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High Maternal Cortisol Levels During Pregnancy Leads to Dysbiosis in Newborn's Gut Microbiome

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SUMMARY Stress during pregnancy can have serious implications for fetal development. Maternal prenatal stress, measured using cortisol concentrations, is a simple measure of stress that is often overlooked when pregnancy outcomes are researched. Heightened stress response through the hypothalamic-pituitary-adrenal (HPA) axis directly affects the maternal gut via glucocorticoid (GR) and mineralocorticoid receptors (MR) on gut epithelial cells. The interaction of cortisol with these intracellular receptors leads to altered epithelial barrier permeability and nutritional availability. Stress results in a shift in maternal gut microbiome composition during pregnancy. This can be transferred to the fetus during birth and prime the newborn's gut microbiome in a manner that leads to gut dysbiosis during early stages. This review will discuss how the HPA axis is involved with stress responses in the body, the impact of stress on the mother's gut microbiome, and the transfer of the disrupted mother's microbiome and/or metabolites to the newborn prior to and during birth.

INTRODUCTION

Humans experience a multitude of perceived stressors, both physical and psychological, which all result in quite similar fluctuations in the levels of key stress hormones, such as adrenaline and cortisol (1,2). Cortisol, which is the primary stress hormone, results in increased glucose levels in the bloodstream, which provides a large supply of glucose to the brain. Cortisol influences the liver, muscle, adipose tissue, and pancreas. In the liver, high cortisol triggers gluconeogenesis which is the energy producing pathway that cells use when they are in need of energy (3). Muscle tissue increases protein degradation to provide gluconeogenesis with amino acids. Lipolysis increases in adipose tissue which releases glycerol and glucagon levels increase in pancreatic tissue which further increases lipolysis (3). The production of cortisol ensures that the body can stay alert for periods of stress, but this mechanism can also result in an overly heightened state of arousal, resulting in mood swings, muscle fatigue, high blood pressure, etc. Cortisol levels are controlled by the HPA axis, however the HPA axis is indirectly influenced by the gut microbiome through the enteric nervous system (4,5). Fluctuations in cortisol levels can lead to dysbiosis, which is an imbalance in the microbiome, due to the interactions with the enteric nervous system. Dysbiosis can involve a loss of diversity among microorganisms, loss of beneficial or "good" bacteria, overgrowth of pathogenic bacteria, etc (4).

This review aims to explore the impact of elevated cortisol levels in pregnant women on the gut microbiome of their newborns. Defining a normal gut microbiome is challenging, there is substantial data on the typical characteristics of a newborn's gut microbiome that are associated with positive health outcomes. This review will utilize those characteristics as the standard for normal. During vaginal births, the infant is exposed to the mother's vaginal microbiome, followed by exposure to the mother's skin microbiome (4,5). At birth, a newborn's gut microbiome has low diversity due to the absence or minimal exposure to

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external microbes (6). Shortly after birth, the gut microbiome of the newborn closely resembles that of the vaginal microbiome of the mother due to ingestion during birth. In the womb, the fetal gut is rich in oxygen due to a rich supply of oxygenated blood provided by the mother. Shortly after birth there is a shift to anaerobic metabolism, as aerobic early invaders rapidly use up the existing oxygen and create an anaerobic environment which selects for primarily anaerobic species (4,5). The infant's gut microbiome, at this time, is unstable and is particularly sensitive to perturbation due to environmental factors and stressors (6).

CORTISOL AND THE GUT MICROBIOME

Dysregulated cortisol levels impact the gut microbiome via the HPA axis. Exposure to psychological or physical maternal stress activates the HPA axis, enabling stress hormones to be produced in sufficient concentrations at the appropriate time (4,5). Cortisol receptors, specifically GRs, are found on almost all cell types in the body but are highly expressed on epithelial, immune, and enteroendocrine cells. MR receptors are specifically found in the brain, kidney, colon, heart, and sweat glands (7). Since these receptors are abundant on epithelial cells within the gut lining, which allows a direct link between stress hormones and the gut (4,5). Cortisol binds to these receptors more readily when levels are high, which can influence intestinal permeability and nutrient availability (4,5). Consequently, this impacts the diversity and composition of the microbes in the maternal gut. An influx of microbes into the bloodstream and alterations in nutrient sources leads to the selection of different microbial species. A change in nutrient supply allows pathogenic bacteria to colonize and enter a wider range of body systems. Coupled with increased epithelial permeability, these alterations elevate the risk of systemic inflammation (7).

