Developmental Programming of Obesity and Metabolic Dysfunction: Role of Prenatal Stress and Stress Biology

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Abstract
Epidemiological, clinical, physiological, cellular and molecular evidence suggests the origins of obesity and metabolic dysfunction can be traced back to intrauterine life and supports an important role for maternal nutrition prior to and during gestation in fetal programming. The elucidation of underlying mechanisms is an area of interest and intense investigation. We propose that in addition to maternal nutrition-related processes, it may be important to concurrently consider the potential role of intrauterine stress and stress biology. We frame our arguments in the larger context of an evolutionary-developmental perspective that supports roles for both nutrition and stress as key environmental conditions driving natural selection and developmental plasticity. We suggest that intrauterine stress exposure may interact with the nutritional milieu, and that stress biology may represent an underlying mechanism mediating the effects of diverse intrauterine perturbations, including but not limited to maternal nutritional insults (undernutrition and overnutrition), on brain and peripheral targets of programming of body composition, energy balance homeostasis and metabolic function. We discuss putative maternal-placental-fetal endocrine and immune/inflammatory candidate processes that may underlie the long-term effects of intrauterine stress.

Introduction
The origins of health and disease susceptibility for many of the complex, common disorders that confer the major, global burden of disease in developed societies as well as societies in rapid transition can be traced back to the intrauter-
ine period of life (i.e. the concept of fetal or developmental programming of health and disease risk [1]). A large number of studies of fetal programming of obesity and metabolic dysfunction have focused on the critical role of maternal nutrition prior to or during gestation and have produced important findings and insights [reviewed in 2]. Questions currently under investigation include those related to mechanisms or pathways by which nutritional programming can exert life-long effects on the developing organism. Some major nutrition-related pathways relate to the effects of nutritional insults on maternal-placental-fetal glucose/insulin physiology and downstream effects on the developing fetal brain and peripheral systems. In this paper, we suggest it may be important to also simultaneously consider the potential role of intrauterine stress and stress biology for the following reasons: (a) From an evolutionary-developmental perspective, energy availability (i.e. nutrition) and challenges that have the potential to impact the structural or functional integrity and survival of the organism (i.e. stress) represent the most important environmental conditions underlying natural selection and developmental plasticity along all times scales. It is therefore likely and plausible that stress represents an important aspect of the intrauterine environment that would be expected to influence many, if not all, developmental outcomes. (b) Stress-related biological factors may exert direct effects on fetal targets of programming of body composition and metabolic function. (c) Many of the effects of nutritional insults (both undernutrition and overnutrition) may be mediated by common stress-related pathways involving the hypothalamic-pituitary-adrenal axis and inflammation. Hence, stress biology may represent a common underlying mechanism. (d) Stress and stress biology is known to alter nutrition at several levels, including caloric intake, selection of food types, and metabolic fate of energy. Conversely, nutritional status is also known to alter stress at multiple levels in the brain and periphery, including appraisals of potentially stressful circumstances, psychological and physiological stress responses, and feedback regulation. Hence, in natural in vivo settings it is likely that the effects of either nutrition or stress are modified by or conditioned upon the state of the other. This issue is particularly important in the human context, since nutritional insults and stress tend to co-occur in populations across the world.

For these reasons, we highlight below the effects of stress and stress biology on fetal programming of body composition, obesity and metabolic function. We review empirical evidence for interactive effects between stress and nutrition, describe findings from some of our own recent studies on prenatal stress and stress biology, and discuss putative maternal-placental-fetal endocrine and immune/inflammatory candidate mechanisms that may underlie and mediate short- and long-term effects of prenatal stress on the developing human fetus, with a specific focus on body composition, metabolic function, and obesity risk.
Rationale for Considering a Role for Stress in Fetal Programming

From conception onwards, the mother and her developing fetus both play an obligatory, active role in all aspects of development. Based on the consideration that environmental conditions that have shaped evolutionary selection and developmental plasticity include not only variation in energy substrate availability (i.e. nutrition) but also challenges that have the potential to impact the structural or functional integrity and survival of the organism (i.e. stress), it is likely and plausible that prenatal stress represents an important aspect of the intrauterine environment that would be expected to influence developmental outcomes [3]. Moreover, we suggest the application of a prenatal stress and stress biology framework offers an excellent model system for the study of intrauterine development and associated developmental, birth and subsequent health-related phenotypes because it is increasingly apparent that the developing fetus acquires and incorporates information about the nature of its environment in part via the same biological systems that in an already-developed individual mediate adaptation and central and peripheral responses to endogenous and exogenous stress (i.e. the neuroendocrine and immune systems [4]).

