

The Role of Epigenetic Regulation in the Development of Obesity: A Comprehensive Review

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1 in 6 children and 1 in 3 adults in the United States are obese. Estimated costs in 2010 relating to obesity exceed \$300 billion. Although the etiology of obesity may be well characterized, the advent of the epigenome has increased the complexity of pinpointing causes of obesity. Ovine and murine models show that maternal overnutrition and maternal undernutrition are implicated in epigenetic dysregulation of endogenous energy-balance mechanisms. Furthermore, retrospective analyses of the Great Dutch Winter Famine and the Great Chinese Famine show an increased risk of obesity and metabolic syndrome in offspring affected by the famines. Studying the epigenetic alterations associated with obesity could afford changes in maternal dietary guidelines, spur new global and individualized therapies for obesity, and could allow nations to obtain fiscal benefits from decreased costs of obesity management. Obesity is a trans-generational disease with a capacity to affect many individuals worldwide.

INTRODUCTION

Obesity has become a worldwide epidemic. The Center for Disease Control (CDC) reported in 2011 that 1 in 3 adults over 20 years old and 1 in 6 children between the ages of 2 and 19 years are obese (Ogden and Carroll 2010; Ogden and Carroll 2010). Worldwide cohort studies have recently determined that there are approximately 1 billion overweight adults and 300 million obese adults (Lillicrop & Burdge 2011). Clinicians continue to debate about a generally acceptable method of measuring overweight and obesity. Most clinicians regard a BMI between 25kg/m² and 30 kg/m² as overweight, while a BMI >30 kg/m² defines obesity (Lee et al. 2012). Trends suggest that the number of obese adults and children will increase in the coming years (Figure 1) (Lillicrop & Burdge 2011). The distribution of obesity is not limited to countries with seemingly unlimited food sources. Obesity also afflicts those who dwell in third world countries (Li et al. 2011). In some societies, women are encouraged to diet during pregnancy in order to reduce fat deposition in the post-pregnancy period (Stevens et al. 2010). Obesity is a disease that should not be taken lightly. Complications resulting from obesity include diabetes mellitus type 2, dyslipidaemia, cardiovascular disease, cancer, and arthritis, among others (Keith et al. 2006). The etiology of obesity has expanded over the past decade and includes many known causative factors: poor diet, sedentary lifestyle, genetic predisposition, medical/psychiatric illness, decreased sleep, endocrine disruptors, socioeconomic status, infectobesity (as a result viral or bacterial infections), late age pregnancies, and decreased smoking (Keith et al. 2006). Despite the known causes of obesity, an accelerated increase in the number of obese individuals over the past two decades has occurred too fast to be

exclusively caused by such factors; this implies the existence of a cause that is not as obvious, a cause that escapes the scrutiny of worldwide cohort studies using multivariate analyses. Extensive research has been conducted in the past decade examining the effects that the perinatal environment can have on development and inheritance of obesity by analyzing the epigenome. This review will highlight obesogenic epigenetic alterations as a result of abnormal maternal nutrition by analyzing animal studies and retrospective analyses, culminating in an increased awareness and understanding of obesogenesis.