Pregnancy is characterized by a natural increase in gut epithelial permeability to ensure that the developing offspring receives proper nutrients via metabolite transfer through the placenta. Metabolites are products of the metabolism of larger molecules, such as aryl sulfates, TMAO (trimethylamine N-oxide), short chain fatty acids, etc. In response to stressful stimuli, such as hormonal changes, anxiety, and physical discomfort, the autonomic nervous system signals for the hypothalamus to release corticotropin-releasing hormone (CRH). CRH then travels to the pituitary gland where it signals the release of adrenocorticotropic hormone (ACTH). ACTH then travels to the adrenal glands to signal the release of cortisol (5).

Activation of the HPA axis leads to altered microbial composition due to the increase in epithelial permeability and cross talk with the enteric nervous system (5). Additionally, more metabolites can be passed to the developing fetus due to altered permeability. If the maternal gut microbes have been altered due to stress response, the metabolites produced by these microbes will also be altered (5). Depending on the degree to which the microbes are altered, the gain and/or loss of certain metabolites can have serious impacts on the pregnancy.

Short chain fatty acids (SCFAs) are a common and incredibly important metabolite in the gut. In short, SCFAs help provide the gut with an innate immune response by promoting barrier integrity, mucus production, and protecting against inflammation (5). SCFAs have also been found to calm the cortisol stress response, which inhibits the dysregulation of the HPA axis during periods of stress (5). There are three key short chain fatty acids, all of which are products of the fermentation of fiber and complex carbohydrates (8). These SCFAs include acetate, propionate, and butyrate. Acetate is essential for proper barrier function and epithelial integrity, which is weakened during activation of the HPA axis (9). Propionate is also involved in barrier function, but it interacts closely with immune cells in the gut which lowers levels of inflammation in the gut epithelium (10).

Butyrate is important for colonocytes (epithelial cells in the colon) as these cells use butyrate as a food source. Therefore, the presence of butyrate ensures proper functioning and integrity of surfaces within the colon (8). As metabolites like SCFAs can produce systemic effects, they are imperative in linking the gut to the brain, partially using the HPA axis. Metabolites such as SCFAs can produce systemic effects, so metabolites are often the messenger that links the gut to the brain, partially with the use of the HPA axis. For example, acetate can facilitate cross-talk between the gut and the brain because it drives microglial maturation in addition to ensuring barrier function in the gut (9). Not all microbes produce

the SCFAs that provide these healthful benefits, so alteration of microbial composition can greatly impede essential immune function and stress regulation in the gut.

MATERNAL INFLUENCE ON INFANT'S GUT MICROBIOME

A fetus does not require a sophisticated stress response during pregnancy, as it is protected in the womb. Throughout pregnancy, maternal stress hormones are able to pass through the placental connection and into the fetus. This means that a maternal stress response could be mirrored in the fetus. Near the end of pregnancy, the fetus does begin to produce stress hormones in response to maternal stress (4). At this time however, the fetus still receives the necessary maternal input to cause the production of these stress hormones. Even when the fetus is capable of producing its own stress hormones the mother still has a significant influence.

Overexposure to cortisol during pregnancy has been associated with various short- and long-term health outcomes in the newborn (4,5). If cortisol levels cause a shift in the microbial species present in the maternal gut, crucial metabolites such as SCFAs will also be altered. The newborn will lack SCFAs and other metabolites, increasing the risk for problems with barrier function and epithelial integrity. Loss of epithelial integrity allows SCFAs to enter the bloodstream, which has been shown to cause diseases such as inflammatory bowel disease.

The placenta has many regulatory mechanisms that mitigate the impact of environmental factors on the developing fetus (5). There is an enzyme expressed by the placenta that keeps concentrations of stress hormones (glucocorticoids) higher in maternal blood, which prevents adverse effects that stem from fetal overexposure to glucocorticoids (5). The enzyme 11β-Hydroxysteroid dehydrogenase type 2 (11β-HSD2), converts maternal corticosteroids into an inactive form (11-keto derivatives) via redox reactions, which prevents high levels within the fetus (5). Although this placental regulatory mechanism protects the fetus from cortisol directly, altered metabolites are still able to transfer from the maternal gut microbiome to the fetal gut (11).

MICROBIAL COMPOSITION AND DIVERSITY IN INFANT GUT

A study published on prenatal stress and its association with the infant intestinal microbiota is focal to this review. The goal of this study was to investigate the presumed link between prenatal stress and the development of the infant intestinal microbiota along with health outcomes over the first 110 days of life (7). This study was done as a follow-up to a study that analyzed a similar concept using Rhesus monkeys. This study was used as a baseline for what microbial compositions were to be expected over the first three years of life in humans. Unlike previous studies, this human-based research utilized a high-throughput phylogenetic microarray to accurately observe and analyze microbial compositions and their relationships (7).

