. Next to the well-studied maternal programming, influences of paternal nutrition on offspring's health is an upcoming research. This essay covers the question how paternal nutrition during preconception influences the offspring's risk of developing the metabolic syndrome.

Research in human mostly cover prospective cohort studies on obesity. The most common risk factor for developing obesity is having obese parents, independent of the offspring's own diet. Human and animal paternal overnutrition shows impaired health up to at least two generations, demonstrated by an increased risk of developing metabolic syndrome. Animal studies show that male rodents fed a high-fat diet are common to have adult offspring with an increased body weight and that paternal obesity increases the risk of developing metabolic syndrome in the offspring, even when the newborns were fed a standard diet. Undernutrition of the father often leads to reduced offspring weight in adulthood. In both over- and undernutrition the long term risk of developing metabolic syndrome is increased.

Paternal programming effects are transmitted by sperm. Oxidative DNA damage in sperm leads to an increased risk of sperm mutations. Besides, changes in sperm epigenetic marks play a crucial role, especially DNA methylation, RNA transcripts, and histone modification. These epigenetic marks are often removed during embryonic and fetal development, but sometimes histones retain at promoters which are important for embryogenesis. This retention as well as histone modifications appear to be at specific locations, programming the next generation, and may be the consequence of environmental influences, like diet. Besides, it is shown that paternally-derived RNA transcripts are involved in the development of obesity and metabolic disorders in the offspring. Moreover, DNA methylation is an important epigenetic mark involved in paternal programming of the next generation. The altered DNA methylation in offspring is found at sites that are related to the metabolic syndrome and are assumed to have escaped the removal of DNA methylation in the zygote or during embryogenesis.

Thus, many studies have shown the impact of paternal nutrition on embryonic and fetal development, as well as on adult metabolic health in the offspring. The epigenetic status of father's sperm plays an important role. Diet interventions in humans and rodents have shown to improve sperm function and resulted in restorations of embryonic and fetal health. Still, many more prospective cohort studies and intervention studies are needed to investigate the possible dietary interventions to reverse or prevent adverse effects on the offspring. In this way, fathers planning to conceive can be advised properly.

Introduction

Metabolic syndrome

Metabolic syndrome is one of the greatest public health concerns and its prevalence is growing worldwide (Smith & Ryckman, 2015). The main characteristics are raised triglycerides, blood pressure and fasting plasma glucose, all of which are related to weight gain and increase the risk of type 2 diabetes and cardiovascular diseases (Han & Lean, 2016). Patients with the metabolic syndrome often display a pro-inflammatory state, which is thought to be related to obesity. Reaven (1997) was the first to describe this clustering of metabolic abnormalities, particularly in overweight individuals. Metabolic syndrome is elicited by weight gain, especially by an increase in abdominal fat which can be observed as a large waist circumference (Han & Lean, 2016). Only limited evidence for a common genetic background of metabolic syndrome can be found, resulting in focus on the genetic background of obesity (Stancáková & Laakso, 2014). Obesity has a sustainable heritability of 40-70%, including more than 20 obesity susceptibility loci of which FTO (fat mass and obesity associated) is shown the most (Herrera & Lindgen, 2010). Simultaneous to a worldwide increase in obesity, the prevalence of metabolic syndrome is increasing. This increase can be partially explained by a population-wide decrease in daily exercise and an increase in the availability of high-fat and high-sugar nutrition nowadays.

Nutrition is an important factor that influences genetic and epigenetic processes during human development, making specific (amounts of) nutrients to have a large effect on key fetal and infant developmental processes (Langley-Evans, 2015). This concept is called 'programming' (Dunford & Sangster, 2017), and also known as the 'fetal origins of adult disease hypothesis' or 'Barker hypothesis'. Barker was the first one to popularize the idea that events during early development have a profound impact on the risk for development of future adult disease (Barker, 1998; Calkins & Devaskar, 2011). Later on, the research group of Roseboom, including Barker, found that people who were small at birth, due to mother's food deprivation during the Dutch hunger winter, had an increased risk of coronary heart disease and chronic bronchitis in later life (Roseboom, Van Der Meulen, Ravelli et al., 2001). On the other hand, children born to an obese parent have an increased risk of developing obesity, even when eating a healthy diet themselves (Whitaker, Wright, Pepe, Seidel, & Dietz, 1997). These two examples indicate the variety of influences of health in embryonic or fetal life on health in later life. Nowadays, it is accepted that maternal prenatal nutrition has a profound impact on the health of the offspring during fetal development (Wu, Bazer, Cudd, Meininger, & Spencer, 2004).

