

The Impact of Lifestyle Factors During Pregnancy on the Epigenome and Subsequent Risk of Disease

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ABSTRACT

While genetic factors have long been known to play a significant role in determining the risk of diseases, less understood is the role lifestyle factors have. Epigenetics is an emerging area of study that seeks to explain how the environment can indirectly influence our genome by causing chemical changes surrounding our DNA. These modifications, collectively called the epigenome, are often permanent and thus can be passed down to future generations. Concerningly, epigenetic modifications have been associated with a wide range of diseases, including cancer, diabetes, and schizophrenia. The rapid development of fetal cells has made pregnancy a time of added worry over the possible implications of such changes, especially considering the dependence of the fetus on maternal decisions. This review focuses on three major lifestyle factors during pregnancy, including diet, stress, and the presence or absence of smoking, and evaluates the risk of each in the development of future diseases. Understanding the connection between lifestyle and disease could help prevent disease development in the future and give a better understanding of the causes of diseases altogether.

Introduction: Epigenetics and its Role in the DOHaD Theory

One of the defining features of prenatal life is the proliferation and differentiation of embryonic cells (1). Such events happen rapidly during embryonic development and impact the development of all organ systems (2). However, due to the widespread development happening in such a short window, the potential for error during this crucial time could lead to lifelong impacts. The Developmental Origins of Health and Disease (DOHaD) theory is one of the earliest theories to suggest that the development of diseases can be traced back to early environmental exposure, noting the connection between prenatal environment and adulthood cases of heart disease and strokes (3). While genetic risk factors have long been known to increase the risk of disease development, genetic variation alone does not explain why groups born under similar, strained environmental conditions often develop similar health problems (4). A look into the specifics of how differences in prenatal environment can impact fetal development opens the door for research into epigenetics.

Epigenetics is the study of chemical changes that happen surrounding DNA that do not interfere with the nucleotide sequence itself but can alter gene expression. These changes can be either permanent or reversible. Generally, these modifications are classified into three different types: DNA methylation, histone acetylation, and non-coding RNA (ncRNA). The first type of modification, DNA methylation, is characterized by the covalent addition of a methyl group to the 5' carbon of a cytosine nucleotide (5). Methylation almost always occurs in cytosines within CpG dinucleotide-containing regions, which has been speculated to serve some regulatory role in gene expression. Methylation of these cytosines generally results in a decrease of gene expression and subsequent mRNA production due to it blocking transcription factors. However, in some cases, methylation can increase transcription by creating an optimal landscape for transcription factors (6). The second class of epigenetic modifications falls under histone acetylation. Histones are the proteins DNA is wrapped around, and the tightness at which they are coiled influences the accessibility of the DNA to bind transcription factors. When an acetyl group is added to a histone, the negative

charge of the acetyl group neutralizes the positivity charge of the histone, reducing the histone's affinity towards the negatively charged DNA (7). The result is DNA is wrapped looser around the histones, increasing transcription factors' availability to bind to DNA and promoting gene expression. The last class of modifications is ncRNA mechanisms. ncRNA are RNA molecules that do not code for a protein. Their function lies in the post-transcriptional regulation of mRNA. ncRNA contains a wide variety of types including microRNA (miRNA), small interfering RNA (siRNA), and piwi-interacting RNA (piRNA), each regulating gene expression in different ways, including recruiting epigenetic modifiers (such as methyl groups) or degrading mRNA before translation (8). These three epigenetic modifications are effective methods for adaptation to environmental factors without altering DNA. If the environmental stimuli signal that the expression of a certain protein should be increased or decreased, signal transduction pathways can trigger epigenetic changes that alter gene expression. A time when this is crucial is during fetal development, in which the embryo is especially prone to environmental stimuli.

Since so many parts of a fetus are being developed during this time frame (such as the immune system, metabolism, and brain), an adverse maternal environment could alter the epigenetic modifications that take place leading to permanent change (9-11). This concept has been termed the "maternal effects" (12). Prenatal environment also serves as the body's understanding of the future resources it may have access to, causing the body to adapt in a way that attempts to serve itself in the future. One theory for the development of diseases is that they are caused by disagreements over the prenatal and postnatal environment, causing the child to be ill-equipped for its external environment (13). If a maternal environment induces stress during fetal development, the child may have an overactive or ineffective response in a healthy postnatal environment. Hence, there is a strong relationship between maternal environment and prenatal development.

