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REVIEW

Endocrine Disrupting Chemicals and the Developmental Programming of Adipogenesis and Obesity

Amanda Janesick and Bruce Blumberg*

Obesity and related disorders are a burgeoning public health epidemic, particularly in the U.S. Currently 34% of the U.S. population is clinically obese (BMI $>$ 30) and 68% are overweight (BMI $>$ 25), more than double the worldwide average and 10-fold higher than Japan and South Korea. Obesity occurs when energy intake exceeds energy expenditure; however, individuals vary widely in their propensity to gain weight and accrue fat mass, even at identical levels of excess caloric input. Clinical, epidemiological, and biological studies show that obesity is largely programmed during early life, including the intrauterine period. The environmental obesogen hypothesis holds that prenatal or early life exposure to certain endocrine disrupting chemicals can predispose exposed individuals to increased fat mass and obesity. Obesogen exposure can alter the epigenome of multipotent stromal stem cells, biasing them toward the adipocyte lineage at the expense of bone. Hence, humans exposed to obesogens during early life might have an altered stem cell compartment, which is preprogrammed toward an adipogenic fate. This results in a higher steady state number of adipocytes and potentially a lifelong struggle to maintain a healthy weight, which can be exacerbated by societal influences that promote poor diet and inadequate exercise. This review focuses on the developmental origins of the adipocyte, the relationship between adipocyte number and obesity, and how obesogenic chemicals may interfere with the highly efficient homeostatic mechanisms regulating adipocyte number and energy balance. **Birth Defects Research (Part C) 93:34–50, 2011.** © **2011 Wiley-Liss, Inc.**

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INTRODUCTION

In the 1940s, the American psychologist William Sheldon and colleagues proposed that people could be grouped into three different somatotypes (Fig. 1), named after the three germ layers (Sheldon, 1940; Sheldon et al., 1954). The ''mesomorph'' and ''endomorph'' had proportionally large muscle and fat mass, respectively, whereas the ''ectomorph'' had neither but allegedly had a more developed nervous system. The further assignment of psychological characteristics to these somatotypes had about as much reliability as attributing personality traits to the signs of the zodiac. Nevertheless, the popularity of the somatotypes has remained because, like some stereotypes, there is a kernel of truth. This is that the somatotype designations recognize that a person's propensity toward building muscle or storing fat is largely predetermined before birth. Investigations into how muscles and fat grow revealed that certain changes in body composition are only associated with early development and rarely persist into adulthood. For example, the number of skeletal muscle fibers was found to be chiefly established during the prenatal period and remained fixed throughout life in mice, cattle, pigs, and chicken (Luff and Goldspink, 1967; Rehfeldt et al., 1987; Wegner et al., 2000), whereas the diameter and length of fibers could be increased with exercise (reviewed in Pearson, 1990). Similarly, adipocyte numbers increased during early development then reached a plateau in adulthood, after which adipose tissue growth became primarily hypertrophic in nature (Hirsch and Knittle, 1970; Knittle, 1972; Salans et al., 1973; Hager et al., 1978; Knittle et al., 1979). In both muscle and fat tissue, the number of cells appeared to be largely predetermined at birth, which partly explains the apparent implacability of somatotypes.

Amanda Janesick and Bruce Blumberg are from Department of Developmental and Cell Biology, University of California, Irvine, California 92697-2300
Bruce Blumberg is from Department of Pharmaceutical Sciences, University of California, Irvine, California 92697-2300

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*Correspondence to: Prof. Bruce Blumberg, Department of Developmental and Cell Biology, University of California, Irvine, CA 92697-2300. E-mail: blumberg@uci.edu

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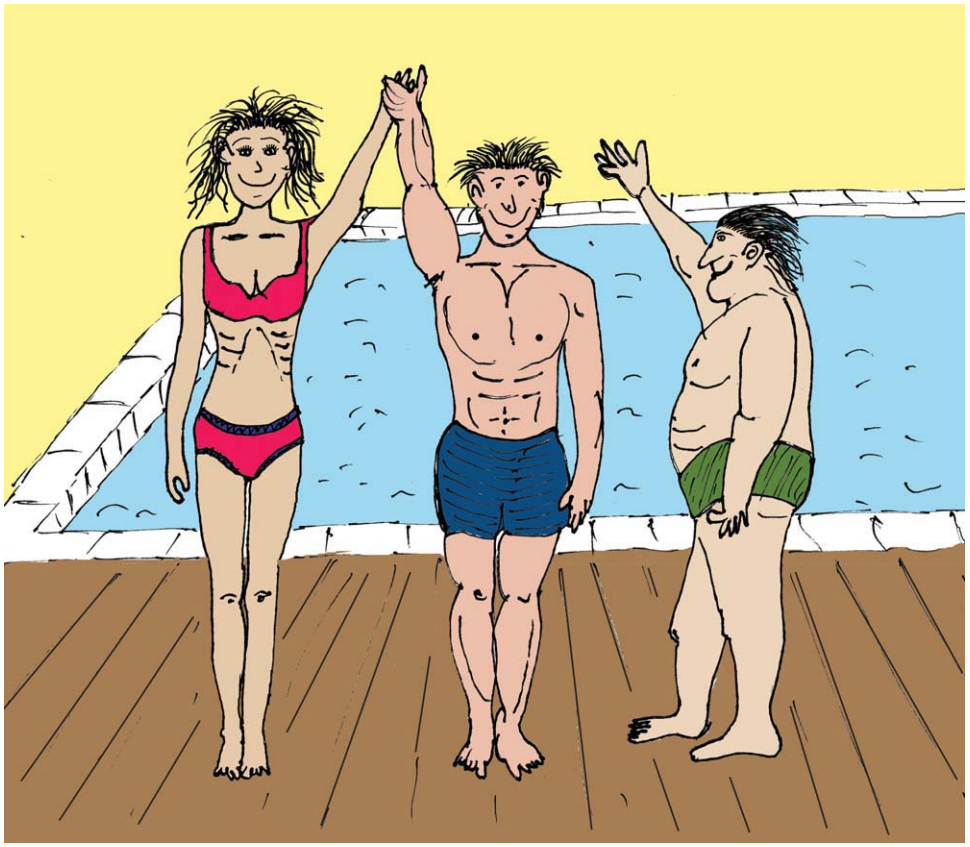

Figure 1. Depiction of the three classical somatotypes (from left to right): ectomorph, mesomorph, and endomorph.

