Introduction

In 1992, Hales and Barker proposed the thrifty phenotype hypothesis to explain epidemiological associations between poor fetal growth and increased risk of developing the metabolic syndrome in adult life [6]. At the crux of this hypothesis was the idea that exposure to fetal malnutrition during a critical prenatal period could program offspring to a long-term "thrifty phenotype." This phenotype included qualities such as relative preservation of brain growth at the expense of visceral growth to reduce energy requirements and predisposition to store metabolic fuel during times of abundant nutrition [13]. Such programmed phenotypic changes were postulated to aid survival in a continuing postnatal environment of poor or cyclic availability of food but deter health in an environment of adequate or overabundant nutrition, predisposing individuals to adult disease [13].

As the concept of the thrifty phenotype hypothesis has gained ground and validity in the field of research, its application to human disease has also broadened. With the current growing epidemic of obesity in the United States [18], studies in the past decades have questioned whether the thrifty phenotype hypothesis may also explain observed associations between intrauterine exposure to malnutrition and subsequent development of adult obesity. Although studies in sheep, rats [2], and even humans have confirmed the association between prenatal malnutrition and increased risk of postnatal obesity [14,15], much research remains to be conducted on the mechanisms mediating this relationship. As a hormone involved in central signaling of satiety [5], leptin is a candidate molecule for potentially explaining mechanisms related to obesity. This paper reviews the potential mechanistic role of leptin in programming of postnatal obesity by prenatal malnutrition based on published studies in animals and humans.

A Potential Role for Leptin

Leptin, produced in both adipose and placental tissue, is a peripheral satiety signal that normally interacts with central regulation of energy homeostasis to promote the stability of fat stores. Discovered in 1994 as the protein product of the *OB* gene in mice, leptin gained attention when it was found that leptin-deficient mice were genetically obese [3]. Studies in the last ten years have detailed that the effect of leptin is to reduce food intake and increase energy expenditure in part by modulating activity of neurons within the arcuate (ARC) nucleus of the hypothalamus [1]. More specifically, leptin suppresses activity of neurons in the ARC nucleus expressing neuropeptide Y and stimulates activity of neurons in the ARC nucleus expressing melanocortin peptides such as α–melanocyte-stimulating hormone. These neurons of the ARC nucleus then project to other areas of the hypothalamus to ultimately reduce food intake and increase energy expenditure [4].

Based on the normal physiological role of leptin, it follows logically that leptin deficiency or leptin resistance disposes to increased food intake, reduced energy expenditure, and ultimately, obesity. However, while leptin-deficient mice and humans have both been shown to exhibit significantly increased appetite and manifest obesity, the reality is that the majority of obese individuals in the United States are not leptin-deficient but rather leptin-resistant and hyperleptinemic [5]. Interestingly, rats exposed to intrauterine malnutrition that later developed obesity have also been found to be hyperleptinemic [2,17]. Thus, in fitting leptin into a paradigm linking fetal malnutrition

and postnatal obesity, a probable and clinically-relevant mechanistic model would involve prenatal programming of leptin resistance by fetal malnutrition.

Evidence in Animal Studies

Offspring of maternal animals fed variations of a low-protein or nutrient-restricted diet that has resulted in intrauterine growth restriction (IUGR) have provided a useful model for studying the effects of fetal malnutrition on postnatal growth. As mentioned previously, studies in sheep and rats have replicated the finding of an association between exposure to fetal malnutrition and increased risk of adult obesity [2]. In murine models, the findings have often been that rats subjected to fetal malnutrition exhibit IUGR and are born with low-birth weight; however, when subsequently exposed to adequate postnatal nutrition, these previously malnourished rats exhibit a rapid postnatal catch-up growth that allows them to catch up and eventually *exceed* the weight of control rats not exposed to fetal malnutrition. In a study by Ozanne et al., it was shown that offspring of dams (i.e. maternal rats) fed a low (8%) protein diet during pregnancy were able to exceed the weight of offspring of control dams fed a normal (20%) protein diet during pregnancy by as early as 7 days of postnatal age [12].