Another compelling rationale for considering a role for in utero stress as a contributor to subsequent risk of obesity and metabolic dysfunction derives from the effort to elucidate and better understand the underlying reason(s) for the well-documented, persistent and large socioeconomic and racial/ethnic disparities in the population distribution of these outcomes in the US and other developed nations. The search for explanations has led to the hypothesis that stress may, in part, independently, or in combination with other factors, account for these disparities, because the experience of social disadvantage and minority racial/ethnic status is characterized by higher levels of stress and lack of resources, and because stress and stress-related biological processes have been implicated in a wide array of adverse reproductive, developmental and other health outcomes [5, 6].

The Role of Context: Potential Interactive Effects between Stress and Nutrition

Maternal nutrition, assessed by indicators of body size (body mass index, BMI), nutritional intake or measures of nutritional biomarkers, is a well-established risk factor for childhood and adult obesity and metabolic dysfunction. Growing evidence supports the concept of a bidirectional interaction between nutrition and stress, such that the effects of nutrition on health may vary as a function of stress, or that the effects of stress on health may vary as a function of nutritional
status. For example, several experimental studies in animals have demonstrated that nutritional manipulations, particularly in the preconception or early pregnancy period, may produce their effects on maternal and fetal outcomes via alterations in stress biology [for example 7, 8]. Conversely, studies in animals and humans of stress induction (by exposure to laboratory-based stressors or endocrine stress analogues) have demonstrated effects on feeding behavior, food choice (high-calorie-dense food preference) and the metabolic fate of food in target tissues [9–13]. For example, chronic stress or cortisol administration motivates people to select high-fat food and to overeat [9, 12], and corticotropin-releasing hormone (CRH) infusion in healthy human adults also increases subsequent food intake [11]. In addition, cortisol increases insulin levels [14, 15]. Although insulin is anabolic and under normal basal conditions can increase both lean and fat mass, coelevation of insulin with cortisol preferentially increases abdominal fat stores [16, 17]. Further evidence of an interaction between stress and nutrition comes from a recent experimental study in humans demonstrating that under conditions of stress the brain’s energy need increases and it actively ‘demands’ energy from the periphery (a concept termed ‘brain-pull’ [10]).

We note that only a small number of studies have examined the relationship between maternal stress and diet or nutritional state in pregnancy. One study found that pregnant women who were more stressed in mid-pregnancy consumed more food (increased macronutrient intake) but concurrently decreased their intake of some micronutrients [18]. Another recent study demonstrated that the level of maternal stress during pregnancy was positively associated with pre-pregnancy BMI [19]. In an animal model, the interactive effects of maternal stress and nutrition on subsequent risk of offspring obesity were investigated [18], and the results suggested that prenatal stress and/or high-fat diet during the intrauterine environment affects offspring in a manner that increases their susceptibility to diet-induced obesity and leads to adverse metabolic consequences. Despite the plausibility of stress-nutrition interaction effects in the context of pregnancy, we are not aware of any human study to date that has examined these interactive effects during pregnancy on offspring body composition and metabolic function.

**Stress-Related Maternal-Placental-Fetal Endocrine and Immune/Inflammatory Processes as Potential Mediators of Fetal Programming of Health and Disease**

The fetal programming hypothesis has led to the search for underlying mechanisms by which disparate intrauterine insults exert a multitude of effects on different physiological systems in the offspring. A question of particular interest
relates to whether these biological mechanisms are exposure and/or outcome specific, or whether there may be some common mechanisms that mediate the effects of various exposures on a range of disparate outcomes. We suggest that stress-related maternal-placental-fetal endocrine and immune processes in gestation constitute an attractive underlying common candidate mechanism because they are responsive to many classes of intrauterine perturbations and they act on multiple targets of fetal programming [4]. Unlike exposure to toxins and teratogens, it is important to appreciate the fact that maternal-placental-fetal hormones and cytokines play an essential and obligatory role in orchestrating key events underlying cellular growth, replication and differentiation in the brain and peripheral tissues [4]. Thus, perturbations in the level and/or time of exposure of these biologic effectors are likely to produce alterations of normal structure and function. Furthermore, it also is important to appreciate that the state of pregnancy itself produces major and progressive alterations in the function of these systems, and that these changes may have important implications for altering the responsivity of these systems to exogenous or endogenous perturbations, and hence their downstream effects on fetal targets of programming.