Not surprisingly, such revelations about muscle growth engendered intense research in agricultural science aimed toward developing particular properties of muscle fibers in livestock that improved the amount, texture, taste, and color of meat (Klont et al., 1998). Researchers in adipose biology still maintained a high level of interest in the factors that increase proliferation and differentiation of fat cells; however, this was seemingly uncoupled from the human predisposition to certain somatotypes. Concurrent with this shift, obesity research became fairly detached from the adipocyte itself, focusing more on broader subjects of human behavior, basal metabolic rate (as measured by heat production and energy consumption) and environment (nutrition and exercise). In the 1990s, adipose tissue was reconnected to human physiology, largely instigated by the discovery of leptin (Zhang et al., 1994) and the peroxisome proliferator-activated receptor gamma (PPARγ) (Tontonoz et al., 1994). It became recognized that adipose tissue is an endocrine/paracrine organ and a central regulator of metabolism (reviewed in Mohamed-Ali et al., 1998; Kershaw and Flier, 2004). Leptin is a protein hormone that is produced in adipocytes and acts in the ventromedial hypothalamus to regulate energy balance (reviewed in Ahima and Flier, 2000). Generally speaking, each individual has a ''personal leptin threshold.'' When leptin levels exceed this threshold, the brain senses energy sufficiency and inhibits the fat storage program (reviewed in Lustig, 2006). Leptin decreases expression of PPARγ in adipocytes, whereas activation of PPARγ increases leptin expression (reviewed in Rosen et al., 2000; Spiegelman and Flier, 2001). Insulin increases both the expression of PPARγ and the generation of endogenous PPARγ ligands in adipocytes, thereby increasing triglyceride storage and adipocyte size (reviewed in Spiegelman and Flier, 2001). This new adipocyte-centric view fostered progress in the understanding of signaling pathways underlying adipogenesis (Rosen and MacDougald, 2006; Kirchner et al., 2010) and how dysregulation within the adipocyte leads to metabolic disease (de Ferranti and Mozaffarian, 2008; Vazquez-Vela et al., 2008).

However, what remains missing is an understanding of prenatal and early life determinants of adipocyte number and morphology, as well as how these relate to metabolic setpoints and the largely unchangeable nature of body composition. Adipose tissue is indeed an organ, albeit a distributed one (Wertheimer and Shapiro, 1948) and must be considered in this light. Advances in stem cell biology and epigenetics allow for a greater understanding of how adipogenesis is regulated and may reveal the molecular basis underlying the

apparent human somatotypes insofar as they exist. In this review, we discuss current concepts about prenatal and early life programming of adipose tissue size and composition, why adipose tissue tends to be resistant to persistent reductions in mass, and how prenatal exposure to obesogenic endocrine disrupting compounds can program the fetus toward an obese, ''endomorphic'' phenotype.

THE OBESOGEN HYPOTHESIS

In 2006, we put forth the ''obesogen hypothesis'' (Grun and Blumberg, 2006), which proposed the existence of endocrine disrupting chemicals (EDCs) that could influence adipogenesis and obesity and that these might be important, yet hitherto unsuspected players in the obesity epidemic. ''Obesogens'' are defined functionally as chemicals (natural, pharmaceutical, or xenobiotic) that promote obesity by increasing the number of fat cells or the storage of fat into existing fat cells. Obesogens can also act on adipocytes indirectly by changing the basal metabolic rate, by shifting energy balance to favor the storage of calories, and by altering hormonal control of appetite and satiety (reviewed in Grun and Blumberg, 2009a,b; Janesick and Blumberg, 2011, in press; Blumberg, in press). Although the obesogen hypothesis was initially controversial, many obesogenic chemicals have been identified in recent years, underscoring the relevance of this novel model. Estrogens such as diethylstilbesterol and genistein (Newbold et al., 2009), organotins such as tributyltin (Grun et al., 2006), perfluorooctanoates (Hines et al., 2009), and bisphenol A (Rubin et al., 2001) are known to be obesogenic in animals. A variety of chemicals have been shown to increase adipogenesis in preadipocyte cell lines such as murine 3T3-L1 cells and in primary multipotent mesenchymal stem cells (Lehmann et al., 1995; Grun et al., 2006; Feige et al., 2007; Yang et al., 2007; Saito et al., 2009; Kirchner et al., 2010; Park et al., 2010; Sargis et al., 2010; Styner et al., 2010; Zhang et al., 2010).

Recent human epidemiological studies have linked the presence of xenobiotic chemicals with increased body mass in humans. For example, the presence of mono-benzyl and mono-ethyl-hexyl phthalate metabolities in urine is associated with increased waist diameter in men (Stahlhut et al., 2007; Hatch et al., 2008). The presence of hexachlorobenzene in umbilical cord blood, independent of whether the mother is overweight, is associated with increased BMI (Smink et al., 2008). Diethylstilbesterol in cord blood was associated with increased BMI in young children, and this effect was exacerbated by maternal smoking (Verhulst et al., 2009). This result was also supported by evidence that higher serum concentration of DDE in mothers was linked to increased weight and BMI in adult female offspring (Karmaus et al., 2009).

In addition to these xenobiotic chemicals, several classes of pharmaceuticals are associated with increased body mass in humans. These include atypical anti-psychotic drugs such as olanzapine (Nemeroff, 1997), selective serotonin reuptake inhibitors (Fava, 2000), tricyclic antidepressants (Berken et al., 1984), and thiazolidinedione anti-diabetic drugs (Larsen et al., 2003; Rubenstrunk et al., 2007). Although, it should be noted that most clinical and epidemiological studies are necessarily only correlational, animal and cell culture models have informed these studies by providing mechanisms through which chemicals might act to create the obesity in humans. For example, thiazolidinediones and phthalates are known to act through the nuclear receptor peroxisome proliferator activated receptor gamma (PPARγ), the dominant regulator of adipogenesis (Evans et al., 2004; Tontonoz and Spiegelman, 2008). Although direct obesogenic effects of chemicals have been observed in animals and humans, the more interesting possibility is that EDC exposure can exert effects during the prenatal period that predispose humans to obesity later in life.