Paternal programming

Next to the well-studied influences of maternal nutrition on offspring's health, paternal programming is an upcoming research topic. Male-mediated developmental toxicity was first described over two decades ago by Olshan and Faustman (1993). The programming effects of father's diet remained underestimated for a long time. It was thought that only exposures in the uterus and during early life could influence its adult health, and that paternal factors can only transmit via Mendelian inheritance (Braun, Messerlian, & Hauser, 2017). Olshan and Faustman reviewed this topic and found accumulating evidence that several chemicals can induce mutations in sperm, resulting in pregnancy loss, growth defects, and neurobehavioral effects. Nowadays it is evident for example that paternal obesity genes are linked to obesity in the offspring and that as early as during puberty paternal nutrition has an influence on the offspring (Li, Tsuprykov, Yang, & Hocher, 2016). Differences between maternal and paternal effects are described. For example, the offspring birth weight is positively correlated with maternal type 2 diabetes and negatively with paternal type 2 diabetes (Li et al., 2016). No consensus is made yet about the proportion of influences of fathers and mothers on their children's health, but it is clear that father's nutrition plays a role as well.

Paternal programming can appear at different periods of father's exposure: during the male's embryonic development, during prepuberty and spermatogenesis, and during the preconception period (Li et al., 2016). The first period comprises actually the effects of a paternal grandmother or grandfather. In the preconception period sperm cell maturation plays a key role. This period of around three months before the time of conception is most noticed and researched in the context of paternal programming (Braun et al., 2017).

Research aim

While mothers receive a list of nutritional advices before and during their pregnancy, fathers are almost excluded from these advices or receive incorrect information (Ritchie, Oakes, Hegedus, et al., 2017). It is becoming clear that fathers have a major influence on their offspring's health as well, but the exact influences and consequences of paternal nutrition are not well understood, as well as the mechanisms behind this concept. So, it is very useful to clarify the nutritional effects of paternal programming on the metabolic health of children. Therefore, this essay covers the following research question: **How does paternal nutrition during preconception influences the offspring's risk of developing metabolic syndrome?**

To answer this question, first human and animal studies covering paternal programming are summarized to state the consequences of specific nutrition on offspring's metabolic health in later life. Subsequently, the (epigenetic) mechanisms behind paternal programming are explained. At the end, the knowledge about nutritional consequences and underlying mechanisms are integrated to discuss interventions and future perspectives.

Nutritional influences

Most people just eat what they are used to eat and what they like. However, our nutrient intake influences our life more than we sometimes may think. Moreover, our diet can influence the health of our children via the concept of programming. Dietary fat is known to be energy-dense and can easily be overconsumed because it causes less satiety than proteins and carbohydrates (Rolls & Hammer, 1995). High-fat diets are known to increase the average body weight and amount of fat stored in the body, and subsequently lead to obesity (Dreon et al., 1988; Flatt, 1995). Furthermore, high-fat diets may lead to several alterations in metabolism such as impairment of mitochondrial metabolism, increased insulin resistance and reduced lipolytic activity in adipose tissue (Coelho et al., 2011). Therefore, in this section the effects of nutrition as well as the effects of the consequences of an unhealthy diet, like obesity, are exhibited. In human, most research is performed on the effects of a specific paternal phenotype on offspring's health or the cause of an offspring's phenotype. In animal research, the effects of a specific diet, like a high-fat or low protein diet, are most often researched. Many epidemiological cohort studies are performed in human, but animal studies are performed even more often since they can be properly controlled (McPherson, Fullston, Aitken, & Lane, 2014). When not mentioned differently, human studies include diet measurements of around 3 months before conception and animal studies include a period of changed diet of around 7-10 weeks before mating.

Human studies

The first and most common risk factor for developing obesity is having obese parents (Whitaker et al., 1997; Danielzik, Langnäse, Mast, Spethmann, & Müller, 2002). This risk factor applies independently for either an obese father or an obese mother (Whitaker et al., 1997). Herewith it is important to bear in mind that parents and children often share the same environment, including dietary habits (Danielzik et al., 2002). However, also when the offspring's diet is healthy, children of obese parents are more prone to develop obesity. vntr Some obesity alleles that regulate the *in utero* expression of the insulin gene and the insulin-growth factor 2 (IGF-2) are only transmitted through the father (Le Stunff, Fallin, & Bougneres, 2001). Paternal BMI is associated to children's BMI as well (Danielzik et al., 2002; Li, Law, Lo Conte, & Power, 2009; Cooper, Hypponen, Berry, Power, 2010). This is independent but additional to the effects of maternal BMI (Cooper et al., 2010). An offspring's increased BMI due to an increased BMI of the father can be attributed to a so-called 'obesogenic' environment and/or genetic predisposition that is shared by father and child(ren) (Wahlqvist, Huang, Lee, et al., 2014).

Strikingly, paternal obesity is also associated with a low birth weight in the offspring (Power, Li, Manor, & Smith, 2003). This phenomenon is contradictory at first sight but consistent with the concept that early postnatal growth restriction is associated with increased incidence of metabolic health problems later in life (Dulloo, Jacquet, Seydoux, & Montani, 2006). Indeed, paternal overnutrition shows impaired health up to at least two generations, demonstrated by an increased risk of developing metabolic syndrome (Danielzik et al., 2002; Ng et al., 2010; Watkins & Sinclair, 2014). Besides, it is seen that paternal obesity increases the offspring's risk of chronic diseases (McPherson et al., 2014) and metabolic pathologies (Fullston et al., 2015) in general. An increased risk of developing type 2 diabetes when born to an obese father is often shown (Abbasi et al., 2011; Fullston et al., 2015).