Not every environmental stimulus has the same risk for epigenetic change however, in large part due to the regulation of the placenta. The placenta is a temporary organ created during pregnancy that allows for the transfer of oxygen and nutrients to the newborn baby from the mother. It serves as a regulated barrier between the mother and baby's environment. While the placenta is normally effective in protecting the baby against pathogens dangerous to the developing immune system, certain chemicals are not as easily recognized and protected by the placenta. Nicotine, for example, easily passes through the placental barrier, essentially bypassing its protective effects (14). Moderate levels of cortisol on the other hand, a naturally produced stress hormone, can be neutralized by the placenta into its inactive form, cortisone and reduce the risk of danger (15). Nicotine and cortisol are only two examples of the numerous chemicals that can fluctuate in their levels based on maternal lifestyle and environmental factors, though they do show the differing roles they can have in relationship to the placenta. Since what enters the placental barrier becomes the environmental stimuli the child responds to, these lifestyle choices can have significant epigenetic implications, especially due to the vulnerable state in the child's development.

As epigenetics is still an emerging area of study, overall literature on its role during fetal development is limited. An understanding of the implications of environmental factors during pregnancy on childhood development is crucial to discover the causes of long-term diseases and abnormalities that may be preventable in the prenatal stage. Similarly, a better understanding of how different factors influence pregnancy could result in more accurate warnings and recommendations. Therefore, this review will follow three different contributing lifestyle factors during pregnancy including nutrition, stress, and smoking, and compare their relative implications on fetal development through epigenetics.

The Impact of Prenatal Nutrition

One factor that has gained significant traction in research is the role of diet during pregnancy, especially considering the rapid rise of noncommunicable diseases (NCDs) and allergies unexplained by simply genetic variation (16). Although living conditions have generally gotten cleaner in recent years and infectious disease rates have fallen in the US from 797 deaths per 100000 in 1900 to 59 deaths per 100000 in 1996 (17), shifting lifestyle factors can largely be attributed to the reverse trend with regards to NCDs (18). One of the biggest lifestyle shifts recently has been with

food consumption, with fast food options only being more accessible to the general public. The creation of ready-made meals has also resulted in rapid changing of the average diet and corresponding nutrient profile (19). Because of these trends, the impact of diet on NCDs has become a huge point of concern. But while adults generally have a choice over their food decisions, the nutrition of a fetus is dependent on that of the mother. This makes prenatal life a vulnerable time for poor nutritional decisions, especially considering the amount of development going on during this period.

An emerging hypothesis is the Barker Hypothesis, which theorizes that susceptibility to many non-communicable diseases is often developed prenatally due to the plasticity of the immune system programming (20). Diet plays a large role in this as certain macro and micronutrients are necessary for the proper development of the immune system. Adequate amounts of nutrients are necessary for optimal function of immune cells and branched-chain amino acids play a significant role in immunity, making them potential biomarkers of many different diseases (21, 22). The diversity of the gut microbiome is also emerging as a determinant for many diseases, with it largely being developed prenatally (23, 24). When the environmental factors governing proper nutrition aren't met, the body is forced to adapt to what it perceives to be the supplies of the environment, which is where epigenetics comes into play.

A series of studies done on rats have investigated the impact of both a protein-restricted (PR) and high fat maternal diet on fetal development. These diets model the corresponding prevalence of two common dietary issues: both malnutrition and a “junk-food” diet. In rats exposed to the PR diet, specific epigenetic changes were seen with hypomethylation in both the GR and PPAR α promoters (25), genes involved in metabolic function, growth and development, and the immune system. Hypomethylation removes methyl groups added to the DNA, resulting in more transcription and altering the normal amount of gene expression. In fetal rats whose mothers had a high fat diet, hypermethylation in the insulin receptor *Irs2* gene was observed, along with hypomethylation in the *Map2k4* gene, a gene important for tumor suppression (26). These same rats also experienced glucose intolerance in their adulthood, increasing their risk of developing diabetes. The liver, important in regulating glucose metabolism, showed altered expression of over 3,900 genes in the offspring of a high-fat maternal diet (27). Importantly, as a result of both the PR diet and high fat diet, the epigenetic changes within the rats were permanent, meaning their bodies carried a “memory” of their prenatal environmental exposure into their adult life. This characteristic has the potential to impact the development of diseases through altered gene expression sustained many years down the line.