OBESOGENS AND THE DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

Current ideas concerning obesogen actions on the endocrine system and hypothalamic-pituitary-adrenal axis have been reviewed recently (Grun and Blumberg, 2009a,b) and will not be emphasized here. Instead, we will focus on the effects of EDCs (Diamanti-Kandarakis et al., 2009) known or suspected to alter the developmental programming of adipogenesis and obesity. EDC exposure can, in principle, predispose an exposed individual to obesity through a variety of mechanisms that act during the specification, differentiation, and maintenance of adipocytes. EDCs can alter gene expression both by modulating developmental signaling pathways directly and by promoting epigenetic changes that produce stably inherited changes in gene expression. Clinical, epidemiological, and experimental studies show that some of the origins of obesity can be traced back to the intrauterine period. The developing fetus responds to suboptimal conditions during sensitive developmental windows by producing structural and functional changes in cells, tissues and organ systems—the concept of fetal programming, or more appropriately, the developmental origins of health and disease (DOHaD) (Gluckman and Hanson, 2004). Epigenetic changes programmed during times of developmental plasticity in adipocyte development are heritable manifestations of environmental exposures whether they are nutritional, physiological, or chemical. Epigenetic changes resulting from EDC exposure could foster the type of swift change in the metabolic profile of a population that has been observed in the United States in recent years (Flegal et al., 2010). Moreover, once an individual becomes obese, it is difficult to lose weight and sustain

weight loss due to highly efficient homeostatic mechanisms regulating energy balance (Butte et al., 2007; Muhlhausler and Smith, 2009).

ORIGIN AND ESTABLISHMENT OF THE ADIPOCYTE COMPARTMENT

Adipogenesis is a differentiation event in the mesodermal lineage wherein multipotent stromal stem cells (MSCs, otherwise known as mesenchymal stem cells) or their more lineage-restricted derivatives give rise to adipocytes (reviewed in Rosen and MacDougald, 2006). Until recently, the identity of adipocyte progenitors has been elusive. Most investigations into adipogenesis used cell lines (e.g., 3T3-L1, 3T3-F442A), or primary cells that were already committed to the adipocyte fate and would not easily differentiate into any other lineage (like bone, cartilage, muscle, or even brown fat) (Cornelius et al., 1994; Timmons et al., 2007; Park et al., 2008; Seale et al., 2008). Therefore, the origins of the adipocyte compartment could only be studied from the point of lineage commitment toward the mature adipocyte. An important breakthrough occurred when MSCs were recharacterized as pericytes, cells that surround vasculature throughout the body (Crisan et al., 2008). Those who study these cells intently have demonstrated that while MSC-pericytes have enormous capacity for multipotency in vitro, they exhibit a much more limited tissue-specific capacity to differentiate in vivo, (Fischbach et al., 2004). MSC-pericytes are known to express different markers, depending on their tissue of origin (Rosenbaum et al., 2008); therefore, MSC-pericytes are probably not a single class of cells. This argument is supported by the observation that pericytes can have different developmental origins. For example, neural crest cells give rise to pericytes in the brain, whereas mesodermal cells give rise to pericytes in the adipose, muscle, and cartilage (da Silva Meirelles et al., 2008).

Tissue-specific, committed progenitors occupy this perivascular niche of any organ. Although it is well established that adipocytes are derived from MSC-pericytes, until recently there was very little known about the process and intermediates through which MSCs become committed to the adipocyte lineage. Friedman and coworkers purified candidate progenitor cells from the stromal-vascular fraction of white adipose tissue (WAT) and verified that they were bona fide adipocyte progenitors by showing that they could generate an entire adipose depot in lipodystrophic mice (Rodeheffer et al., 2008). In adipose tissue, this progenitor is a PPARγ-expressing cell (Tang et al., 2008) displaying the following suite of markers: lin$^-$, CD29$^+$, CD34$^+$, Sca1$^+$, and CD24$^+$ (Rodeheffer et al., 2008). In support of the vascular niche argument, these cells are also positive for pericyte markers (e.g., SMA, PDGFRβ and NG2) (Tang et al., 2008). Moreover, adipose tissue is highly vascularized, and anti-angiogenic agents reduce adipose mass (Rupnick et al., 2002; Kahn, 2008). Armed with new knowledge of the origins of the adipose compartment, we can now understand how the development of adipose (from MSC-pericyte to the mature adipocyte) can be dysregulated by EDCs.

ADIPOCYTE NUMBER IS REFRACTORY TO CHANGE ONCE ESTABLISHED

It was once generally believed that the root cause of obesity was a lack of will-power (Newburgh and Johnston, 1930) that was either due to individual personality defects or perhaps a disruption of the hypothalamus that interfered with satiety (Hetherington and Ranson, 1942). In May 2009, John Birkbeck, a professor at Massey University in New Zealand, re-energized this notion that obese individuals are wholly responsible for their condition. Noting that there were no ''fattys'' in Nazi concentration camps, he suggested that obesity is the singular result of a positive energy balance that any obese individual will lose weight under coercion and that governments are too merciful with the obese populus (Johns and Leask, 2009). This logic is flawed on many levels, but mostly because it conflates capacity with reality and assumes that obesity only results from disordered eating and inactivity. Indeed, as Birkbeck argues, obese individuals can lose an appreciable amount of weight given a forced, calorie restricted diet; however, the metabolism of obese people is fundamentally deranged compared with normal individuals.

Jules Hirsch and coworkers first characterized the ''reduced obese'' state (Glucksman and Hirsch, 1968; 1969; Glucksman et al., 1968) where a small group of obese patients were given strictly 600 kcal per day, for three months. On average, these individuals, achieved and maintained a $\sim$30% reduction in weight, but this was not a homeostatic state (Glucksman and Hirsch, 1968; 1969; Glucksman et al., 1968). They exhibited depression, lethargy, and a manic, obsessive craving for food, indicative of a starvation response. Conversely, a study involving healthy male prisoners in a Vermont penitentiary demonstrated that gaining weight through overconsumption of calories could not ''be undertaken as a secondary occupation'' (Sims and Horton, 1968). Although gaining weight was difficult, almost all participants were able to return to their former weight quite easily, after the period of overfeeding was over. These studies suggest that while the potential for weight loss (or gain) always exists, it is not necessarily the case that forcing obese patients to lose weight with a strict dietary regiment improves their quality of life. The implication is that obese and normal individuals respond differently to increased or decreased caloric intake and that forcing lean people to gain weight or obese people to drastically lose weight is leading people to struggle against their basic nature.