Not only the effects of a consequence of an unhealthy diet, obesity, but also the effects of such unhealthy diet itself are investigated. A high-fat diet seems to have an effect on offspring's metabolic health, independent of the glucose homeostasis (Fullston et al., 2013; Ng et al., 2010). The effects of paternal exposure to a high-fat diet are weaker than those of in utero (maternal) exposure, but it is additional to the effects from mothers (Masuyama, Mitsui, Eguchi, Tamada, & Hiramatsu, 2016).

On the other hand, the effects of paternal undernutrition are barely explored. Data from cohorts born in 1890, 1905 and 1920 in Överkalix in Sweden include clear historical records about food abundancy and famines. In 2001 it was published that children from paternal grandfathers that had abundant food during their puberty had a lower survival rate (Kaati, Bygren, & Edvinsson, 2001). Strikingly, paternal undernutrition due to a famine season during their puberty resulted in a low cardiovascular mortality in the offspring (Kaati, Bygren, & Edvinsson, 2002). Thus, no substantiated adverse effects on offspring's adult metabolic health in humans are reported regarding paternal undernutrition.

Animal studies

In animals a high-fat diet is often used to induce obesity-like phenotypes. Male rodents fed a high-fat diet are common to have adult offspring with an increased body weight (Fullston et al., 2013; Ng et al., 2010; Zhang, Li, Fu, & Di, 2017). On the other hand, rats' weight at prenatal day one tended to be reduced (Ng et al., 2010), while other rats' weight at 27 weeks of age was also less (Chowdhury, Lecomte, Erlich, et al., 2016) when from obese fathers compared to normal fathers. The latter result came from a study that shows an increase in renal injury in the offspring, but was unable to explain the absent of lipid accumulation.

Many animal studies show that paternal obesity increases the risk of non-communicable diseases in offspring (McPherson et al., 2014). An increased risk of metabolic syndrome and impairment of the reproductive health is found through at least two generations (Fullston et al., 2013; McPherson et al., 2014; Ng et al., 2010). These effects are seen while the newborns were fed a standard diet (Fullston et al., 2013). This influence can be explained by altered insulin and glucose metabolism. High-fat induced obesity in fathers leads to impaired glucose tolerance and insulin secretion in the next generation (Fullston et al., 2013; Lucas & Watkins, 2017; Ng et al., 2010; Pentinat, Ramon-Krauel, Cebria, et al., 2010). Beta-cell islets in the pancreas are reduced and expression of genes involved in regulatory pathways associated with insulin and glucose metabolism are altered, resulting in an overall impairment of the pancreatic function (Ng et al., 2010; Sharma, Conine, Shea, et al., 2015). Some researchers only found altered insulin and glucose metabolism in either male offspring (Pentinat et al., 2010) or female offspring (Lucas & Watkins, 2017; Ng et al., 2010). This may indicate sex-specificity, but both sexes are mentioned alternately. So, probably an increased risk of alterations exist which do not happen always. Ng et al. (2010) also found that glucose intolerance worsened with increasing age of the offspring.

Moreover, adiposity is often mentioned as a risk factor when born at a (high-fat diet induced) obese father (Figueroa-Colon, Arani, Goran, et al., 2000; Lucas & Watkins, 2017; Ng et al., 2010), even with resembling adiposity levels between father and offspring (Figueroa-Colon et al., 2000). However, Ng et al. (2010) found that a paternal high-fat diet did not alter adiposity in female offspring.

Some other specific effects of high-fat diet induced obesity in fathers on their progeny include increased liver fat deposition, increased expression of sterol regulatory element-binding protein-1 (Srebp1) and fatty acid synthase, and reduced expression of peroxisome proliferator-activated receptor-γ coactivator-1α (Pgc1a), which may be related to increased de novo synthesis of fatty acid and reduced liver function (Zhang et al., 2017). In the kidney, triglyceride content was significantly increased and the protein Acat1, involved in entry of fatty acid for beta-oxidation, was significantly upregulated, possibly to counteract increased triglyceride storage (Chowdhury et al., 2016). Besides, paternal obesity was associated with renal triglyceride accumulation, suggesting a mild renal insult in offspring, who may be at risk of developing chronic kidney disease (Chowdhury et al., 2016). At last, increased levels of leptin were found in the bloodstream of male offspring from obese fathers (Lucas & Watkins, 2017). All these effects from paternal overnutrition are related to characteristics of metabolic syndrome. Moreover, metabolic and fertility disturbances in offspring from high-fat diet fathers are exacerbated by a similar obesogenic environment postnatally (Fullston et al., 2015). Offspring fed a same high-fat diet as their obese fathers gained even more weight and had significantly more adiposity than offspring fed a

standard diet, showing an additional effect. Perhaps this shows a predictive adaptation response of children from obese fathers.