Current Knowledge

Chromatin modeling and epigenetic alterations

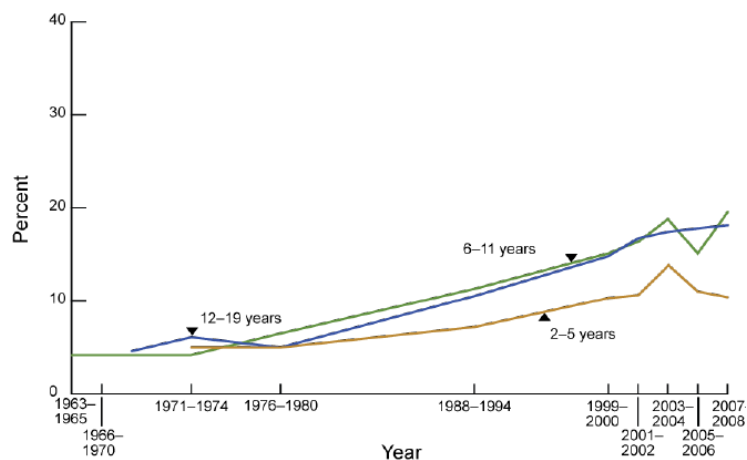
Understanding the structure and alterations of chromatin are critical to comprehend the complex epigenetic regulations associated with obesity. Chromatin has been likened to "beads on a string" due to its appearance in electron micrographs (Szerlong & Hansen 2011). It is a 3-dimensional structure consisting of deoxyribonucleic acid (DNA) wound around histone octamers and DNA linker histones (Szerlong & Hansen 2011). The spatial structure of the chromatin dictates whether transcription or repression occurs (Szerlong & Hansen 2011).

The packing of chromatin and transcription of DNA is a dynamic process. To tightly compact DNA, processes known as histone methylation and histone deacetylation occur (Szerlong & Hansen 2011). Histone methylation involves the recruitment of histone methyltransferases (HMTs) while histone deacetylation involves the recruitment of histone deacetylases (HDACs; Murawska & Brehm 2011). HMTs methylate certain residues on the exposed histone tails (Figure 2; Murawska & Brehm 2011). HDACs cleave acetyl groups off histone tails (Szerlong & Hansen 2011). Methylation and deacetylation cause a conformational change from a 10nm chromatin fiber to a compact 30nm fiber, due to decreased static repulsion of the negatively charged acetyl groups and negatively charged phosphate backbone of DNA (Murawska & Brehm 2011). Compaction of chromatin prevents transcriptional machinery from binding to unique consensus sequences, which prevents

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DNA transcription (Pauler et al. 2012). Conversely, a conformational change from inactive 30nm chromatin to transcriptionally active 10nm chromatin requires histone demethylases (HDMs) and histone acetyltransferases (HATs; Murawska & Brehm 2011). The addition of negatively charged acetyl groups and removal of methyl groups causes static repulsion between the acetyl groups and phosphate backbone of DNA (Murawska & Brehm 2011).



NOTE: Obesity is defined as body mass index (BMI) greater than or equal to sex- and age-specific 95th percentile from the 201 CDC Growth Charts. SOURCES: CDC/NCHS, National Health Examination Surveys II (ages 6-11), III (ages 12-17), and National Health and Nutrition Examination Surveys (NHANES) I-III, and NHANES 1999-2000, 2001-2002, 2003-2004, 2005-2006, and 2007-2008.

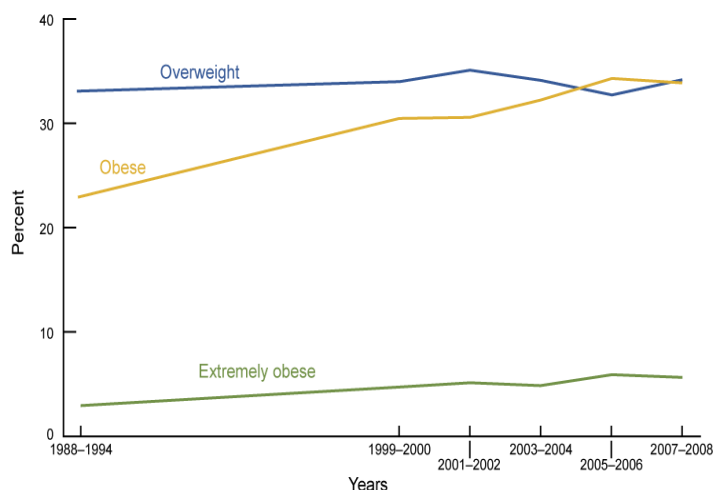


Figure 1. Trends from 1963-2008 of overweight, obesity, and extreme obesity in the United States among children aged 2-19 years (top panel) suggest an imminent increase in obese children aged 6-19 years.¹³ The bottom panel depicts the trend from 1965-2008 of overweight, obese, and extremely obese adults over 20 years of age (Ogden and Carroll 2010; Ogden and Carroll 2010).