In a recent study looking at plasma leptin levels in rats exposed to fetal malnutrition, Desai et al. showed that Sprague Dawley rats born to dams 50% food-restricted during pregnancy had lower birthweight and lower levels of plasma leptin at birth than control rats born to dams fed adequately during pregnancy. In this study, both fetally-malnourished and control rats were subsequently nursed by adequately-fed dams and then later weaned onto ad libitum feed at 3 weeks of age. Similar to other studies, the rats exposed to fetal malnutrition exhibited rapid growth in the postnatal period that allowed them to catch up and eventually exceed the weight of controls to become significantly heavier as adults. Plasma leptin levels in the rats exposed to fetal malnutrition were found to be significantly greater than controls by 3 weeks of age and remained significantly greater when re-evaluated at 9 months. It was also found that rats exposed to fetal malnutrition exhibited relative hyperphagia (defined as quantity of food consumed adjusted for body weight) when compared to controls that manifested from approximately 4 weeks of age until 8 weeks of age.

The findings by Desai et al. of concurrent hyperleptinemia and hyperphagia in rats exposed to fetal malnutrition were consistent with previous findings by Vickers et al. in 125 day-old adult Virgin Wistar rats born to dams 30% food-restricted during pregnancy and subsequently fed a standard postnatal diet. Due to the fact that increased levels of plasma leptin act to reduce food intake in a normal physiologic state, persistent hyperphagia in these rats in spite of high plasma levels of leptin implies resistance to the normally satiety-inducing effects of leptin. Thus, the combined studies by Desai et al. and Vickers et al. suggest that fetally-malnourished rats are leptin-resistant as early as 28 days of age and as late as 125 days of age. Interestingly, Vickers et al. was also able to show that leptin resistance in fetally-malnourished rats was exacerbated when rats were fed a hypercaloric diet instead of a standard diet postnatally.

Whether leptin resistance in fetally-malnourished rats is programmed in utero or whether it develops as a secondary consequence of other physiological phenomenoa programmed by intrauterine malnutrition remains largely uncertain. However, intrauterine programming of leptin resistance could explain why fetally-malnourished

rats develop a relative hyperphagia in comparison to controls and thus subsequently grow and exceed the weight of controls postnatally. Definitive proof that fetal malnutrition programs leptin resistance in the prenatal period would require evidence of evoked epigenetic (i.e. permanent) modifications of genes associated with leptin or leptin-signaling. Based on recent literature searches on PubMed, there is currently very little, if anything, known about the epigenetics of leptin, let alone the epigenetics of leptin as influenced by fetal malnutrition. However, though the scientific field is far from providing definitive proof of mechanisms of programmed leptin resistance, there is animal evidence to support several biologically plausible mechanisms of programming of leptin resistance at the level of the hypothalamus.

One potential mechanism involves the finding that rats exposed to intrauterine malnutrition are exposed to high levels of physiological glucocorticoids in utero due to downregulation of a glucocorticoid-inactivating enzyme (11β-hydroxysteroid dehydrogenase type 2) expressed in the placenta [8]. Interestingly, high levels of glucocorticoids in fetal rats (as simulated by maternal dexamethasone treatment) have been shown to downregulate leptin receptors in the placenta [16]. If high levels of glucocorticoids in fetal rats exposed to intrauterine malnutrition also downregulated leptin receptors in the hypothalamus, this would lead to a diminished hypothalamic response to a given amount of leptin. If this effect was retained throughout postnatal life, this would lead to effective leptin resistance that would particularly manifest during times when a capacity to respond to high plasma levels of leptin was needed (i.e. times of adequate or overabundant nutrition).