Also undernutrition in the form of food deprivation or low amounts of a specific nutrient like protein or folate, can have influences on children's health. Father's undernutrition often leads to reduced adult weight in the offspring (McPherson et al., 2016) and an increased pre- and postnatal weight (Watkins & Sinclair, 2014; Watkins, Sirovica, Stokes, et al., 2017). A low protein diet of the father increases the risk of developing cardiovascular and metabolic diseases in later life (Lambrot, Xu, Saint-Phar, et al., 2013; Radford, et al., 2014; Watkins & Sinclair, 2014).

It is seen that these next generations from undernourished fathers show increased adiposity, dyslipidemia, hypotension and heart rate in their adulthood (Lucas & Watkins, 2017; McPherson et al., 2016; Watkins & Sinclair, 2014). Similar to overnutrition, paternal undernutrition increases the glucose intolerance of female offspring, but it lowers plasma glucose concentrations of male offspring (Anderson et al., 2006; Ng et al., 2010; Watkins & Sinclair, 2014). These effects may occur from altered pancreas gene expression (McPherson et al., 2016). Besides, insulin-growth factor 1 and corticosterone levels are lowered in offspring from undernourished fathers (Anderson et al., 2006; Carone et al., 2010). Moreover, plasma lipid concentrations and hepatic cholesterol content are increased, and cholesterol esters are reduced (Carone et al., 2010). This may in part be due to altered expression of hepatic lipid and cholesterol biosynthesis genes, like Srebp and Ppara, a putative enhancer for a major lipid regulator. In male offspring, adiponectin levels are lowered while levels of the pro-inflammatory cytokine TNF-alpha are elevated, which result in impaired glucose homeostasis as seen in offspring from low protein fathers (Watkins & Sinclair, 2014). Low levels of adiponectin are associated with cardiovascular and metabolic disorders like diabetes and high blood pressure (Gu & Xu, 2013). On the other hand, increased levels of TNF-alpha are associated with insulin resistance (Aroor, McKarns, Demarco, et al., 2013). Clear correlations are found between postnatal body weight, adult body weight, and adiponectin and TNF-alpha levels in offspring from fathers fed a low protein diet (Watkins & Sinclair, 2014). Strikingly, in female offspring these levels of adiponectin and TNF-alpha seems to be opposite and may protect them from pathologies.

Furthermore, low protein results in decreased offspring expression of genes involved in calcium signaling and metabolism (Watkins & Sinclair, 2014). One of the reduced metabolism genes is Fto, the fat mass and obesity associated gene, a mRNA demethylase. Some Fto gene variants are correlated with obesity in humans (Loos & Yeo, 2014).

Thus, many human and animal studies have shown the impact of paternal nutrition on embryonic metabolism and fetal development, as well as on adult cardiovascular and metabolic health (Binder, Mitchell, & Gardner, 2012; Carone, Fauquier, Habib, et al., 2010; Lambrot et al., 2013; Ng et al., 2010; Watkins & Sinclair, 2014). Although around birth the effects of paternal undernutrition are mostly opposite to the effects of paternal overnutrition, the long-term risk of developing metabolic syndrome is increased in both malnutrition situations. Often the pancreas and liver seem to be involved. But how can paternal nutrition have all these effects on offspring's development and thus on later life health? The mechanisms known this far, are discussed in the next section.

Mechanisms

The fact that the nutritional status of fathers have an effect on the health of the offspring, especially the sons, indicates important intergenerational and transgenerational paternal programming (Braun & Champagne, 2014; Kaati et al. 2002; Pembrey et al. 2006). Transgenerational programming includes effects through at least two generations, and effects on multiple generations are indeed often observed (Braun & Champagne, 2014; Siklenka et al., 2015). Since most effects are seen in sons and are also seen in the postnatal absence of the father, the programming effects are suggested to be transmitted through the paternal line (Dunn & Bale, 2011). Father's environmentally-induced information must be transmitted at the time of conception and stably incorporated into the offspring's genome (Lucas & Watkins, 2017).

Sperm alterations

Paternal transmission during conception makes it therefore reasonable to think about sperm as the transmission vector. Programming namely occurs during natural conception as well as during assisted reproductive technologies like in vitro fertilization where interactions with the female tract and seminal fluid are avoided (McPherson et al., 2014). The testis of a man contains a pool of spermatogonial stem cells, which can undergo self-renewal to maintain the undifferentiated stem cell pool or can differentiate into spermatocytes, spermatids and finally spermatozoa. During fetal development the first progenitor sperm cells, called primordial germ cells, are developed into spermatogonial stem cells. The maturation from these stem cells to spermatozoa, called spermatogenesis, lasts from puberty until death. Mature sperm cells need high motility and a highly condensed head to be transported through the male and female tract (Jenkinks & Carrell, 2012). The process of spermatogenesis is probably more sensitive to paternal programming than the spermatogonial stem cell, because of the absence of many permanent programmed spermatogonial stem cells and the presence of specific programmed spermatozoa (Lucas & Watkins, 2017).