Conformational changes of 10nm chromatin to 30nm chromatin and *vice versa* are examples of epigenetic regulation, because they alter gene expression without changing the genetic sequence. An additional mechanism of epigenetic regulation is the direct methylation of cytosine residues in cytosine-phosphate-guanine (CpG) islands. CpG islands are regions that lie upstream of the transcription start site (TSS) and range from 200-2000 base pairs in length with a G:C > 60% (Hashimoto et al. 2010; Szerlong & Hansen 2010). DNA methyltransferases (DNMTs) catalyze *de novo* methylation and maintenance methylation (for inheritance or cell identity; Hashimoto et al. 2010; Szerlong & Hansen 2010). Methylation may occur in the promoter region, the gene body, or the intergenic region (Hashimoto et al. 2010). Although the location of the methylation relative to the TSS may be different, the outcome is the same: incomplete transcription of DNA into primary mRNA (Murawska & Brehm 2011; Szerlong & Hansen 2010).

Endogenous regulation of energy balance and food intake

The physiological processes governing food intake and energy balance are extremely complex, and are currently the subject of investigation by many researchers. Evolution has conferred humans with an increased propensity to store energy in preparation for a famine (Stevens et al. 2010); prenatal fetal programming (*via* acetylation, methylation, etc.) allows for development of energy regulating pathways so that a fetus may survive in a food-restricted environment. The primary site responsible for energy homeostasis is the hypothalamic-pituitary axis (HPA), although other organs also secrete factors responsible for regulating food and energy intake (Figure 3; Stevens et al. 2010). A hypothalamic region that is strongly implicated in normal metabolism is the arcuate nucleus (ARC; Stevens et al. 2010).

Two general classes of hormones are responsible for the regulation of food/energy intake, orexigenic hormones and anorexigenic hormones (Plagemann et al. 2009). Orexigenic hormones (*e.g.*, neuropeptide Y [NPY] and agouti-related peptide [AgRP]) are endogenous substances which promote a state of hunger and increase energy storage (Plagemann et al. 2009). Orexigenic neurons, such as NPY-releasing and NPY-sensitive neurons, will activate when the organism is in a low-energy state (Plagemann et al. 2009). Conversely, anorexigenic hormones (*e.g.*, leptin, insulin, and proopiomelanocortin [POMC]) are hormones which cause a feeling of satiety and decrease energy storage (Plagemann et al. 2010); anorexigenic neurons, such as POMC-releasing and POMC-sensitive neurons, will activate when the organism is in a high-energy state. In other words, when in a fast or state of hunger, the orexigenic hormones will predominate, whereas after a meal, the anorexigenic hormones will predominate and suppress the actions of orexigenic substances until the organism is hungry (Ravelli et al. 1976). Glucocorticoids have also been implicated in negatively regulating the HPA (Ravelli et al. 1976). Maternal and fetal HPAs contain high amounts of glucocorticoid receptors (GRs) which act in a similar way as the orexigenic hormones, causing hunger and increases in energy storage (Stevens et al. 2010).

lead to altered metabolism and abnormal food-intake rhythm (Stevens et al. 2010).

Principal Approaches

In vivo studies: ovine and murine models

Many research groups have chosen to use *in vivo* animal models to examine the periconceptual changes in phenotype associated with different nutritional insults. Ovine models are a well-regarded standard for use in examination of perinatal alterations due to the similarity between ovine and human fetal development and maturation (Stevens et al. 2010). The periconceptual conditions to which the animals were exposed have varied amongst research groups. Stevens et al. (2010) chose to examine the epigenetic effects of periconceptual hyponutrition on the expression of GRs, POMC, and NPY. Three maternal undernutrition groups were utilized: 60 days pre-conception to day of conception, 2 days pre-conception to 30 days post-conception, and 60 days pre-conception to 30 days post-conception. The experimental groups represented different periods in which