A recorded instance of this was with the Dutch Winter Hunger Famine during World War II, in which food supplies were suddenly cut off from a specific part of the Netherlands from November 1944 to May 1945. Due to the nature of this instance and its well defined timeline, it quickly became an important study on the impacts of sudden malnutrition on fetal development. The epigenetic changes appeared to be once again long lasting, one notably in the *IGF2* gene (responsible for growth and development,) still persisting in a follow up study done six decades later (28). The specific health effects of malnutrition also appeared to depend on the timing at which the organs and systems were developing, once again highlighting the plasticity of fetal development (29).

Prenatal diet also may have long term impacts on metabolism. A portion of the DOHaD theory centers around this concept, arguing that an unbalanced maternal diet influences the epigenetic and developmental changes that govern metabolism, which can be explained evolutionary as the offspring would be able to adapt early to its perceived postnatal environment (30). Studies done on fetal rats whose mothers were fed a PR diet have observed many characteristics of metabolic disease, including increased fat deposition and preference towards fattier foods in adulthood (31). Concerningly, this altered metabolic function may only be worsened by better dietary availability in the future. Malnutrition in fetal development, for example, may adapt the metabolism to be more conservative in its energy expenditure in the future and store more energy as fat. If energy availability remains low the body would be best suited for the demands of the environment, though if the same food restriction no longer applied in adulthood, the body would be at an increased risk of the development of many diseases. A test of this theory on rats showed that rats fed a high fat diet prenatally did not observe the same health complications on the same diet postnatally as rats who switched from a standard prenatal diet to the high fat one (32). Similarly, rats who were fed a 30% global nutrition

restriction diet experienced a variety of health problems including higher blood pressure, hyperleptinemia, and obesity. These symptoms worsened when they were switched to a high-fat postnatal diet (33). It's important to note that while an adverse prenatal diet may increase the risk of health complications on its own, the body appears to have some evolutionary mechanism to adapt metabolism development to limit these effects. A mismatched prenatal and postnatal diet may only be more telling in the development of diseases, though there have not been enough controlled studies to truly understand the impact of pre- and postnatal diet discrepancies.

An area where the role of maternal nutrition on fetal development is still very much in question is with regards to allergies. Allergies are a reaction by the immune system to an otherwise harmless substance, so due to the programming of the immune system during this time, it seems plausible maternal nutrition could impact their development. One explanation for its role comes from T helper cells. T helper 2 (Th2) cells play a large role in the allergic phenotype, responsible for the initiation of allergic cells such as mast cells and eosinophils. T helper 1 cells (Th1), on the other hand, inhibit the Th2 cell pathway, blocking the development of such cells (34). Therefore, a balanced amount of Th1 and 2 cells is required for appropriate, yet not overactive, immune system function. There appears to be a short window during prenatal life in which T helper cells are differentiated, largely influenced by the surrounding environment. A better understanding of this timeline could help diagnose kids with allergies at a young age or even provide a window for preventing allergies. Recently, fish oils have shown some impact on allergic development by supplying N-3 LC-PUFA, a fatty acid that causes acetylation of histone 3 in the PRKCZ gene and encouraging cell differentiation towards Th1 cells, limiting immune system hyperactivity (35). While some trials have shown a decrease in allergic reactions in response to a maternal diet high in fish oils, results have been too inconsistent and require further investigation to validate (36).

Maternal diet plays a significant influence on the epigenetic development of a child, especially in cases where the fetus lacks necessary nutrients or is being overfed. It also appears the body has an adaptive ability in the development of its metabolism, which may be harmful if one's postnatal diet is significantly different. The data supporting a link between prenatal diet and the development of allergies is of interest for prevention but requires further research.