A key finding in Hirsch's studies was the observation that weight loss was primarily due to a reduction in fat cell size, not number. In

the reduced obese patients, the amount of triglycerides per cell was reduced about 25% whereas the number of adipocytes did not change (Stern et al., 1972). The ''reduced obese'' population's weight loss was hypotrophic, whereas the prisoner's weight gain was hypertrophic in nature. These results were confirmed by subsequent studies showing that weight loss after bariatric surgery or cancer-related cachexia reduces adipocyte size but not number (Spalding et al., 2008; Dahlman et al., 2010). Thus, adults are limited mostly to changes they can make in adipocyte volume, and it is this component of adipose mass that can be altered most easily by disciplined diet and exercise. In contrast, for the developing fetus, child, and adolescent, the number and distribution of adipocytes is not at all constant. Human babies have about 15% body fat (mostly white adipose) at birth (Kuzawa, 1998), which nearly doubles during early development (to about 28%), then declines during early childhood (Hager et al., 1977). From about 8 years of age until the end of puberty, adipocyte number increases as the result of hyperplastic expansion (Spalding et al., 2008). Obese children possess more fat cells, which multiply at a faster rate, than nonobese children (Knittle et al., 1979) and obesity, particularly early-onset obesity, is linked to hypercellularity of adipose tissue (Hirsch and Knittle, 1970; Knittle, 1972; Salans et al., 1973). Adolescent girls experiencing a relatively large increase in adipose cell number over a period of 1.5 years were the most resistant to treatment (e.g., restrictive diet and exercise regime) (Hager et al., 1978).

Hence, it is likely that obese individuals either acquire more adipocytes before reaching adulthood, which may be causal for their obesity, or create more adipocytes and associated obesity as adults through poor diet and exercise. Although diet may be the driving force for adipose hypertrophy, it is unlikely to cause the hypercellularity observed in obesity under all but the most extraordinary levels of dietary excess. Adipocytes are capable of reaching a certain triglyceride limit (e.g., in the case of a high fat diet) and spawning the generation of more fat cells through paracrine signaling, but this only occurs when adipocyte capacity for lipid storage is completely exhausted (Shillabeer et al., 1989; Lau et al., 1990; Marques et al., 1998). In general, once early adulthood is reached, the total number of adipocytes becomes largely stable in both males and females (Spalding et al., 2008). Using the same [^{14}C]-labeling method used to prove that adults have the capacity to produce new brain cells, it was found that adipocytes are continually undergoing apoptosis and being replenished (Spalding et al., 2008). The generation of new adipocytes was greater for the obese population, because they were replenishing an already-established, larger pool of cells (Spalding et al., 2008). When comparing the turnover rate of adipocytes between normal individuals and those with early onset obesity, no significant difference was observed (Spalding et al., 2008). Taken together, increased adipocyte number is the primary feature shared among obese individuals, particularly those who developed the disease early or during adolescence, compared with those of normal weight. This suggests that obese individuals possess a pool of MSCs that is intrinsically biased toward the fat cell lineage. More fat cells need to be generated from precursors to maintain a steady state level of adipocytes that is higher than in nonobese people. We argue (below) that such a bias in the stem cell compartment could be regulated by epigenetic changes due to exposure to environmental cues experienced during critical developmental windows.

DO ADIPOCYTES CRAVE TO BE FILLED?

The observation that obese individuals have more adipocytes leads naturally to the hypothesis that obesity is a consequence of more adipocytes. Although it is unclear at present whether such a causal connection exists between adipocyte number and obesity, it is known that mice with hyperplastic obesity become morbidly obese (Kim et al., 2007), and that humans whose diabetes is being treated with rosiglitazone (a drug that activates PPARγ) develop more adipocytes and gain weight (Shim et al., 2006). Obese subjects (in particular, those with early onset obesity (Salans et al., 1973)), are also predisposed to have more adipocytes. Although the evidence is incomplete, there appears to be a critical minimum of triglycerides that adipocytes are programmed (or ''want'') to maintain. In normal weight individuals, there is an inverse relationship between the number of fat cells within an adipose depot and the size of each cell (Arner et al., 2010). Thus, a reduction in cell volume can only be carried out to a point. If an obese individual possesses both adipose hypertrophy and hyperplasia, he can comfortably consume fewer calories to reduce cell size somewhat; however, to achieve further permanent weight reduction, the adipocyte population must be reduced. Since adipocyte number is refractory to change (as stated above) and adipocytes are resistant to apoptosis (Sorisky et al., 2000), the obese individual can reach a point of physiological starvation while still being fat. The sensation of starvation results when smaller fat cells secrete less leptin (Van Harmelen et al., 1998), a hormone that plays a role in informing the brain about how much fat is stored, which in turn suppresses food intake and upregulates the sympathetic nervous system.

These data all converge on the conclusion that a hypercellular adipose mass, in essence, ''craves'' to be filled with a minimum amount of lipid, per cell. This argues in favor of therapeutic strategies that aim to alleviate adipose hyperplasia. It has been suggested that altering the ''birth–death balance'' of adipose tissue

might be an effective treatment for obesity (Spalding et al., 2008; Arner et al., 2010). Of course, encouraging the death of adipocytes or increasing the triglyceride load on existing adipocytes might counterproductively generate an inflammatory response (Gustafson et al., 2009; Alkhouri et al., 2010). Nevertheless, an obese individual who has successfully reduced adipocyte volume through diet and exercise but reaches a barrier due to his fat-craving, hypercellular adipose mass, might benefit from therapeutic strategies that target the mechanisms regulating adipocyte turnover (Arner, 2010). Alternatively, from a preventative standpoint, one might attempt to understand early developmental events that created the hypercellular adipose mass. For example, factors, which encourage the commitment of MSCs to the adipocyte lineage or promote differentiation of progenitors into mature adipocytes, could create such a hyperplastic adipose mass that an individual might be stuck with for life. We discuss (below) how EDCs have the potential to disrupt both the commitment and differentiation phase of adipogenesis to create hyperplastic obesity.

ENDOCRINE DISRUPTION DURING THE COMMITMENT PHASE OF ADIPOGENESIS

Mature adipocytes are generated from multipotent stromal cells (MSCs) found in almost all fetal and adult tissues (da Silva Meirelles et al., 2006). What remains largely unknown is the transcriptional program that turns MSCs into preadipocytes, a subset of stem cells that retains some degree of multipotency but which typically gives rise to adipocytes, in vivo. One important player in the process of lineage commitment is bone morphogenic protein (BMP) signaling. BMP signaling is well known for its importance in skeletal development and BMP4 was also shown to divert the fibroblastic cell line C3H10T1/2 from an MSC-like state into preadipocytes (Tang et al., 2004). This suggests that BMP signaling might be important for commitment to the preadipocyte lineage. In support of this idea, it was recently shown that the zinc finger transcription factor, Zfp423, a downstream target of BMP signaling, was upregulated in preadipocyte cell lines with high adipogenic potential compared with those (e.g., NIH 3T3) that had a low adipogenic potential (Gupta et al., 2010). Interestingly, Zfp423 loss-of-function severely impaired but did not eliminate adipogenesis in mouse embryos, which suggests that Zfp423 is not absolutely indispensable (Gupta et al., 2010). In addition, although Zfp423 expression was noted to increase in preadipocytes compared with precursor cells, its expression remained unchanged during the preadipocyte to adipocyte transition. This further suggests that Zfp423 may be involved in the commitment of MSCs to the adipocyte lineage but might not be required for the maintenance of the preadipocyte phenotype (Gupta et al., 2010).