a nutritional insult could occur – early pre-conception, post-conception, and periconception respectively. Undernutrition was achieved by initially subjecting the ewes to a two day fast, followed by a diet permitting a consistent ten to fifteen percent reduction in body weight compared to the control. Shortly before birth, the ewes and fetuses were sacrificed and analyzed for deviations in normal levels or function of GR, POMC, and NPY. The results indicated that preconceptional and periconceptional malnutrition resulted in hyperacetylation of H3K9 in the POMC promoter region as well as hypomethylation in the POMC and GR promoter regions. Together, the epigenetic changes indicate a change in the late gestation hypothalamic genes, resulting in a dysfunctional HPA energy-regulating system and an increased risk of developing adult-onset obesity. Although the study was completed using an *in vivo* calorie-restricted ovine model, one could expect a similar outcome in a human model (with appropriate changes in feeding schedule/caloric restriction in accordance with a more prolonged gestational period in humans).

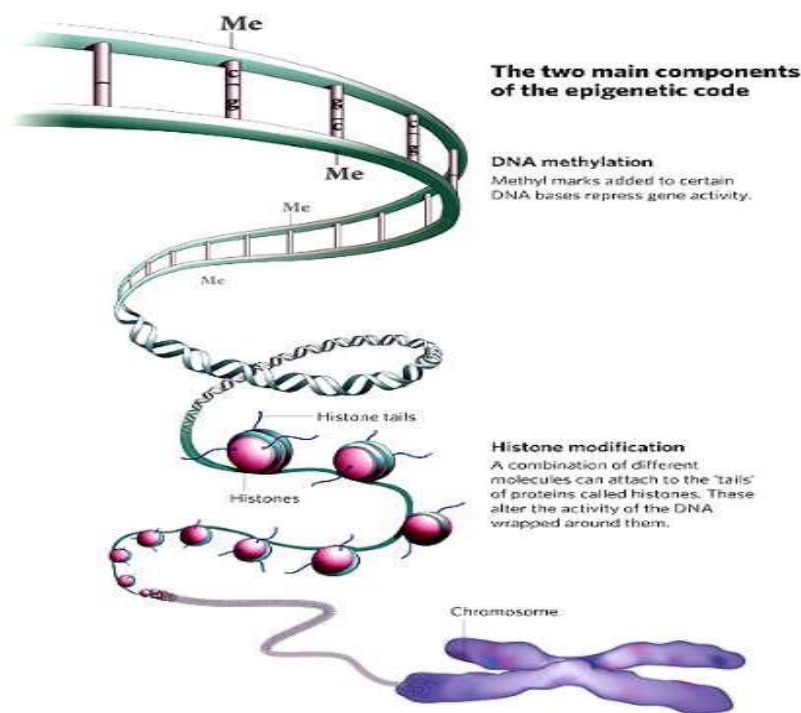


Figure 2. The precise structure of DNA base-pairing and the subsequent packing of the chromatin to form chromosomes (Qiu 2006).

Together, the dynamic balance of orexigenic and anorexigenic hormones and their importance in organism development make the HPA extremely susceptible to nutritional insults.

An insult disrupting the balance of orexigenic/anorexigenic hormones or an insult desensitizing the body's normal response to food intake and energy regulation can have long-term consequences. A particularly notable example of a prenatal insult occurred during the Great Winter Dutch Famine of 1944. Retrospective analyses of birth weights, metabolic phenotypes, and gestational insults has revealed that low metabolic activity affects individuals in a temporal-dependent manner (Rhodes et al. 2009). Periconceptual malnutrition, as seen in the Great Winter Dutch Famine, has also been linked to hypomethylation of *POMC* and *GR* and hyperacetylation of *NPY* (Stevens et al. 2010). Dysregulation of *POMC*, *NPY*, and *GR* can