Stress Biology in Human Pregnancy

Stress biology refers to the set of biological adaptations in response to challenges or demands that threaten or are perceived to have the potential to threaten the stability of the internal milieu of the organism. The nervous, endocrine, immune and vascular systems play a major role in adaptations to stress. There are no direct neural, vascular or other connections between the mother and her developing fetus – all communication between the maternal and fetal compartments is mediated via the placenta, an organ of fetal origin. Based on the physiology of stress, parturition and the evidence linking maternal stress to earlier delivery, we have previously proposed a biobehavioral framework of stress and adverse birth outcomes [4] that may also be applicable in the present context.

Pregnancy produces major alterations in neuroendocrine and immune function, including changes in hormone and cytokine levels and control mechanisms (feedback loops), that are crucial in providing a favorable environment within the uterus and fetal compartment for growth, differentiation and maturation and conveying signals when the fetus is ready for extrauterine life. Glucocorticoid physiology (cortisol in humans) has received extensive and well-placed consideration as a critical endocrine mediator of fetal programming, with an emphasis on not only hormone production but also hormone action mediated by tissue-specific glucocorticoid receptor expression, sensitivity and affinity,
and by maternal-fetal transfer mediated by the activity of the placental 11β-hydroxysteroid dehydrogenase enzyme system [20]. Less well recognized is the potential and perhaps equally important role of the peptide CRH. In primates, but not other mammals, the placenta synthesizes and releases CRH in large amounts into the fetal and maternal circulations, with actions on central and multiple peripheral target systems in both compartments [reviewed in 4]. With respect to the immune axis, a major endeavor of pregnancy-related alterations in immune function is to achieve and maintain the optimal balance between tolerating the fetal semi-allograft while not suppressing maternal immune responses to an extent that increases maternal or fetal susceptibility to infection. Thus, a generalized reduction of maternal immune responsiveness occurs during pregnancy, mediated by hormonal changes (e.g. increased levels of progesterone), trophoblast expression of key immunomodulatory molecules, and a progressive switch from a TH1/TH2 balance to a predominantly T-helper 2 type pattern of cytokines [21].

Prenatal Stress and Maternal-Placental-Fetal Endocrine and Immune Function

The well-demonstrated link between stress exposure and activation of the neuroendocrine system and exaggerated inflammatory responses cannot be assumed to also be present in the pregnant state because the above-described changes in endocrine and immune physiology have consequences for attenuating the responsivity of these systems to stress. However, we and others have shown that despite the large pregnancy-associated changes in maternal physiology, the system is responsive to maternal psychosocial states (such as high stress and low social support), that maternal psychophysiological stress responses are progressively attenuated with advancing gestation, and that after accounting for the effects of other established risk factors, the degree of attenuation is a significant predictor of shortened length of gestation and earlier delivery [reviewed in 3]. Studies by other groups have reported that elevated psychosocial stress and depressive symptoms in pregnant women are associated with changes in immune and inflammatory markers (in vivo and in vitro evidence, summarized in [3]).

In addition to psychosocial stress, substantial in vitro and in vivo evidence indicates that maternal-placental-fetal endocrine and immune processes during pregnancy respond to a variety of other maternal and intrauterine perturbations, including biological effectors of stress, obstetric risk conditions such as preeclampsia, pregnancy-induced hypertension, gestational diabetes, infection,
reduced uteroplacental blood flow, and behavioral factors such as the constituents of maternal diet, over- and undernutrition, and smoking [reviewed in 22]. Thus, based on these observations, it is apparent that measures of maternal-fetal endocrine and immune/inflammatory stress markers capture physiological responses to a wide range of intrauterine perturbations including, but not limited to, prenatal stress.

Long-Term Effects of Prenatal Stress Exposure on Human Adult Physiology and Health

The majority of human epidemiological studies of the fetal programming hypothesis have operationalized unfavorable intrauterine environments using indicators such as low birthweight. However, the long-term effects on child or adult disease-related phenotypes of interest may not necessarily be mediated by adverse birth outcomes. Only a very small number of studies have investigated this issue in humans. As a first step to addressing this question, we conducted a retrospective case-control study in a sample of healthy young adults born to mothers with healthy pregnancies and normal birth outcomes. One half of the study population of young adults was born to mothers who had experienced a major stressful life event during the index pregnancy (prenatal stress group), whereas the other half was a sociodemographically matched population with no history of maternal exposure to prenatal stress (comparison group). We selected a study population of younger as opposed to older adults in order to focus on pre-disease markers of physiological dysregulation of metabolic, endocrine and immune systems as early predictors of disease susceptibility. The potential effects of other established obstetric, newborn and childhood risk factors on adult health were controlled using a stringent set of exclusionary criteria.