Implications of Prolonged Stress During Pregnancy

Another major contributing factor to epigenetic change during pregnancy is stress. Although stress is an unavoidable part of life, prolonged or chronic stress can be especially dangerous to a developing fetus. Much of this has to do with the placental barrier. While the placenta has a mechanism of neutralizing cortisol into its inactive counterpart, cortisone, prolonged stress can disrupt this protective placental mechanism and allow high levels of cortisol to enter the fetus (15, 36). This has the potential for detrimental effects such as lower birth weight, itself a risk factor for many other diseases (37). A proper understanding of this topic is important for understanding the role traumatic stress can have during pregnancy, as well as the impact of maternal anxiety or depression on fetal development. The possible implications are widespread as between 8% and 13% of women are diagnosed with anxiety or depression during pregnancy, non-inclusive of those who already have a diagnosis beforehand (38).

Significant interest in the impact of stress during pregnancy arguably began in the 1960s after observations of clinical problems in the offspring of Holocaust survivors, providing the first evidence to support the idea of inherited trauma (39). Although adult offspring of Holocaust survivors were not more likely to experience traumatic events, they had a higher risk of developing depression, anxiety, and conduct disorder amongst other issues (40). The reason for this connection remains hard to pinpoint as it is likely influenced by a multitude of factors. While genetics may play a role, so could fear induced by traumatic war stories told by parents. However, as maternal PTSD has shown a higher association with PTSD development in offspring than paternal PTSD, the theory that risk to PTSD could be developed from the intrauterine environment remains strong (41). Epigenetics plays a large role in this as it sets the foundation for how environmental exposure by the mother could lead to changes surrounding fetal DNA that manifest themselves long after the original exposure. In mice, prenatal exposure to restraint stress led to specific methylation

within the genes *Gpm6a*, *RELN*, and *GAD1* that were associated with an increased risk of a schizophrenia phenotype (42). Schizophrenia itself is recognized by significantly altered methylation patterns in the brain, so while the disease may not manifest itself until later in life, an analysis of the epigenome early in life could evaluate the risk of developing the disease decades later (43).

What makes prenatal stress so impactful is the influence it can have on the development of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis serves as the body's response system to stress, using the interactions between the hypothalamus, pituitary gland, and adrenal glands to turn perceived stress into a stress response. This axis begins developing rapidly early in prenatal life, so an abnormally stressful prenatal environment could permanently adjust the development of the axis to prioritize survival in the near term (44). Epigenetic alterations in genes associated with the HPA axis can influence the glucocorticoid signaling response that takes place when encountering a stressful situation, simultaneously altering the role of glucocorticoids in normal immune system function. Because of this, proper HPA axis development is dependent upon proper environmental conditions.

In the wake of chronic stress, epigenetic changes in the HPA axis can accumulate over time. These epigenetic changes have been found to arise from a variety of stressful situations. Individuals prenatal during the First Congo War (1996-1997), for instance, showed increased methylation in the *CRH* gene and decreased methylation in the *FKBP5* and *NR3C1* genes (45). All of these genes play a central role in maintenance of homeostatic HPA axis control, and altered expression of them could influence how the body responds to stress. The implications for such a problem can come in multiple ways. For one, hyper or hypo reactivity of this system is associated with depression and PTSD, respectively (46). In the case of PTSD, this could cause enhanced responsiveness of glucocorticoid receptors even in comparison to low levels of cortisol (47). On the other hand, altered HPA axis expression can also cause a misalignment of the body's hormonal and neural response to even acute stress, resulting in poor coping practices that contribute to numerous other neurological diseases (48).

The First Congo War wasn't the only stressful event to show epigenetic modifications in genes within the HPA axis. Rather, a wide variety of other stressors prenatally, including maternal domestic violence, natural disasters, and low socioeconomic status, all have found similar modifications within these genes (49-51). This provides evidence for the fact that fetal development to stress may not be as variable in its impact as other contributing factors during pregnancy, though as a whole it can still contribute to a wide variety of health conditions.