Author & Year	Year	Subjects	Results
Lusanna et al	2008	730 men and women from the Dutch Famine Birth Cohort	No change in fat:carbohydrates:protein. Individuals exposed in early gestation have 2.1 odds ratio of consuming a high fat diet. All individuals have lower physical activity
Tobi et al.	2009	60 men and women exposed to the Dutch Famine periconceptionally and 62 men and women who were exposed late gestation to the famine	Hypomethylation of <i>INSIGF</i> in individuals who were periconceptionally exposed to famine. Hypermethylation of <i>IL10</i> , <i>LEP</i> , <i>ABCA1</i> , <i>GNASAS</i> , and <i>MEG3</i> in individuals who were periconceptionally exposed to famine. Gender differences between methylation status of <i>INSIGF</i> , <i>LEP</i> , and <i>GNASAS</i> were noted. Individuals who were exposed to famine late gestation had altered methylation patterns in <i>GNASAS</i> and <i>LEP</i> . Key changes in metabolic genes predispose individuals to obesity.
Ravelli et al.	1976	300,000 19 year old patients from Dutch Famine	Mothers exposed to the famine during the first two trimesters of pregnancy gave birth to heavier babies who subsequently developed obesity. Mothers exposed to famine during the last trimester gave birth to normal-sized babies who had a lower chance of developing obesity later in life.
Painter et al.	2008	855 Dutch Famine cohort members (F1) and their offspring (F2) (n=1496)	No significant changes in F2 birth weight were noted. Decreases in F2 birth length, increased F2 ponderal index, and increased F2 adiposity were discovered.
Zheng et al.	2012	5040 individuals either fetally or postnatally exposed to the Great Chinese Famine	Women had increased incidence of metabolic syndrome regardless of exposure group. Men displayed no significant increased risk of disease.
Li et al.	2011	7874 adults born between 1954 and 1964 in China. Groups were split in to fetal exposure and early childhood exposure	Those born in "severe famine" areas and fetally exposed were 3 times more likely to develop metabolic syndrome compared to control. Individuals exposed during early childhood also had an increased risk of developing metabolic syndrome. Higher increases in risk of developing metabolic syndrome were noted in individuals who adapted a Western diet.

Table 1. Retrospective analyses of the Great Dutch Famine and the Great Chinese Famine. Increases in metabolic syndrome and/or obesity were globally noted.

Similarly, Adam et al. (2008) examined midgestational (day 81) fetal ARCs for expression and sensitivity of NPY, agouti-related peptide (AGRP), POMC, and cocaine and amphetamine related transcript (CART). Experimental groups used in the study included a control group, a hypercaloric group, a control group + growth hormone, and a hypercaloric group + growth hormone. Results from the study indicated a strong positive correlation between serum glucose levels and POMC and CART expression. Orexinergic peptide expression was found

to be glucose independent. Increased expression of anorexigenic hormones early in gestation and prior to birth can lead to desensitization of the energy-regulating pathways, creating a potential for the development of obesity later in life.

Although Stevens et al. (2010) and Adam et al. (2008) pinpointed keystone epigenetic alterations following pre- and periconceptional undernutrition, the groups did not examine the result of the epigenetic alterations in offspring. Rhodes et al. (2009) explored the long-term implications of ewe hyponutrition on metabolic flexibility and obesity development in offspring. Using three experimental groups (control, low energy early [days 1-65 postconception], and low energy late [days 65-128]), the group simulated low energy by a 70% of control maintenance diet. Offspring were tested at 1.5 years for glucose and insulin tolerance in addition to body composition analysis via dual energy x-ray absorptiometry (DXA). Body composition was rated on a scale from 1 to 5, 1 being emaciated and 5 being obese. All sheep at 1.5 years were considered to be lean at a body rating of 2.4. At 1.5 years of age, males grew faster, and had a greater absolute fat mass compared to females. All metabolic parameters were normal between male and female offspring. Following metabolic and body composition analysis, the offspring were fed a 150% maintenance diet for 6 months and subjected to reduced activity (simulated by non-free range living). Glucose/insulin tolerance tests and DXA were repeated. In an obesogenic environment, global increases were seen in serum