Our results indicated that the young adults exposed during intrauterine life to maternal psychosocial stress consistently exhibited significant dysregulation in key physiological parameters, thereby placing them at increased risk for developing complex common disorders. Specifically, individuals in the prenatal stress group exhibited: higher BMI and percent body fat, primary insulin resistance, and a lipid profile consistent with the metabolic syndrome [23]; altered immune function with a TH2 shift in the TH1/TH2 balance (consistent with increased risk of asthma and autoimmune disorders [24]; altered endocrine function, with an increased ACTH and reduced cortisol levels during pharmacological and psychological stimulation paradigms; accelerated cellular aging (as indexed by shortened leukocyte telomere length that extrapolated to approxi-
mately a 3.5-year increase in the rate of cell aging [25]), and impaired prefrontal cortex–related cognitive performance (impairments in working memory performance after hydrocortisone administration) [26].

Taken together, our findings suggest that in utero exposure to prenatal psychosocial stress may confer increased long-term risk of a range of negative physiological and cognitive health outcomes in humans; these effects are independent from those of other established obstetric and childhood risk factors, and these long-term effects are not necessarily mediated by unfavorable birth outcomes such as low birthweight.

**Fetal Programming of Body Composition, Metabolic Function and Obesity Risk**

At the individual level, obesity (or, more precisely, adiposity) results when energy intake exceeds energy expenditure. However, there is wide variation among children or adults at identical levels of excess energy intake in their propensity to gain weight and accrue fat mass. This variation across individuals defines susceptibility for developing obesity/adiposity. Once an individual becomes obese, it is difficult to lose weight, and even more difficult to sustain weight loss, because of the remarkable efficiency of energy balance homeostasis mechanisms. For these reasons, it is important to gain a better understanding of the origins of individual differences in the propensity for weight and fat mass gain in order to predict obesity risk and develop strategies for primary prevention. Continuing with the theme of a common underlying biological mechanism, in this section we address the issue of the potential impact of intrauterine stress biology on multiple targets of fetal programming related to body composition, metabolic function and obesity risk.

**Targets of Programming of Obesity: Potential Role of the Maternal-Placental-Fetal Endocrine and Immune/Inflammatory Pathway**

It is well established that the primary targets of programming of body composition, metabolic function and obesity risk are the neural networks that regulate energy balance (appetite, feeding and basal energy expenditure) and peripheral organs and tissues involved in fat synthesis/breakdown, storage and metabolic function (adipocyte, liver, pancreas, muscle). We recently reviewed findings that pertain to the potential role of prenatal stress biology in programming these major targets of interest [see 22].
For example, we have reported that placental CRH concentrations in human pregnancy significantly predict the rate of fetal growth and size at birth [27], which, in turn, is a significant predictor of childhood and adult adiposity [28]. Other researchers have found a positive association between CRH levels in pregnancy and an increase in central adiposity [29] and alterations in adiponectin levels in 3-year-old children [30]. Yet others have reported a positive association between maternal levels of interleukin-6 in pregnancy and neonatal adiposity [31]. A recent, large epidemiological study in humans found an association between maternal bereavement from death of someone close during pregnancy and an increased risk of overweight in the offspring in later childhood [32]. Furthermore, many animal studies have demonstrated long-term effects of prenatal stress exposure on increased bodyweight in the offspring [18, 33].

Neural Circuits
A growing body of literature suggests that intrauterine perturbations can produce reorganization of these neural pathways that regulate energy intake and expenditure in ways that enhance the development of obesity. Several studies have convincingly demonstrated that biological (endocrine, immune) stress during gestation, triggered by a variety of nutritional, inflammatory, vascular, behavioral or psychosocial perturbations, can promote obesity in the offspring by reorganizing central neural pathways through programming of energy balance ‘set points’ [see 34 for recent review]. One key system involved in the regulation of energy balance is the hypothalamic (CRH)-pituitary (ACTH)-adrenal (cortisol) neuroendocrine stress axis, which forms a network of neuronal pathways capable of interacting with brain circuits controlling energy balance [35]. For instance, the adipogenic hormone leptin which is the afferent loop informing the hypothalamus about the states of fat stores, participates in the expression of hypothalamic CRH, interacts at the adrenal with ACTH, and is regulated by cortisol. Cortisol increases leptin secretion and limits CNS leptin-induced efferents [36].