Another key developmental pathway involved in adipogenesis is the Wnt signaling pathway. It was shown some time ago that preadipocytes do not differentiate into adipocytes in the presence of noncanonical Wnt signaling (Ross et al., 2000). The reciprocal relationship between adipocyte and osteocyte commitment and differentiation is well documented and is thought to involve a shift in the flow of MSCs from osteoblastic to adipogenic lineages (Shockley et al., 2007). The balance between the osteogenic and adipogenic lineages is thought to be mediated by the presence of PPARγ, and it was shown that Wnt-5a expression inhibited the expression of PPARγ, thereby diverting MSCs toward the osteogenic lineage (reviewed in Takada et al., 2009). Thus, repression of noncanonical Wnt-5a signaling together with active BMP signaling is required for MSCs to proceed toward the adipogenic and away from the osteogenic lineage (Bilkovski et al., 2010).

The confluence of several signaling pathways to allocate stem cells between adipogenic and osteogenic fates provides multiple possibilities for disruption. However, there are only very few studies testing how EDCs might influence MSC fate. The pesticides chlorpyrifos and carbofuran inhibited the ability of MSCs to differentiate into bone (Hoogduijn et al., 2006). Although one might expect that a decrease in the allocation of MSCs to the bone lineage would result in an increase in adipogenic progenitors, this possibility was not tested. We found that prenatal treatment with the environmental obesogen, tributyltin, or the pharmaceutical obesogen, rosiglitazone, altered lineage allocation in MSCs. Pregnant dams were treated with a single dose of tributyltin or rosiglitazone and MSCs harvested from WAT at 8 weeks of age. WAT-derived MSCs were enriched in preadipocytes and also in cells predisposed toward the adipocyte lineage. MSCs differentiated into adipocytes about twice as frequently in culture and the frequency was further increased by in vitro treatment with tributyltin or rosiglitazone (Kirchner et al., 2010). The ability of these cells to differentiate into bone was correspondingly inhibited (Kirchner et al., 2010). MSCs harvested from the tributyltin or rosiglitazone-exposed pups were preprogrammed to prefer the adipogenic fate. Remarkably, MSCs from pups exposed prenatally to TBT or rosiglitazone and then treated with a bone differentiating cocktail instead differentiated into adipocytes at high frequency (Kirchner et al., 2010).

This observation is closely correlated with clinical findings. Indeed, osteoporosis has been called ''obesity of bone'' to reflect the observation that bone marrow from osteoporotic women is often filled with adipocytes (Verma et al., 2002; Rosen and Bouxsein, 2006). Moreover, MSCs cultured from postmenopausal women with osteoporosis or low bone density accumulate twofold more lipid, and twofold less type I collagen (part of the bone extracellular matrix) compared with

women with healthy bones (Rodriguez et al., 2000). Considering these observations, it is perhaps not surprising that women taking thiazolidinedione anti-diabetes medications (such as rosiglitazone) that activate PPARγ are at increased risk for bone fractures (Habib et al., 2010). These results lead to the prediction that obesogens that act through PPARγ, such as tributyltin, will have a similar effect on osteoporosis.

As noted above, much of the most recent evidence supports the model that adipocytes are regenerated from an existing population of MSCs that reside in the vasculature of adipose tissues (Rodeheffer et al., 2008; Tang et al., 2008). As MSCs are known to be mobile, it is possible that they circulate beyond adipose depots and/or can be recruited to WAT from other niches such as the bone marrow. The majority of studies on MSC migration focus on their ability to escape from bone marrow, migrate along blood vessels toward an injury site following chemotactic signals then moving through the extracellular matrix to flood an area of injury (Orlic et al., 2001; Hofstetter et al., 2002; Liu et al., 2009). It is not unreasonable to hypothesize that a similar mechanism might lead MSCs to migrate to adipose depots from bone marrow on receipt of an appropriate signal (perhaps adipokines). A recent study tested this hypothesis by transplanting green fluorescent protein labeled MSCs into irradiated wild-type mice and asking whether green fluorescing cells could be found in adipose depots (Crossno et al., 2006). It was found that rosiglitazone or a high fat diet increased migration of these MSCs to omental or dorsal intrascapular fat depots (Crossno et al., 2006). However, their interpretation was challenged by a subsequent study that claimed the bone marrow derived cells did not differentiate into adipocytes, because the green fluorescing, lipid-containing cells were not unilocular as is typically the case for adipocytes (Koh et al., 2007). Further research will be required to establish the fate and nature of the transplanted cells and the potential effects of EDC exposure on this process. It is also currently controversial whether MSCs derived from tissues outside the adipose vasculature can differentiate into adipocytes, in vivo. Some researchers believe that adipose progenitors are exclusively localized in the bone marrow and adipose vasculature (Rodeheffer et al., 2008; Tang et al., 2008), whereas others favor a model wherein MSCs from many tissues can give rise to adipocytes in situ under appropriate stimulation (Crisan et al., 2008). If the latter possibility is true, whether or not MSCs can migrate from their point of origin to generate adipocytes in remote parts of the body may be a completely moot point. Overall, it remains to be demonstrated where adipogenic MSCs are located in the body, whether or not they migrate to points beyond their origin in response to dietary or other stimuli and to what extent obesogen exposure influences these processes.

ENDOCRINE DISRUPTION DURING THE DIFFERENTIATION PHASE OF ADIPOGENESIS

In contrast to the relative paucity of data regarding the commitment of MSCs to become preadipocytes, there is much known about the process of adipocyte differentiation (reviewed in Rosen and MacDougald, 2006; Tontonoz and Spiegelman, 2008). The central regulator in this process is the peroxisome proliferator activated receptor gamma (PPARγ), which associates with the retinoid X receptor (RXR) and binds DNA targets as a heterodimer to directly regulate the expression of its target genes at the transcriptional level (Tontonoz et al., 1994). PPARγ is considered to be the master regulator of adipogenesis (reviewed in Evans et al., 2004) and plays key roles in nearly all aspects of adipocyte biology (reviewed in Tontonoz and Spiegelman, 2008). Figure 2 summarizes the important events in adipocyte differentiation, focusing on their origin from multipotent precursors, how they commit to the adipocyte lineage instead of the other potential pathways downstream of MSCs and how EDCs might affect the generation, function, or apoptosis of adipocytes.