It is observed that obese human show impaired sex hormones, sperm function and molecular composition. Testosterone levels are reduced while estrogen levels are increased, which directly impairs spermatogenesis (Sermondade, Faure, Fezeu, et al., 2013). The sperm function is impaired by affected sperm concentration, motility and morphology, and increased sperm DNA damage (Kasturi, Tannir, & Brannigan, 2008). Also in animals, a high-fat diet is associated with reduced sperm motility, increased sperm DNA damage and sperm binding (Bakos, Mitchell, Setchell, & Lane, 2011; Fullstone et al., 2015; Hammoud, Gibson, Stanford, et al., 2009; Kort, Massey, Elsner, et al., 2006; Mitchell, Bakos, & Lane, 2011; Zhao, Zhai, Liu, et al., 2014).

Mutations by ROS

Comparable to overnutrition, undernutrition also shows increased sperm DNA damage (McPherson et al., 2016). Normally, DNA damage can undergo DNA repair. However, when cells are replicating this DNA damage can result in mutations that can be transmitted to the next generation. This DNA damage is often caused by an increased release of reactive oxygen species (ROS), which also influence plasma membrane integrity in sperm (Agarwal, Nandipati, Sharma, et al., 2006; Fullston et al., 2015). Sperm is known to be highly susceptible to ROS since they have lost scavenging enzymes during spermatogenesis and have lost the protection of the blood-testes barrier when released into the epididymis (McPherson et al., 2016). Oxidative DNA damage in sperm may be more prone to mismatch repair and increase the mutation load (Fullston et al., 2015), changing the gene function or regulation of gene expression. Testicular (Ghanayem, Bai, Kissling, et al., 2009) as well as hepatic (Carone et al., 2010) gene expression is altered in offspring from obese high-fat diet induced obese fathers. The oxidative stress in the sperm ultimately results in adverse embryo and fetal growth (Bakos et al., 2011; Binder et al., 2012).

Epigenetics

However, the average baseline mutational rate frequency appears to be too low to account for all transgenerational phenotypic inheritance (Curley, Mashoodh, & Champagne, 2011; Soubry, Hoyo, Jirtle, & Murphy, 2014). Therefore, next to the hypothesis of the origin of paternal programming by increased sperm DNA damage resulting in de novo mutations, changes in sperm epigenetic marks play a crucial role (McPherson et al., 2014). These marks alter the access, transcription, and translation of paternally derived genes during early embryogenesis. The role of epigenetics is also established by the fact that paternal programming occurs in isogenic species and thus is unlikely to be attributed to inherited genetic variation only (Braun & Champagne, 2014). In addition, it is seen that although the gene is not transmitted, genes can have an influence on the offspring's phenotype, emphasizing the role of epigenetics (Li et al., 2016). Thus, a major role is suggested for epigenetic mechanisms of gene expression regulation rather than stable, heritable modifications to DNA sequences (Gallou-Kabani & Junien, 2005). Epigenetic modifications encoded in sperm are heritable and therefore influence offspring phenotypes (Braun et al., 2017). These heritable epigenetic marks can be induced by mutations and DNA damage (Curley et al., 2011) and can on their turn induce DNA damage (Braun et al., 2017). DNA methylation, RNA transcripts, and histone modification, associated with chromatin protamination, appear to be important in the epigenetic state of sperm (Bohacek & Mansuy, 2015; Fullston et al., 2015; Fullston et al., 2015; Jenkins & Carrell, 2012; Siklenka et al., 2015), and will be explained in the next sections.

Histones

Normally, a high degree of sperm compaction is assumed to be achieved by histones, which wrap DNA to form chromatin (Ooi & Henikoff, 2007). During spermatogenesis, histones need to be replaced by protamine proteins, resulting in a ten times higher compaction of the spermatozoan DNA (Jenkins & Carrell, 2012; Terashima et al., 2015). This compact sperm DNA is necessary for sperm motility enabling the sperm to transport through the male and female tracts. Chromatin changes in sperm contribute to many functions of sperm cells during spermatogenesis and as mature spermatozoa (Jenkins & Carrell, 2012). However, some histones are not removed and remain at the promoters important for embryogenesis, like developmental gene promoters, microRNAs (discussed later) and imprinted genes (also discussed later) (Hammoud et al., 2009; Jenkins & Carrell, 2012). Retained histones appear not to be random but specific, programming the next generation. The way chromatin packs sperm DNA is influenced by the environment (Carone et al., 2010). For example, sperm with low protein levels tended to lack genes encoded for chromatin regulators.