Other mechanisms of programming of effective leptin resistance include malformation of leptin-sensitive neural pathways. Recent evidence suggests that a surge of leptin during a critical period of early postnatal life is necessary for normal development of leptin-sensitive neurons in the ARC nucleus in rodents [1]. It is possible that fetally-malnourished rats that are hypoleptinemic at birth are unable to induce a proper surge of leptin for normal development of leptin-sensitive neural pathways in the early postnatal period. This would lead to inadequate responses to leptin in later postnatal life that would predispose to obesity. Although this would be a potential mechanism for programming rodent obesity in the neonatal as opposed to prenatal period, rats are born at a more immature stage than humans. Thus, potential mechanisms of obesity programming in the early postnatal period in rats may have implications for the third trimester, or prenatal period, in humans [2].

Evidence in Human Studies

Though the association between prenatal malnutrition and postnatal obesity has been reported in humans by historical studies such as the Dutch Famine study [14,15], well-controlled human studies on the effects of fetal malnutrition have been reasonably limited by the ethics of such trials. However, some interesting parallels can be drawn between data in rodents and that obtained from human infants born with intrauterine growth retardation (IUGR). IUGR is defined as a condition in which a fetus is unable to grow to its genetically determined potential size [7]; though protein-calorie malnutrition has been cited as a cause of IUGR, the numerous alternative causes of IUGR make it an imperfect model for studying postnatal consequences of prenatal malnutrition. Interestingly,

however, similar to fetally-malnourished rats, human IUGR infants are born with lower birthweight and lower levels of leptin in umbilical cord blood [9,11].

In a 1999 prospective cohort study by Jaquet et al. of 70 IUGR infants followed up to 2 years of age, it was shown that IUGR infants, though small and hypoleptinemic at birth, exhibited a dramatic catch-up growth in their first year of life (-2.58 ± 0.52 SD score at birth *vs* –0.94 ± 0.64 SD score at 1 year) [9]. Interestingly, when evaluated at 1 year of life, these infants were found to be hyperleptinemic in comparison to cross-sectional controls ($p<0.0001$). However, this relative hyperleptinemia in IUGR infants was noted to disappear at 2 years of age when the study ended [9]. In a separate cohort study by Jaquet et al. (2001) evaluating 26 IUGR infants at a later stage in life, serum leptin levels between IUGR infants and age-matched controls at 24 years of age were also noted to be statistically similar [10]. In this second study, however, although 24-year-old adults born with IUGR had comparable body weight ($p=0.51$) and BMI ($p=0.59$) as controls, they were shown to possess significantly more body fat mass (27.2 ± 7.6% *vs* 22.0 ± 7.3%, $p=0.02$), demonstrating a pattern of growth toward obesity reminiscent of fetally malnourished rats [10]. Though these studies lost subjects to follow-up (e.g. only 30 out of 70 IUGR subjects remained at the end of the 1999 study by Jaquet et al.) and did not control for gender differences when evaluating weight, BMI, and body fat mass, the parallels that they suggest between human infants born with IUGR and fetally malnourished rats are striking and give credence to the idea that the association between prenatal malnutrition and postnatal obesity in rats and humans may be mediated by similar principles of metabolic programming. Unfortunately, hyperphagia is much more difficult to define in humans than in animal models where food intake may be carefully and accurately monitored. As a result, although human infants born with IUGR exhibit hyperleptinemia in the early postnatal period, it is not yet clear whether this hyperleptinemia is a manifestation of leptin resistance.

Conclusion

While the human evidence for a potential mechanistic role of leptin in central programming of postnatal obesity by prenatal malnutrition is limited, sufficient animal evidence exists to support several biologically-plausible mechanisms involving hypothalamic programming of leptin resistance that merit further research. Although a variety of physiologic changes likely contribute to the true relationship between fetal malnutrition and postnatal obesity, the role played by leptin, a central regulator of energy homeostasis, is likely an important one in both animals and humans. The research field has not yet generated enough conclusive evidence about the role of leptin in prenatal programming of obesity to be equipped to make any recommendations for clinical practice. However, with the current epidemic of obesity, future findings may have widespread implications for both medicine and public health.

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