triglycerides & cholesterol levels, decreases were seen in serum

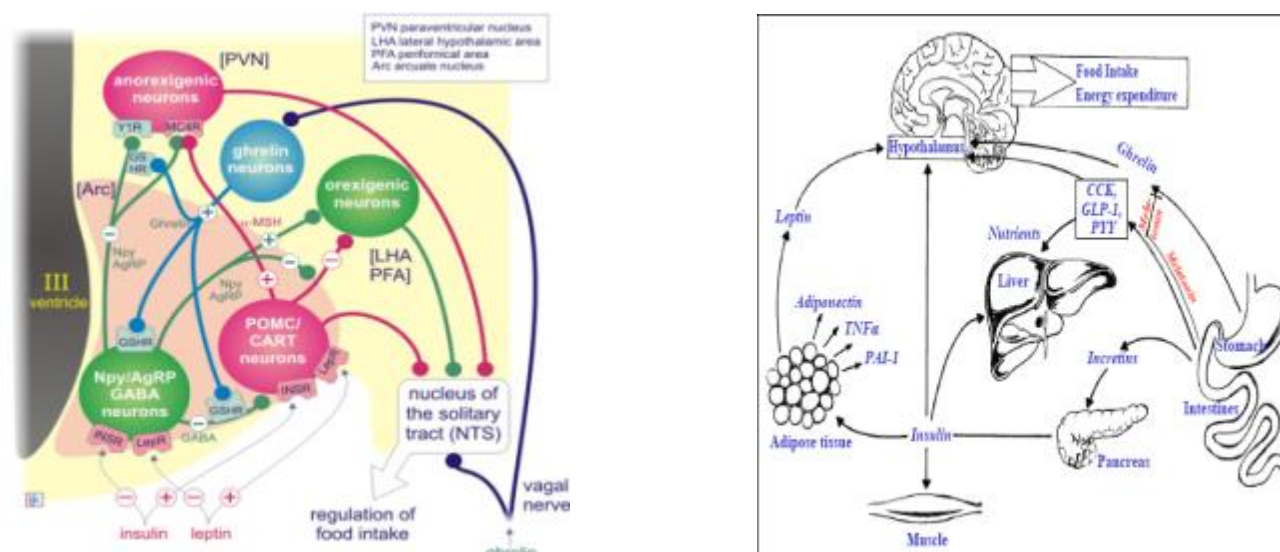


Figure 3. The primary hypothalamic nuclei responsible for energy balance and food intake (left panel) (Authessere et al. 2009). Anorexigenic neurons are responsible for inhibiting energy storage/food intake while orexigenic neurons function to increase energy stores/food intake (Authessere et al. 2009). The right panel shows the complex interplay between multiple organs in regulating food and energy intake (Konturek et al. 2007).

urea, and metabolic alkalosis indicated significant changes in fat metabolism, protein metabolism, and metabolic flexibility respectively. Decreased insulin sensitivity and a decreased fasting glucose level further indicated reprogramming of the energy regulating mechanism.

The use of an ovine model is suitable for examining fetal development; however the sheep genome is not as well characterized as the murine genome. Several groups have utilized rat models, a significant example being a study conducted by Plagemann et al. (2009). Typical litters of rats contain eight to twelve rats; raising rats in small litters promotes overfeeding due to the lack of competition between pups. Plagemann et al. (2009) sought to determine whether offspring reared in small litters (three rats/litter) had altered methylation patterns in NPY or POMC gene promoters by using bisulfite sequencing. Specific focus was placed on methylation patterns in consensus sequences of transcription factor binding sites (*i.e.* Sp1, NGF, NF- κ B, STAT3, AR-1, AP-2 α , NGFI-A, nGRE, and FoxO1). A metabolic panel and body analysis was also used to assess the phenotypic changes associated with small litter rearing. Results showed that from postnatal day 7 until postnatal day 21 (when the rats were sacrificed), small litter rats were significantly overweight and contained twice as much fat compared to controls. Small litter rats also displayed hyperleptinaemia, hyperglycaemia, hyperinsulinaemia, and an abnormal insulin:glucose ratio. The abnormal metabolic profile suggests early development of metabolic syndrome and insulin resistance. Bisulfite sequencing of NPY and POMC promoter regions revealed no significant change in methylation status of the NPY promoter; however, the POMC promoter displayed hypermethylation in the CpG islands containing the consensus