While much of the current research on the epigenetic effects of stress focus on its role in changing the HPA axis, there is another front opening on investigating its role in the immune system and inflammation. Stress and inflammation are highly correlated. Glucocorticoids released by the stress response are able to increase or decrease inflammatory activity. Prolonged inflammatory activation of the immune system by these stress-related responses has negative health results in the long term (52). Excessive cortisol can also counter the role of insulin in moving glucose, resulting in insulin resistance (53). The Quebec Ice Storm of 1988 provided an optimal controlled occasion to study the impact of objective stress on epigenetic modifications. While previous studies focused on the differences in genes associated with the HPA axis, a more recent study focused on 1675 altered CG sites found within 957 predominantly related to immune system function (54). With the sudden excessive cortisol that the ice storm most likely prompted, insulin resistance and its corresponding health problems would be expected years down the line for people who were still prenatally developing during this time. Indeed, follow up studies done of the children years after the storm found both increased insulin secretion (a warning sign for insulin resistance) and an increased risk of obesity (55, 56). While the Quebec Ice Storm remains one of the most controlled and studied instances of the effects of stress on a developing immune system, a series of other studies have found increased inflammation during stressful pregnancies (57).

The impact of stress during pregnancy remains a developing field of study. While the resulting epigenetic modifications appear to show less variety in their implications than that of nutrition, the impacts of stress are still diverse, connecting to both mental and immune disorders. Stress also rarely acts in isolation. A famine or natural disaster, for example, can cause both nutritional and psychological stress that may amplify the problems caused by

epigenetic modifications. Because of this, the role of stress during pregnancy should remain a topic of investigation for the future.

Consequences of Maternal Smoking During Pregnancy

Unlike diet and stress which are unavoidable and can influence epigenetic modifications on a spectrum, smoking is a lifestyle factor individuals have much more control over. While research connecting smoking to adverse health effects such as cardiovascular disease, cancer, and respiratory complications is abundant, lesser known is the role smoking during pregnancy can have on the child (58). The importance of research in this field cannot be overlooked considering the sheer prevalence of it. In 2016 in the US, 7.2% of women reported smoking during their pregnancy, though in other countries, such as Ireland (which reported 38.4% of women smoking during pregnancy), and Uruguay and Bulgaria (which reported 29.7% and 29.4% respectively), it is even more common (59, 60). With such popularity, the potential risk of smoking on fetal development becomes a global issue.

The role of the placenta in regulating the impact of the environment on epigenetic modifications cannot be understated. Nicotine is a substance able to easily cross the placental barrier, giving no system of defense against even the smallest amount of exposure. Due to this, placental nicotine concentrations can exceed maternal ones by over 15%, only amplifying the potential issues smoking may cause in a mother into the child (61). In a developing fetus, this vulnerable state only worsens the chance for detrimental epigenetic changes made. It is unsurprising, therefore, that maternal smoking during pregnancy has been associated with numerous epigenetic changes thought to contribute to the development of a wide variety of diseases.

Among the genes affected by epigenetic modifications are BDNF (important for brain development), AHRR (associated with cancer risk) and FRMD4A (contributes to future nicotine dependence) (62, 63). These genes often show abnormal methylation in their promoter regions, altering their expression and consequentiality, increasing the risk of disease with time. Unlike the role of diet and stress, the impact of smoking is noticeably less defined with the potential epigenetic consequences being much more diverse. This likely has to do with the vast consequences nicotine itself has been associated with including its ability to stimulate nicotinic acetylcholine receptors (nAChRs) in the lungs and other organs associated with mutations and pathways that could increase the risk of cancer, as well as impairing signal transduction pathways that mediate proper immune response (64). Smoking during pregnancy has shown much of the same adverse health effects, though only developing earlier in the fetus. Children of mothers who smoked during pregnancy already were beginning to experience the same reduced lung and immune function as their parents (65, 66). This in part may be due to the alterations in miR-31 expression that smoking has been associated with, an important miRNA for regulating cell proliferation and apoptosis, the very function that would be detrimental if turned cancerous (67).