Histone tails can be modified by epigenetic marks, like acetyl- and methylgroups, resulting in acetylated or methylated histones. Specific histone modifications are found at retained histones (Bale, 2014; Brykczynska, Hisano, Erkek, et al., 2010; Hammoud et al., 2009; Terashima et al., 2015). In sperm from fathers fed a high-fat diet, Terashima et al. (2015) found altered histone variant 3 at genes involved in the regulation of embryogenesis. Moreover, histone variant 3 mono-methylation at the lysine residue number 4, named H3K4me1, was enriched at promoters and enhancers of genes involved in the regulation of the testis and liver during embryogenesis. The histone modification H3K27me3 may contribute to gene suppression in the early embryo (Erkek, Hisano, Liang, et al., 2013; Jenkinks & Carrell, 2012) and persists in the embryo with the lack of his demethylase (Puschendorf, Terranova, Boutsma, et al., 2008), making it possible to transmit to the offspring. H3K9 methylation promotes adipogenesis through the peroxisome proliferator-activated receptor mediated pathway (Wakabayashi, Okamura, Tsutsumi, et al., 2009). Besides, H3K4me2 changes alter genes in spermatogenesis and are correlated with H3K4me3 changes (Jenkins & Carrell, 2012; Siklenka et al., 2015), which are known to control embryogenesis (Erkek et al., 2013). Thus, father's diet can modulate the sperm histone composition at regulatory genes which are important in embryo development (Siklenka et al., 2015; Terashima et al., 2015). Other histone tail modifications beside methylation, are acetylation, ubiquitination and phosphorylation (Jenkins & Carrell, 2012). Palmer et al., (2011), for example, found increased histone acetylation in mature sperm cells upon paternal high-fat diet. The replacement of histones by protamines is dependent of histone

acetylation regulated by histone deacetylases and acetylases (McPherson et al., 2014), and the obese mice from the experiments of Palmer et al. (2011) indeed had decreased levels of histone deacetylase. This could perturb the retention of histones and is associated with an increase in sperm DNA damage. In general, histone H2B ubiquitination, H3 acetylation, H3K4 methylation and H4 acetylation promote gene transcription and H2A ubiquitination, H3K9 methylation and H3K27 methylation tend to inhibit transcription (Jenkins & Carrell, 2012).

RNA transcripts

Since RNA transcripts co-localize with regions where histones are retained, probably histone retention is regulated by these RNA transcripts, possibly inhibiting protamination and maintaining the histones (Jenkins & Carrell, 2012). This link between histone retention and RNAs is debated, but many researchers agree on the fact that RNA transcripts play a role in paternal transmission (Braun et al., 2017; Curley et al., 2011; Fullston et al., 2015; Li et al., 2016); Rassoulzadegan, Grandjean, Gounon, et al., 2006). It is as well shown that paternally-derived RNAs are involved in the development of obesity and metabolic disorders (Braun et al., 2017; Grandjean et al., 2015).

Various cytoplasmic RNAs including messenger RNAs, short interfering RNAs, microRNAs and long non-coding RNAs are shown to be transmitted by germ cells (Curley et al., 2011). They carry epigenetic information which is essential for embryonic development. Long non-coding RNAs (lncRNAs) are found to play a role in mature sperm in adipogenesis and metabolism (Chen, Cui, Shi, et al., 2015), adipocyte differentiation and development (Chen, Liu, Lu, et al., 2016). It seems that lncRNAs express differentially between sperm from healthy and diabetic individuals (Jiang, Teng, Tian, et al., 2016). An et al. (2017) found the largest difference in lncRNA expression between fathers fed a high-fat diet and fathers fed a normal diet. The targets of these differentially expressed lncRNAs are mainly important in metabolic processes and associated with obesity-related pathogenesis. Therefore, An et al. suggest lncRNAs as hereditary vectors that induce paternal transmission of obesity. However, Siklenka et al. (2015) doubted whether such random RNA could induce the observed paternal transmission. They argue that the fate of a germ cell is specified many cell divisions after fertilization, making it more plausible to loose these RNAs when they seems to be meaningless to amplify. Next to lncRNAs, microRNAs are associated with paternal programming. MicroRNAs are small non-coding RNAs that degrade gene transcripts and suppress protein translation by specifically binding to target mRNAs. Upon injection of sperm RNA from fathers fed a Western-like diet into one-cell embryos, microRNAs showed increased expression (Grandjean et al., 2015). The injection also led to the Western-like diet-induced phenotype in the offspring. This effect was not seen upon injection of RNAs from healthy controls, making it reasonable that the health effects are induced by sperm RNA transfer during fertilization.

DNA methylation

Next to histones and RNA transcripts, DNA methylation is an important epigenetic mark involved in paternal programming. In humans, normally around 96% of CpG sites are methylated (Jenkins & Carrell, 2012). The regulation of DNA methylation is essential to normal cell function in somatic cells, gametes, and the embryo (Jenkins & Carrell, 2012), especially to spermatogenesis (Fullston et al., 2013). DNA hypomethylation promotes gene transcription and DNA hypermethylation tends to inhibit transcription (Jenkins & Carrell, 2012). Only few changes in the methylation pattern are necessary to have profound effects on the development of offspring (Carone et al., 2010). Diet-induced paternal obesity affects sperm methylation and causes metabolic disorders in two generations of mice (De Carso Barbosa, Ingerslev, Alm et al., 2016).