sequence for NF- κ B and in the CpG islands upstream of the Sp1 binding site. Metabolic testing revealed decreases in the ratio of POMC/leptin and POMC/insulin. In a rat with a normal metabolic phenotype, increased levels of insulin and leptin should cause a subsequent increase in POMC; no such event occurred in the small litter rats. The results suggest that obesogenic environment rearing, even for a limited amount of time, can cause reprogramming of the energy regulating system in the hypothalamus.

Although hypercalorism in a murine model appears to induce metabolic rats, what the study conducted by Plagemann et al did not demonstrate was a persistence of the phenotype from young to old rats. A study conducted by Breton et al. (2009) examined the ability of metabolic inflexibility to persist into adulthood using an FR30 murine model, FR30 referring to a food restricted 30% maintenance diet (a seventy percent reduction in food intake compared to control). Rats were sacrificed at 4 months of age following a two day fast. Rat tissues and blood were subjected to a metabolic panel. The eating habits of rats were also recorded during the four months. FR30 diet-subjected rats displayed growth retardation, hyperleptinaemia, and hypercorticonaemia at four months. The insulin:glucose ratio was abnormal as well, suggestive of a mild glucose intolerance. With weight corrected, FR30 rats had an increased food intake in addition to abnormal day-night feeding cycles - Rats are nocturnal animals, feeding primarily during the night hours. In this study, the rats had an increased food intake during daylight hours. The results indicated a change in POMC regulation and subsequent metabolic syndrome characteristics. Hypercorticonaemia, a finding not seen in many other studies, is unique due to the fact that corticosteroids are

responsible for increasing gluconeogenesis. An increase in gluconeogenesis could account for the abnormal insulin:glucose ratio. Severe hyponutrition in a murine model results in aberrant metabolism and feeding cycles, leading to an increased risk of developing obesity later in life.

Similar to the study conducted by Breton et al., Lukaszewski et al. (2011) used an FR30 murine model and high fat diet to specifically examine fat tissues of four month old male rats. Specifically, the group looked at body weight gain, endocrinology, gene expression, and fat distribution. Experimental groups included control, control + high fat, FR30 control, FR30 + high fat. White and brown adipose tissue sample were collected for histology and gene expression analysis. Results indicated that FR30HF rats displayed catch-up growth, while all HF rats displayed greater adipocytic area, adipocyte hypertrophy, and marked gene expression differences. Maternal prenatal undernutrition significantly alters male offspring adipose gene expression, thus predisposing the offspring to obesity development.

Retrospective analyses

In addition to *in vivo* models, research groups also have also chosen to utilize retrospective analyses to assess whether there is an increased risk of epigenetic reprogramming to an obese phenotype associated with abnormal maternal nutrition. The benefit of using retrospective analysis is that the subjects are humans. A significant caveat of using animal models is that results obtained in animal models do not necessarily hold true in humans. One of the most commonly analyzed events for maternal undernutrition and offspring obesity is the Dutch Winter Famine of 1944. One analysis of 730 men and women from the Dutch Famine Birth Cohort demonstrated a two-fold increase in the propensity to develop a high fat diet in those individuals who were exposed to the famine in early gestation (Lussana et al. 2008). An analysis of 60 individuals exposed to the famine revealed timing-specific and sex-specific methylation patterns in 15 loci implicated in metabolism and growth (Tobi et al. 2009). The largest cohort study to date was conducted by Ravelli et al. in 1976. The group concluded that mothers who experienced the famine during the first six months of pregnancy gave birth to obese offspring, whereas mothers impacted by famine during the last trimester gave birth to normal weight offspring (Ravelli et al. 1976). Table 1 shows several retrospective analyses and cohort studies addressing the implication of maternal malnutrition on the development of obesity.