Exposure to smoking during pregnancy also increased the susceptibility of nicotine dependence in the child, likely due to both the epigenetic changes induced in the brain that mediate decision-making and to genes important to future nicotine dependence (62, 65, 68). This creates a cycle of nicotine dependence in which adverse health effects may be ignored due to the feeling of addiction that is passed on through generations. Indeed, epigenetic changes, such as the ones induced by smoking, are transmissible to the next generation and may be permanent. One study found that a grandmother smoking during her pregnancy still could carry the same health risk and epigenetic changes of smoking in a granddaughter, independent of whether or not the mother smoked (69). This reinforces the idea that nicotine dependence is significantly shaped by genetic factors, and may be a hard cycle to get out of if present in family history.

One well-documented effect of maternal smoking during pregnancy appears to be in birth weight. Repeatedly, exposure to smoking prenatally has been associated with a higher risk for a low birth weight (70, 71). Nicotine use has been tied to increased blood vessel constriction, reducing blood flow to the fetus which delivers important nutrients and oxygen (72). Because of this, smoking is thought to be a risk factor for intrauterine growth restriction (IUGR) in which a fetus's growth is significantly less than what would be expected. IUGR itself is a

condition marked by significant epigenetic changes (mostly methylation) from a normal placenta (73). The role nicotine may have in inducing these placental changes is still being refined.

A reasonable question regarding the role of smoking on epigenetic modifications would be whether it is more shaped by direct epigenetic changes from placental exposure or from transmissible ones the mother accumulated from her own experience with smoking. If transmissible epigenetic factors appeared to be more significant, then the timing of smoking surrounding pregnancy would be less important than the intensity of it beforehand. On the other hand, if epigenetic changes in the fetus from placental exposure played a larger role, quitting smoking for the span of a pregnancy should be significantly less detrimental for possible health complications. Based on current research, it is clear both factors play a role. Both smoking before and during pregnancy have been found to play similar roles in the development of birth defects, however quitting smoking earlier into pregnancy (before 15 weeks) shows strong benefits in preventing the development of certain health complications (74, 75). So while any maternal exposure to smoking throughout her lifetime has the potential to appear in an offspring's epigenome, exposure during pregnancy appears to be a time of heightened vulnerability. However, this is not to overlook the risks of significant parental smoking at any time during their lives. As epigenetic changes can accumulate from lifestyle factors before pregnancy and still appear present in a child's epigenome, quitting smoking for the span of a pregnancy alone may not prevent the development of smoking-related diseases. Because of this, it is not just the lifestyle of the mother that needs to be considered in understanding the risks of disease development in a child, but also the father's smoking habits. Paternal smoking has a strong correlation with the risk of diseases in children and their epigenome, associated with 33 altered CpG sites (76). Hence, while smoking during pregnancy carries excessively high risk due to the closely tied maternal and fetal environments, parental smoking habits before conception should not be ignored as risk factors as epigenetic changes are still accumulating during these times.

Of all the factors discussed, smoking is the most preventable, yet also potentially the most detrimental to fetal development. It has been associated with a wide array of diseases and birth abnormalities caused by epigenetic modifications. Although parental smoking in general can contribute to adverse health effects, smoking during pregnancy appears to hit at an especially vulnerable window of time.

Discussion

As more research is coming out, the role of prenatal environment on the development of diseases is constantly being refined. However, recent studies have repeatedly shown a connection between different environmental factors and their role in inducing epigenetic modifications associated with disease and health complications later in life. While maternal diet, stress, and smoking are some lifestyle factors that may affect a developing fetus, they are far from the only factors that can influence the epigenome. While the impact of some factors like drug and alcohol use during pregnancy are relatively well documented, there are still an infinite number of other factors that could contribute to epigenetic changes that are still undiscovered (77). More research into the role different lifestyle factors such as sleep, exercise, and hydration during pregnancy have on the fetal epigenome could give a better understanding into the interplay between different factors.