Offspring of obese fathers show altered DNA methylation (Bohacek & Munsey, 2015; Dunford & Sangster, 2017; Fullston et al., 2015), mostly detected as hypomethylation (Fullston et al., 2013; Tunc & Tremellen, 2009), especially at IGF2 (Dunford & Sangster, 2017) and at the interleukin-13 receptor α2 (Il13ra2) (Ng et al., 2010). Hypomethylated IGF2 leads to increased insulin secretion and subsequent obesity (Dunford & Sangster, 2017). Il13ra2 is shown to be upregulated in various tumors (Park et al., 2016), the effects of hypomethylation are yet unknown. Another lipid-related

gene that is often found to be differently methylated upon paternal diet is PPARa (Carone et al., 2010). This putative enhancer for a major lipid regulator showed increased methylation in offspring from fathers on a low protein diet. In general, undernutrition also shows altered DNA methylation (Lambrot et al., 2013). Paternal intake of the methyl-group donor betaine is positively associated with paternal DNA hydroxymethylation, where a hydroxymethyl group is added to a CpG site, and cord blood DNA methylation (Pauwels et al., 2016). The intake of another methyl-group donor, methionine, is positively associated with methylation of the IGF2 in cord blood. Overall, the altered DNA methylation in offspring is found at sites that are related to the metabolic syndrome, obesity, hypertension, lipid regulation and fat deposition (Carone et al., 2010; Dunford & Sangster, 2017; Radford et al., 2014) or, in general, related to developmental processes (Jenkins & Carrell, 2012). This can be confirmed by the fact that DNA methylation negatively correlates with adiposity (Morgan et al., 1999; Lucas & Watkins, 2017). Also oxidative stress, caused by obesity, is associated with sperm DNA hypomethylation (McPherson et al., 2014). Knockouts and/or mutations of some specific DNA methyltransferases in mice resulted in global hypomethylation (Jenkins & Carrell, 2012). This hypomethylation caused a decrease in spermatogenesis and increased the risk of embryo lethality. Mice treated with the methylation inhibitor 5-azacytidine showed decreased fertility and increased risk of embryo lethality as well. It should be noted that some experiments did show no changes in sperm DNA methylation upon certain paternal diet (Siklenka et al., 2015; Terashima et al., 2015).

Normally, DNA methylation is erased at two major time points, this process is often called reprogramming (Braun et al., 2017; Curley et al., 2011; Li et al., 2016; Lucas & Watkins, 2017; Pauwels et al., 2016). The first moment is in the zygote shortly after fertilization, and the second moment is in the primordial germ cell during embryogenesis. However, sometimes epigenetic marks escape this removal resulting in maintained information to transmit to the next generation (Curley et al., 2011; Li et al., 2016; Lucas & Watkins, 2017). Most retention is seen at imprinted genes and retrotransposable elements (Braun et al., 2017; Curley et al., 2011; Li et al., 2016; Lucas & Watkins, 2017; Pauwels et al., 2016). Imprinted genes are genes where only the paternal or maternal allele is transcribed through epigenetic silencing of the other allele, often by DNA methylation (Curley et al., 2011; Lucas & Watkins, 2017). In sperm from mice that were fertilized by artificial reproductive technologies altered methylation patterns of specific imprinted genes (*Snrpn* and *H19*) were observed up to the third generation, demonstrating the escaped reprogramming and transgenerational inheritance (Stouder, Deutsch, & Paoloni-Giacobino, 2009). Retrotransposable elements are DNA sequences that can amplify themselves in the genome and mostly origin from remnants of ancestral infections (Curley et al., 2011). Most of these elements are silenced by DNA methylation over time. For example, Intracisteral-A particle elements, long terminal retrotranspons, have the capacity to escape demethylation and regulate the transcription of adjacent genes (Curley et al., 2011).

Finally, additional or novel epigenetic regulators such as prions are possibly also involved in paternal programming of metabolic health (Carone et al., 2010). Besides, we should bear in mind that paternal transmission can also be influenced or executed by the mother. Factors within the oocyte are capable of removing or restoring epigenetic marks (Curley et al., 2011). And the male phenotype can have direct influences on, for example, the hormone levels of the female which can have profound effects on offspring's development. Also compensatory processes of the mother can alter paternal transmission. However, it is clear that the epigenetic status of father's sperm is of major influence on the regulation of gene transcription and therefore the protein expression during embryogenesis, fetal growth, childhood, adolescence and eventually adulthood, creating a large impact on the risk of developing metabolic disorder in later life (Lucas & Watkins, 2017). Despite this major role of epigenetics, the role of genetics should not be forgotten, and even more the interaction between genetics and epigenetics (Curley et al., 2011; McPherson et al., 2014).

Discussion

The research question of this essay was: **How does paternal nutrition during preconception influence the offspring's risk of developing the metabolic syndrome?** The preconception period seems the most vulnerable and most investigated time period of paternal influence on offspring's health. Comparing overnutrition and undernutrition, the effects of overnutrition on the next generation is investigated most and most present in the western society. Hardly any studies examine one specific nutrient, since most studies examine the consequences of an overall overnutrition. Furthermore, the most investigated subject is the consequences of paternal programming on fetal development and adult metabolic health, especially obesity. Many studies clearly show that paternal overnutrition has a profound effect on the offpsring's health.