PPARγ sits at the center of a complex web of interacting signaling pathways that regulate its expression (see Fig. 3 in Tontonoz and Spiegelman, 2008). In 3T3-L1 cells, PPARγ is first induced at the transcriptional level by CCAAT/enhancer binding protein (C/EBP) β and δ (Wu et al., 1996; Shao and Lazar, 1997) and then engages in a feed-forward loop with C/EBPα, amplifying the adipogenic signal (Rosen et al., 1999). To moderate the effects of this feed-forward loop, C/EBPα also induces Sirt-1 (Jin et al., 2010), which curbs adipogenesis via inhibition of PPARγ target genes (Picard et al., 2004). Studies of adipogenesis in cell culture generally require that MSCs, or preadipocytes be treated with a sensitizing cocktail that stimulates the differentiation process (Student et al., 1980), then with a PPARγ ligand that strongly promotes the differentiation process (Tontonoz et al., 1994). Such cocktails often contain differentiation agents such as insulin, glucocorticoids, methylisobutylxanthine, and indomethacin, which act through the PI3K/AKT, glucocorticoid receptor, cAMP protein kinase, and PPARγ signaling pathways, respectively. Generally speaking, the function of the induction cocktail is to increase the expression levels of C/EBPα or PPARγ to levels where adipogenesis is favored, rather than in the direct ligand activation of PPARγ. The insulin-induced transcription factor, sterol regulatory element binding protein 1c (SREBP1c), can also lead to the synthesis of fatty acids that can bind PPARγ, and it is known that high levels of insulin are sufficient to fully stimulate adipogenesis (Kim et al., 1998).

It was recently proposed that PPARγ may function in adipogenesis without requiring the ability to be activated by ligand (Walkey and Spiegelman, 2008). When the

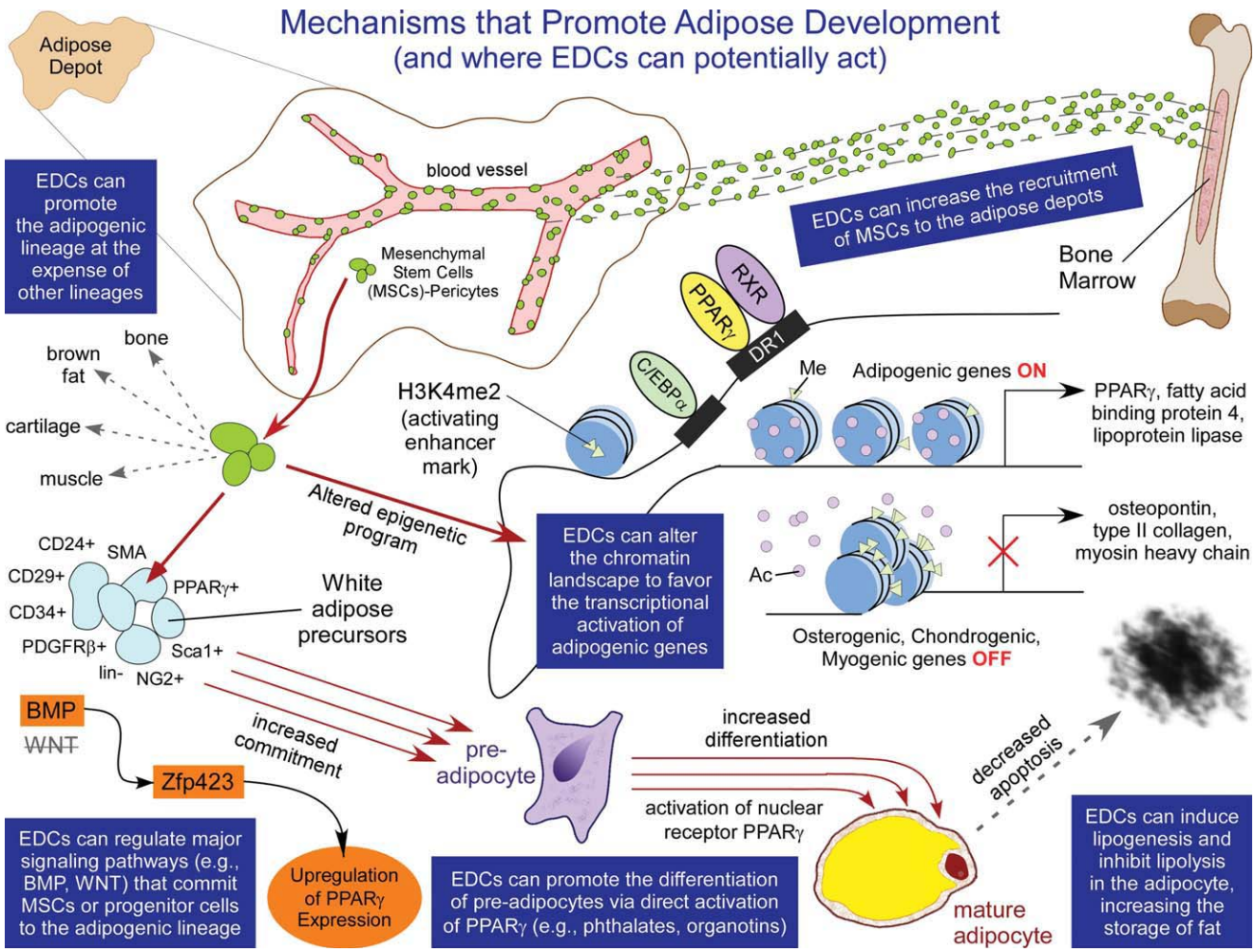

Figure 2. Diagram of the potential interactions of endocrine disrupting chemicals during various stages of adipose tissue development.

ligand binding domain of PPARγ was mutated such that the receptor was unresponsive to known agonists, the ability of preadipocytes to differentiate into adipocytes in cell culture was unaffected (Walkey and Spiegelman, 2008). In contrast, deletion of the activation function 2 region of the PPARγ ligand-binding domain rendered the receptor unable to support adipogenesis (Walkey and Spiegelman, 2008). The most reasonable interpretation of these data is that either PPARγ can act as an unliganded transcription factor to mediate adipogenesis, or that an as yet unknown endogenous ligand is being produced in response to the induction cocktail.