Understanding the interplay between environmental factors, epigenetic modifications, and the development of diseases is especially important considering the implications it could have for disease prevention. Since epigenetic changes do not interfere with the nucleotide sequence of DNA, potential therapies for diseases caused by them carry far less of a risk of causing accidental mutations. In fact, recent epigenetic treatment approaches against cancer have shown some success, opening the door for research into epigenetic therapies for other diseases (78). Many of these treatments are in the beginning stages of development and trial, so more time is needed to determine the success of epigenetic treatment options as a whole.

A possible caveat to epigenetic treatment approaches comes if not all epigenetic modifications are reversible. The permanency versus reversibility of epigenetic modifications remains a largely unknown subject. A common thread amongst the role of the three environmental factors explored in this paper was the changes they could still show

in the epigenome decades, or even generations, after the exposure. If these changes were reversible, it would beg the question of why the body doesn't have a system better able to regulate changes to the epigenome. If these changes were permanent on the other hand, the underlying reason for what could turn an epigenetic change permanent would need to be explored considering the wide variety of demethylase and deacetylase enzymes that should otherwise be able to modify these chemical groups. Some studies have pointed towards the length or intensity of an environmental exposure as a contributing factor into its permanence, but more research would need to be done to fully explain the process behind what might be happening. The reversibility of epigenetic modifications also appears to be heavily correlated to its type. The vast majority of the modifications highlighted in this review were DNA methylation, and there has been some research to support that methylation is more chemically stable than acetylation, which may factor into its increased prevalence in changes in the epigenome years down the line (79). However, there is still a lack of research exploring this topic, which may interfere with or set a timeline on the efficiency of epigenetic treatment approaches.

It is hard to directly compare the effects of different lifestyles, especially considering they rarely act in isolation. Famine, for example, has both a nutrition and stress related response, making it hard to tell which factor is more telling in any epigenetic modifications that may result. However, of the three lifestyle factors explored, smoking generally seemed to have the most diverse negative implications for disease, while research surrounding the impact of nutrition was the most inconsistent. This can be explained partly because any one person's diet is dependent on an infinite combination of different foods in comparison to smoking which introduces the same chemicals, allowing for a more controlled study. Of the most studied diets, results were much more consistent, showing an increased risk of high blood pressure, obesity, and hyperleptinemia among other things. The role of epigenetics in allergies is still too inconsistent to pinpoint the significance of its impact.

At the placental barrier, cortisol and nicotine also show very different behaviors. While the body is used to experiencing cortisol and can neutralize it into an inactive form, it has no defense against nicotine. Despite this, in times of excessive stress when the body is overwhelmed by cortisol, cortisol can behave similar to nicotine, able to cross the placental barrier with ease and contribute to epigenetic modifications.

Unlike diet, a wide variety of different stressful situations can lead to similar epigenetic changes in the HPA axis. This likely has to do with the way the body responds to stress, releasing cortisol as needed as opposed to nutrition which introduces a variety of nutrients and minerals the body responds to. The effects of stress on the development of diseases are a little more refined in terms of the direct contributions it can have towards PTSD and depression. There is some, but limited, evidence to support that it can also lead to changes in immune function, which requires further investigation.

Smoking has the most variable changes in the epigenome, affecting genes ranging from ones important to brain development to cancer and cardiovascular disease. It also has the most evidence for transgenerational transmission. Unlike traumatic stress during pregnancy which may happen during a specific timeline, it is unlikely someone would pick up smoking for just the course of a pregnancy. The role of smoking in parental lifestyle before pregnancy is also a significant risk factor for epigenetic changes.

Understanding the impact of different lifestyle factors on epigenetic changes and the development of diseases is necessary for future prevention of them. Even if epigenetic treatment approaches are still far in the future, more accurate recommendations and warnings can be given out now to mediate the risk of disease in the future.

The connection between prenatal exposure and the development of diseases decades later is a topic not fully understood. However, considering its global significance and vast implications for public health, more research into the topic is necessary to get a better grasp of the role it can play. Specifically, more funding and trials into epigenetic treatments would provide a new approach to cures for rapidly rising NCDs and a better understanding of the permanence of epigenetic modifications. The risk of developing life-altering NCDs in adulthood has the potential to be discovered at a young age.

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