The study followed 192 Dutch children from the third trimester of pregnancy until day 110 of life and had the following inclusion criteria, (1) uncomplicated, singleton pregnancy, (2) fluency in Dutch, (3) no drug use, and (4) no physical health problems (7). Fecal samples were collected at 9 time points but only 5 time points were selected. To select participants based on stress level, five variables were used to measure maternal stress levels (7). The five variables were general anxiety, pregnancy-related anxiety, daily hassles, pregnancy-related daily hassles, and maternal prenatal cortisol (7,11). A median score was calculated and anyone scoring above the median on 4 or 5 variables were considered the high maternal prenatal stress group (7). People scoring above the median on no more than 2 variables were considered the low maternal prenatal stress group. The exclusion criteria were as follows, (1) children missing three or more fecal samples and (2) born via cesarean delivery (7). Fecal samples were collected at home and transported to a laboratory where DNA was extracted using the repeated bead-beating method (7). The Human Intestinal Tract Chip was used to analyze the microbiota composition. All statistical analyses were done using R (7).

Reported stress and cortisol levels were not associated with each other, indicating that these two ways of measuring stress are independent of each other (7). Stress and cortisol levels were not associated in this particular study because the participants often reported stress levels that were lower than what their cortisol levels were indicating. The effects on the infant microbiome peaked at day 80 and were still significant at day 110. As shown in Figure 1,

there was a significant difference in beta diversity between the low stress group at 14, 28, and 80 days (7). The most significant source of individual variation was a ratio of a group of Proteobacteria 1 to a group of lactic acid bacteria to Actinobacteria (1,7).

In the low prenatal cumulative stress group, the diversity of the infants' microbiota decreased at first but increased throughout the study (7). The newborn gut microbiome had a high abundance of *Streptococci* and Proteobacteria for the first month, however this was replaced by Actinobacteria, lactic acid bacteria and a different Proteobacteria strain (2,7). In the high cumulative stress group, the overall diversity was higher (7). As seen in Figure 2, the relative abundance of Proteobacteria 1 was higher and the abundance of Actinobacteria, lactic acid bacteria, and Proteobacteria 2 were lower (1,7). Lastly, *Akkermansia* decreased suddenly and did not replenish in the high stress group while Bacilli stayed high compared to the low stress group (7).

The authors concluded that maternal reported stress and cortisol concentrations are associated with shifts in the infant intestinal microbiota that are present until at least day 110 (7). As seen in Figure 3, high reported stress and high cortisol concentrations resulted in an increased abundance of Proteobacteria (Escherichia and Enterobacter) and decreased abundance of lactic acid bacteria and Actinobacteria (1,7). Proteobacteria are often associated with disease in humans (12). They are gram-negative and therefore contain lipopolysaccharides (LPS) in their cell membranes (12). In previous studies, a connection was found between LPS, inflammation, and metabolic disorders (12). Gram negative bacteria, like Proteobacteria, uphold LPS production and subsequently enhance inflammation (12). Levels of Proteobacteria are commonly found to be elevated in individuals with inflammatory bowel disease as a result of their role in inflammation (12).

Lactic acid bacteria are essential for the breakdown of substances that humans can't digest on their own. They can produce SCFAs from pyruvate which are incredibly important for the health of the gut in both the mother and the newborn (13). Actinobacteria are a Gram positive family of bacteria that include Bifidobacteria (14). The SCFAs that are produced by Bifidobacteria ensure proper epithelial integrity within the gut (14).

The authors concluded that there are three possible ways in which maternal stress is influencing the newborn's gut microbiota: (1) through bile acid production, (2) cortisol transfer via the placenta, and (3) through immune cells in the gut (7). High maternal stress has been shown to cause an increase in bile acid production, as cortisol mediates this process. Increased bile acid production alters the "normal" maternal gut microbiota which gets transferred to the infant during birth. Maternal cortisol can pass through the placenta and raise cortisol levels in utero, in turn impacting the development of the HPA axis (4,5,7). Cortisol can interact with gut immune cells which can disrupt barrier integrity, thus altering the newborn gut microbiota (6).