Future Directions

The study of epigenetic reprogramming in obesity is very complex due to the dynamic interplay between multiple organs and multiple feedback loops in the body. As evident, conflicting results are possible and are common when comparing animal models to various retrospective human analyses. Certain animals possess similar neonatal development, anatomy, and physiology

when compared to humans. However, this similarity does not guarantee equivalent results across phylogenetic classes. Even the minutest differences (*e.g.*, enzyme levels, brain structure) may have substantial effects on experimental outcomes, especially when transgenerational data is analyzed.

More focus needs to be placed on sequencing the epigenome and discovering the significance of certain histone modifications or CpG methylations in each gene, as understanding epigenetic signatures could afford easier detection of obesity or metabolic syndrome in those individuals who are genetically predisposed. Comprehension of the epigenetic alterations surrounding obesity could also provide researchers with insight into development of novel therapeutics (*i.e.* antibodies, exogenous engineered proteins, (an)orexigenomimetic drugs) targeting certain epigenetic marks, with outcomes designed to alter levels of expression of orexigenic and/or anorexigenic proteins. By understanding the significance of epigenetic signatures, individualized therapy for those suffering from obesity would be made possible.

In addition to early detection of an individual's predisposition to obesity and individualized therapy, significant changes in pregnant mothers' diets could also be made. An increase in public service announcements highlighting the dangers of dieting and the importance of a balanced diet in the periconceptional period can serve as a starting point for informing the public of this disturbing trend. The public should be educated about the fact that the heritability of epigenetic signatures can extend far beyond the first filial generation, resulting in a vicious cycle of obese offspring. Societal pressure on women to maintain a thin stature is, in fact, harmful, especially to pregnant mothers. Likewise, disregard for one's diet and nutritional health could have the same impact on several generations of offspring. Comprehending the association of diet and risk for obesity could lead to significant alterations in the pregnant mother's diet both before and during pregnancy.

Finally, understanding the epigenetic signatures associated with the development of obesity could provide fiscal benefit for both for affected individuals and governments worldwide. According to a publication by Trogdon et al. (2012), obesity costs the public sector of the United States approximately \$147 billion, as 23% of cases covered by Medicare and 19% covered by Medicaid (Trogdon et al. 2012). By 2018, the cost of obesity will exceed \$338 billion (Behan et al. 2010). By better understanding obesity and having more affordable treatments for it, the costs related to obesity can be decreased in the long-term.

Conclusion

The etiology of obesity has evolved into a more complex disease process, given the advent of the epigenome. Altered epigenetic profiles and periconceptional reprogramming play key roles in the pathway leading to development of obesity in offspring. Direct *in vivo* evidence highlighting significant epigenetic alterations in the appetite-energy regulatory mechanism following maternal hyponutrition and hypernutrition have been demonstrated in ovine and murine models. While no *in vivo*

model can yet accurately replace a human model, there is conclusive evidence underscoring the role of periconceptional maternal dysnutrition in the development and propagation of obesity. Retrospective analyses have revealed a strong correlation between maternal hyponutrition during famines and metabolic syndrome and obesity. The role of the epigenome in modulating the development of obesity can help explain the staggering global increase in the number of obese individuals. Challenges facing researchers include the constantly changing environmental influences and human tendencies over time. The potential development of early obesity detection methods, the engineering of novel therapies for obesity, and the potential impact of maternal dietary guidelines can help assuage the enormous physical and financial costs associated with obesity. The societal and clinical consequences of obesity are far-reaching. Vicious cycles spanning many generations are created; a poor environment for a mother results in entrapment in the same environment for her as well as her future generations. One offspring may branch out and be exposed to a better, fiscally well-off environment; however, periconceptional reprogramming subjects the well -doing offspring to obesity and other metabolic disorders. Thus, obesity is a pervasive medical crisis that must be dealt with, as the ramifications of obesity are trans-generational.

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