These effects are executed through genetic and epigenetic changes in the sperm. These affect transcription and therefore fetal development and adult health. The epigenetic changes are induced by certain nutrients and food patterns. So, a future father who is planning to reproduce has to realize the impact his food habits can have on his future children. Well-substantiated information is needed, so that a man together with his partner can prepare themselves when it comes to the effects of their diet on their children. This needs more attention than it gets nowadays, because a child can eat very healthy, but still can experience health issues by the influence of its father's diet. Many animal intervention studies support the idea that fathers can indeed reverse the adverse effects.

Interventions

Since it is seen that paternal epigenetics play an important role in offspring's health, these mechanisms are integrated to discuss possible interventions. Some human and many animal diet interventions have shown to improve sperm function and resulted in restorations of embryonic and fetal health (Hakonsen, Thulstrup, Aggerholm, et al., 2011; McPherson, Setchell, Owens, & Lane, 2013; McPherson et al., 2014; Palmer, Bakos, Owens, et al., 2012). The negative effects on offspring have been prevented by these interventions , indicating that paternal programming seems reversible (Lucas & Watkins, 2017). Masuyama (2016) showed that a control diet of the father diminished the metabolic effects of a high-fat diet. Only after two generations of a control diet the effects were totally abolished. Also when human fathers lost weight, sperm function was improved in the form of improved motility, morphology, count and DNA integrity (Hakonsen et al., 2011; Palmer et al., 2012). This resulted in a healthy increased fetal weight (McPherson et al., 2013). Negative effects from paternal undernutrition on offspring were prevented when fathers supplemented their diet with vitamins and antioxidants (Lucas & Watkins, 2017). Their offspring's growth restriction, fat accumulation, dyslipidemia and altered pancreas gene expression were reversed (Lucas & Watkins, 2017; McPherson et al., 2016). Probably this restoration is due to reduced amounts of sperm ROS and normalized global sperm methylation. Besides, in many interventions exercise also played a substantial role in reversing the negative paternal programming effects (McPherson et al., 2014).

Ma & Hardy (2012) stated that dietary interventions that target transcription factors like nuclear receptors would be most promising in reversing adverse metabolic outcomes in children born from mothers with metabolic disorders. It is possible to influence the epigenetic structure of specific genes that are mutated in the father, to prevent the induction of heritable epigenetic marks (Curley et al., 2011). Another target is the relatively recent discovered resistance to DNA methylation (Hamet, 2016). Maybe it is possible to specifically increase demethylation in sperm. The last mentioned ideas yield a tremendous amount of further research, but will be more specific than dietary interventions. On the other hand, it is much more easy to advise potential parents, especially during the three months before trying to conceive, about a healthy diet and a healthy weight to reduce the risk of metabolic syndrome in offspring (Dunford & Sangster, 2017).

Future

Like Braun et al. (2017) state, many more prospective cohort studies are needed to investigate the possible dietary interventions to reverse or prevent adverse effects on the offspring. Cohort studies possess useful information to turn into advises for potential parents (Li et al., 2016). However, substantial limitations come along with these studies (Braun et al., 2017). First, recruiting couples that are actively trying to conceive at a certain time, which are willing to participate and let their child be followed for a period of time, is a difficult task. Often the sample size is reduced during the study, due to for example unsuccessful conceptions or canceled follow-ups. Besides, prospective cohort studies may be exposed to a selection bias, like more easily selection of intended pregnancies compared to unintended, which can go together with socioeconomic factors, or selection from fertility clinics, which include many infertility problems that can influence offspring's health. Moreover, these studies need to be supported by animal studies to distinguish between transmission of obesity genes and environmentally modified epigenetic transmission from the father's sperm (Hamet, 2016). To demonstrate that a phenotypical change is transmitted through the germline, this phenotype also has to be exhibited by the first non-exposed generation, the F3 generation (Curley et al., 2011). When no effect is seen in the F3 generation, the transmission is only multigenerational instead of transgenerational. This can be due to fading away of the phenotype in the absence of the environmental stimulus or the removal of specific epigenetic marks, cohort studies a difficult task, although desperately needed.

Beside more prospective cohort studies, the mechanisms behind paternal programming should be explored more abundantly by animal studies. To invent interventions it is needed to examine exactly how and when epigenetic marks in the sperm are changed as well as their effects on which proteins and therefore which steps in the development. Thereafter, human intervention studies are able to be designed.

The final goal, as in almost every biomedical field, it to find proper clues for personalized medicine. This is not only needed for clinical patients but also during prenatal planning and conception (Hamet, 2016). Every couple, but especially their future child, needs and deserves a specific advise about the consequences of diet on the offspring's health, based on solid-grounded research. Although most researchers find a stronger transmission of maternal effects compared to paternal effects (Vaag, Lehtovirta, Thye-Rönn, & Groop, 2001; Karter, Rowell, Ackerson, et al., 1999; Groop, Forsblom, Lehtovirta, et al., 1996), which can possibly be explained by genomic imprinting, mutations in mitochondrial DNA, which are maternally inherited, and metabolic programming in the uterus (Abbasi et al., 2011), it is undeniable that paternal effects on offspring's health cannot be excluded.

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