Several pharmaceutical compounds and xenobiotic EDCs are known to affect PPARγ activity and induce adipogenesis. The thiazolidinedione class of anti-diabetic agents including rosiglitazone and pioglitazone are well-known synthetic PPARγ agonists (Lehmann et al., 1995). These drugs not only directly increase insulin sensitivity thereby ameliorating diabetes but also encourage new fat cell growth. This has the benefit of relieving inflammatory stress associated with hypertrophic adipocytes but has the disadvantage of promoting hyperplastic obesity, which increases insulin sensitivity. Several classes of environmental EDC are agonistic PPARγ ligands. Notable among these are organotins such as tributyltin and triphenyltin (Kanayama et al., 2005; Grun et al., 2006; Hiromori et al., 2009) and certain phthalates (Hurst and Waxman, 2003; Bility et al., 2004; Feige et al., 2007). Perfluoroalkyl acids either activate PPARγ weakly (Vanden Heuvel et al., 2006) or not at all (Takacs and Abbott, 2007), despite activating PPARα or PPARδ. Flavanone (Saito et al., 2009), bixin (Takahashi et al., 2009), and emodin (Yang et al., 2007) activate PPARγ and induce adipogenesis in cell culture models. Phthalates and triorganotins also have the ability to induce adipocyte differentiation in a variety of cell culture models (Hurst and Waxman, 2003; Inadera and Shimomura, 2005; Kanayama et al., 2005; Grun et al., 2006). Importantly, tributyltin can induce adipogenesis, in vivo. Mice treated prenatally with tributyltin

are born with more fat stored than controls and *Xenopus laevis* tadpoles exposed to tributyltin exhibited replacement of testicular tissue with adipocytes (Grun et al., 2006). It was inferred that tributyltin is likely to be promoting the development of new adipocytes at the expense of other cell types.

Other EDCs are known to promote adipogenesis but probably do not act through PPARγ. These include bisphenol A (Rubin and Soto, 2009), nicotine (Somm et al., 2008), organophosphate pesticides (Slotkin, 2010), monosodium glutamate (Matsuyama et al., 1973; Bunyan et al., 1976), and polybrominated diphenyl ethers (PBDEs) (Hoppe and Carey, 2007). Coplanar polychlorinated biphenyls (PCBs; e.g., PCB-77) bind the aryl hydrocarbon receptor in adipocytes and increase adipogenesis (Arsenescu et al., 2008). Bisphenol A and alkylphenols stimulate adipogenesis in 3T3-L1 cells (Masuno et al., 2005), and bisphenol A diglycidyl ether was recently shown to induce adipogenesis in human and mouse bone marrow-derived MSCs (Kirchner et al., 2011).

Hence, EDCs can influence adipocyte differentiation; however, the mechanisms are not always clear. Because PPARγ is such an important regulator of adipogenesis, it is common to associate this receptor with the obesogenic phenomenon observed. It should be noted, though, that numerous other nuclear receptors and their cofactors are important during adipogenesis (Feige et al., 2007; Fu et al., 2005; Rosen and MacDougald, 2006). These include positive regulators of adipogenesis such as the glucocorticoid receptor (GR) (Sargis et al., 2010), the liver ''X'' receptor (LXR) (Seo et al., 2004), COUP-TFII (Li et al., 2009), estrogen-related receptors alpha and gamma (ERRα, ERRγ) (Kubo et al., 2009; Morganstein et al., 2010), Rev-ERB (Kumar et al., 2010; Wang and Lazar, 2008), and the NR4A family (Zhao and Bruemmer, 2010). Negative regulators of adipogenesis include the thyroid hormone receptor (TR) (Lu and Cheng, 2010), RORα (Ohoka et al., 2009), Vitamin D (VDR) (Wood, 2008), DAX-1 (Kim and Surh, 2008), and the nuclear receptor corepressor SMRT (Sutanto et al., 2010). Furthermore, the retinoid X receptor, RXR, is also upregulated during adipogenesis and is activated by the obesogens tributyltin and triphenyltin (Grun et al., 2006; le Maire et al., 2009). RXR is an obligate heterodimeric partner for PPARγ, as well as some of the receptors noted above (TR, LXR, PPAR, VDR). RXR itself can be activated in a subset of these heterodimers. In principle, any of these receptor-mediated pathways could be targeted by EDCs and this area is ripe for future studies.

ENDOCRINE DISRUPTOR ACTION THAT ALTERS METABOLISM OR LIPID HOMEOSTASIS

Although several EDCs are associated with adipogenesis and obesity in animal models, tributyltin is the only EDC known to cause in utero effects on adipocytes via activation of PPARγ (Kirchner et al., 2010; reviewed in Janesick and Blumberg, in press). Prenatal exposure to tributyltin in mice led to a substantial increase in the amount of triglycerides in newborn tissues that normally have little to no fat at all (Grun et al., 2006); although, the experiments did not distinguish whether more lipid was stored in existing cells, more cells were produced, or both. Other EDCs are likely to promote adipogenesis, in utero, although it is possible that this is secondary to broader metabolic imbalances. For example, certain PCBs and PBDEs reduce thyroid function (Hallgren et al., 2001) as does the antibacterial compound triclosan (Paul et al., 2010; Rodriguez and Sanchez, 2010). The mechanisms of action are not completely certain, but possible modes include interference with thyroid hormone synthesis, transport, metabolism, or clearance (Diamanti-Kandarakis et al., 2009). High levels of maternal PCBs and PBDEs are correlated with reduced total and free T_4 levels in infant cord blood (Herbstman et al., 2008) and thyroid hormone stimulates lipolysis in adipocytes (Van Inwegen et al., 1975; Smith et al., 1991). Thyroid hormone also inhibits lipogenesis by downregulating expression of SREBP1c (Viguerie et al., 2002). Taken together, these results lead to the inference that exposure to EDCs such as triclosan, PCBs, and PBDEs can reduce circulating thyroid hormone levels leading to a consequent increase in adipocyte lipid accumulation.

Estrogens are another class of EDCs that can globally affect energy metabolism (reviewed in Chen et al., 2009). Estrogens are antiobesogenic in adults; they protect against adiposity by promoting exergonic, energy-consuming, reactions in glycolysis, fatty acid oxidation, and electron transport (reviewed in Chen et al., 2009). However, perinatal exposure to low doses of estrogens such as diethylstilbestrol (DES) or genistein leads to obesity in mice (Newbold et al., 2009). Perfluoroaklyl sulfonate exposure in mice led to increased body weight in offspring, along with higher serum insulin and leptin levels (Hines et al., 2009). There is evidence both in support of (Sakurai et al., 2004; Hugo et al., 2008; Ben-Jonathan et al., 2009; Rubin and Soto, 2009) and against (Ryan et al., 2010) bisphenol A as an obesogen. Nicotine, which promotes weight loss in adults, increases adipose hypertrophy in young rats prenatally exposed to nicotine via osmotic pump (Somm et al., 2008). This has a parallel in humans where epidemiological studies of maternal smoking show that the adjusted odds ratio for obesity is between 1.5–2.0 fold greater if children were exposed during, but not prior or after, the pregnancy (Power and Jefferis, 2002; Oken et al., 2005; Al Mamun et al., 2006).