Adipocytes
Obesity is impacted by increases in fat cell number, size, or both. Fetal adipose tissue development is regulated by the complex interaction of maternal, endocrine, and paracrine influences that initiate specific changes in angiogenesis, adipogenesis, and metabolism [37]. Adipogenesis, the process of adipocyte development from mesenchymal stem cell precursors, occurs primarily during late fetal and early postnatal life in humans, and the number of adipocytes is relatively fixed after young adulthood [37–39], supporting the notion that fetal and early postnatal periods are crucial windows in the development of adipose depots. Adipogenesis is highly sensitive to the intrauterine biological environment,
in particular to concentrations of insulin-like growth factors, glucose, insulin and glucocorticoids [37, 38]. In vitro studies could show that the differentiation of human adipocyte precursor cells in the presence of insulin is stimulated by cortisol in a dose-dependent manner and occurs at physiological concentrations [40, 41]. Furthermore, in vitro exposure of isolated human adipocytes to insulin and corticosteroids synergistically induces peroxisome proliferator-activated receptor-γ mRNA expression [42].

CRH seems to be an important regulator of adipocyte function, and CRH receptors are expressed in both white and brown adipocytes [43]. The role of cytokines as regulators of adipose tissue metabolism is well established. Proinflammatory cytokines are elevated in obese individuals, and they seem to modulate leptin secretion from adipocytes [44]. Prenatal exposure to proinflammatory cytokines or dexamethasone in animals has been shown to increase offspring fat depots [45].

Liver and Pancreas

The liver controls the production and fate of metabolic fuels through the action of hepatic enzymes. Phosphoenolpyruvate carboxykinase (PEPCK), a key enzyme in hepatic gluconeogenesis, is under potent glucocorticoid regulation. In animals, prenatal exposure to dexamethasone produces an increased expression of hepatic glucocorticoid receptors as well as increased levels and activity of PEPCK [46], thereby predisposing these animals to glucose intolerance later in life. Furthermore, manipulation of diet during pregnancy is associated with epigenetic changes in the promoter regions of the genes encoding peroxisome proliferator-activated receptor-α and the glucocorticoid receptors in the liver in offspring after birth, thereby altering their metabolic phenotype [47, 48]. Insulin is produced by the β-cells in the pancreas in response to elevated blood glucose levels. Increased glucocorticoid exposure and malnutrition during fetal development have the potential to permanently reduce the pancreatic β-cell mass and lower pancreatic insulin content, thereby increasing the risk for metabolic disease later in life [reviewed in 49]. For example, in humans prenatal exposure to glucocorticoids or stress was associated with higher insulin resistance in the adult offspring [23, 50].

Genes, Gene-Environment Interactions and Epigenetic Mechanisms

Although weight and body composition are highly heritable, known genes account for only a modest proportion of their variance. Genetic makeup alone cannot explain the rapid increase in obesity prevalence in the population because the genetic characteristics of the human population have not changed in the last three decades, but the prevalence of obesity has tripled during that time
Estimates of maternal transmission of heritability are stronger than those for paternal transmission, which argues in favor of intrauterine effects and/or mitochondrial DNA effects. Moreover, the strongest genetic associations seem to vary as a function of the environment (e.g. effects are seen at specific times but not other times in the life cycle). These observations suggest gene-environment interactions are particularly relevant in the context of the obesity phenotype. Interestingly, a variant in the gene encoding the glucocorticoid receptor gene has been associated with increased body fatness in children [52], and we and others have described the association of the same variant with altered physiological stress responses [53]. Potential epigenetic mechanisms are areas of great interest in this context. A detailed review of epigenetics in the context of stress- and nutrition-related programming is beyond the scope of the current paper, and we and others have elaborated on this issue elsewhere [54–56].

Conclusions

Based on the conceptual framework and empirical findings presented here, we suggest in addition to maternal nutrition it may be important to also consider the potential role of intrauterine stress and stress biology in arriving at a better understanding of developmental programming of health and disease susceptibility. Moreover, we submit that stress-related maternal-placental-fetal endocrine and immune processes in human gestation represent a potentially attractive underlying candidate mechanism for elucidating the common biological basis (pathway) for mediating not only the long-term effects of prenatal stress but also those of a host of other intrauterine perturbations including maternal over- and undernutrition that have been implicated in this area. This framework and related empirical findings add further support and highlight the critical importance of the early developmental period (i.e. the first 1,000 days) in child and adult health outcomes.

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