DISEASE IMPLICATIONS IN THE INFANT

Altered gut microbial composition and diversity in newborns can be especially devastating due to the fragility of their development. One of the most lethal inflammatory intestinal conditions associated with dysregulated gut microbiota is necrotizing enterocolitis. Necrotizing enterocolitis, or NEC, is an inflammatory intestinal disease that results in over-colonization of pathogenic bacteria (15). Due to its severity, it usually results in tissue death that spreads to the intestine and colon (15). Since this condition is so difficult to treat and predict, finding predictive measures is of utmost importance. The most significant risk factors for NEC are low birth weight and premature birth (15). NEC is characterized by a reduction in microbial diversity and an increase in pathogenic anaerobes (15). Maternal stress has been shown to impact the immune system by altering fecal IgA levels in mice. Normally, IgA increases during pregnancy but in mothers with high stress, this does not occur which puts the offspring at greater risk for pathogenic invasion of the gut (15).

Another group of increasingly prevalent diseases linked back to the gut microbiome are allergic diseases, specifically asthma. An altered maternal gut microbiome significantly impacts priming of the fetal immune system because it can cause an increase in cytokine production due to a lack of anti-inflammatory response provided by certain microbes (16). Failure to correctly train the immune system results in a hypersensitivity to certain stimuli that normally would be recognized as harmless (7). If cytokine production is altered in the

fetus, then it can favor the selection of certain types of immune cells over others (16). Altered cytokine production often results in T helper (TH2) cells instead of T regulatory cells (Tregs), which causes an inappropriate immune response (16). T regulatory cells are crucial because they allow the immune system to recognize self from non-self and remember antigens that the immune system is exposed to so that proper immune responses are produced later in life (16). Without proper Treg production, allergic disease ensues and asthma develops when Tregs are lacking within the respiratory tract (17).

ALLERGIC AIRWAYS DISEASE

One study found evidence that asthma is influenced by maternal diet and bacterial metabolites by looking at how a high fiber diet impacts gut microbiota and subsequent asthma development (18). This study took stool samples from infants at three weeks of age that had been exposed to either a high fiber or no fiber diet. The microbiota composition of the samples was determined via 16S sequencing and significant phylum differences were observed between the control and no fiber diet samples (18). In the high fiber samples, Bacteroidetes dominated with an especially high prevalence of an acetate-producing strain (*Bacteroides acidifaciens*) (18). Next this study concluded that a high fiber diet protected against the development of allergic airways disease (AAD). Researchers used the house-dust mite model of AAD where mice were fed the same three diets from above to test the immune regulatory effects of diet (18). In addition to diet, the mice were also provided water with added acetate. In mice that consumed the high fiber diet and acetate, asthma-like symptoms did not develop and they did develop in mice of both other diets (18).

There was the lowest number of eosinophils and mucus-secreting cells in mice fed a high fiber diet and even less in mice also given additional acetate. This is direct evidence that a high fiber diet is protective against asthma due to the increase in SCFAs. A high fiber diet during pregnancy positively alters the maternal microbiome and therefore also alters the infant's microbiome. A high fiber diet is just as important in infancy and early childhood as it is before birth since the microbiome continues to be colonized for the first few years of life and the immune system is primed during those years as well (19). There has been recent work using probiotics during pregnancy in an attempt to prevent asthma post-birth. There have not been significant results but the studies are still in their infancy. There have been a few trials that did show some reduction in asthma and eczema when probiotics were administered during pregnancy and then to the infant after birth (20).

Conclusions There is almost certainly a link between maternal high cortisol levels and altered microbial composition and diversity in the infant gut. Although there is not enough information to determine a causal relationship, maternal prenatal stress is a very viable indicator of infant health and an important link for future medical strategies. Whether the link is between the mother's altered HPA axis and subsequent microbial differences that are transferred to the infant during late pregnancy and birth or excess cortisol exposure before birth, the association between prenatal stress and microbial species in the infant gut should definitely be an area of continued research. Research on this topic could have numerous implications for the medical community and could help lower infant mortality rates in the United States.

In the future, there needs to be more research done on the effects of chronic and acute stress, since prolonged stress is thought to impact the body more significantly. It will be important to determine at what level stress starts producing negative health outcomes either in the mother or the newborn. The majority of current research focuses on the first few months after birth and in order to truly understand the impact of prenatal stress and determine a causal relationship, there needs to be more research done on the long-term effects of varying cortisol concentrations on the infant. Finding productive stress management strategies specific to pregnancy, and a way of testing how high risk a pregnancy is in terms of hormone levels would greatly improve health outcomes for both the mother and the newborn.

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