Taken together, the studies presented above support the contention that obesogens can act on adipocyte commitment, adipocyte differentiation, by altering metabolic setpoints, modulating lipid homeostasis, and by other mecha-

nisms not yet characterized. We will next consider the possible mechanisms through which obesogen action during critical windows of development might permanently alter the phenotype of exposed individuals.

EPIGENETIC MODIFICATIONS DURING EARLY LIFE

One seemingly perplexing aspect of the burgeoning obesity epidemic is the very rapid increase in the rates of obesity, particularly in the U.S. As recently as the 1980s, ~15% of the population was obese compared with 34% in 2008 (Flegal et al., 2010). Although genetic variability is sufficient to explain why some people may have the propensity to become obese, it is inconceivable that the rapid increase in the rate of obesity in the U.S. has any genetic basis. In contrast, epigenetic phenomena can occur very rapidly and could easily become established within a population within a single generation (Gluckman et al., 2005). Therefore, epigenetic changes mediated by dietary and environmental factors, rather than genetic changes, are a more plausible explanation for the ''epidemic'' of obesity in Western countries. Moreover, epigenetics can also explain the types of rapid metabolic adaptations described in the DOHaD studies (Barker, 1994; Hales and Barker, 2001; Gluckman and Hanson, 2004). It is likely that epigenetic changes also underlie adaptations made during other critical developmental time windows, like adolescence, because the epigenome changes substantially during life, even in monozygotic twins (Fraga et al., 2005).

At the chromatin level, EDC exposure can alter the expression of proteins required for DNA accessibility or structure. Changing the expression levels of DNA methyltransferases, histone acetyltransferases, histone deacetylases, and histone methyltransferases will have significant and sustained effects on gene expression. For example, prenatal exposure to DES led to a significant increase in the mRNA expression of DNA methyltransferase 1 (Dnmt1) and DNA methyltransferase 3b (Dnmt3b). In turn, this led to hypermethylation of the homeobox gene HOXA10 and region-specific alterations in its expression levels in the uteri of exposed animals (Bromer et al., 2009). Exposure to the commonly used fungicide vinclozolin caused a decrease in the expression of Dnmt1, Dnmt3a, Dnmt3L, and euchromatic histone methyltransferase (Ehmt1) in the testes of male rats, and these changes were shown to persist for at least three subsequent generations without further exposure (Anway et al., 2008). What remains an important question is how EDCs can lead to epigenetic alterations on particular metabolic pathways to the exclusion of others. There are relatively few DNA and histone methyl transferases, compounding the problem even further. One possibility is that DNA methyl transferases such as Dnmt1 and Dnmt3 are escorted by specific transcription factors to the target DNA sequences that they, themselves recognize. In this case, target gene selectivity resides in the interaction between transcription factor and the chromatin remodeling factor and the specificity of the EDC for a particular pathway would be conferred by the transcriptional programs in individual cells (Robertson et al., 2000; Burgers et al., 2002).

Epigenetic changes related to adipogenesis and obesity originate within the stem cell compartment. The observation that diet and exercise primarily alter adipocyte volume rather than adipocyte number in adults probably results from the developmentally programmed bias in the stem cell population that causes the adipocyte pool to be replenished to its ''set point.'' Understanding how adipocyte number is programmed at the genomic level will be of critical importance in understanding this set point phenomenon, and how it is modified by EDCs, dietary factors, or the intrauterine environment. At least one example of EDC-induced changes in MSC fate has already been identified. MSCs from mice exposed to tributyltin in utero exhibited alterations in the methylation status of the CpG islands of adipogenic genes such as AP2 and PPARγ. This led to an increase in the number of preadipocytes at birth and an increased propensity of MSCs to differentiate into adipocytes on adipogenic stimulation (Kirchner et al., 2010). It is likely that other such examples will be identified in the future.

The observation that EDCs, such as tributyltin, can modify DNA methylation to alter the expression of adipogenic genes in MSCs (Kirchner et al., 2010) is only the beginning of possible EDC-derived epigenetic modifications within the stem cell compartment of adipose tissue. Recent studies using stem cells, including MSCs have revealed the influence of histone methylation on lineage programming. MSCs, such as embryonic stem cells (Azuara et al., 2006; Bernstein et al., 2006), naïve T cells (Wei et al., 2009), and neural progenitors (Mikkelsen et al., 2007), exhibit bivalent chromatin marks on histone H3 proteins associated with promoters of lineage specific genes (Noer et al., 2009). That is, both activating marks such as tri-methylation of H3 at lysine 4 (H3K4me3) and repressive marks, such as H3K27me3, are present in the same histone molecules (Azuara et al., 2006; Bernstein et al., 2006; Roh et al., 2006). These opposing histone modifications are thought to ''prime'' genes such that they can be quiescent but ready to be quickly activated when differentiation is induced simply by demethylating H3K27 (Bernstein et al., 2006). A similar phenomenon has been identified in the PPARγ promoter in adipose-derived MSCs. Both marks are present in the MSCs, but when differentiation is stimulated, H3K27 is demethylated completely, leaving only the activating mark on the PPARγ promoter (Noer et al., 2009). It would be readily possible for obesogen exposure to alter early program-

ming events when these lineage-specific histone modifications are established. In addition, inappropriate temporal or spatial stimulation of the demethylases that remove the H3K27me3 marks, JMJD3 and UTX (Lan et al., 2007), could lead to inappropriate activation of PPARγ and the adipogenic program.

An intriguing situation occurs with genes such as PPARγ, which are important for multiple differentiation processes. For example, PPARγ is expressed in monoblasts (Greene et al., 2000) and promotes macrophage differentiation (Tontonoz et al., 1998), but PPARγ is also expressed in preadipocytes where it is required for the adipogenic pathway (Tontonoz et al., 1994). Adipocytes and macrophages diverge from a common progenitor early in development, but both share a requirement for PPARγ expression. In order for PPARγ to promote a cell-specific transcriptional program, an additional layer of regulation is required: differential recruitment of PPARγ to enhancer elements. Similar to the estrogen receptor, PPARγ has an affinity for gene regulatory regions in introns and distal to the promoter (Nielsen et al., 2008). The PPARγ/RXR heterodimer binds to direct repeats separated by one nucleotide (DR1) with PPARγ binding 5′ to RXR (Jpenberg et al., 1997; Chandra et al., 2008). Enhancer-specific histone methylation increases the probability that a potential DR1 element is an actual, functional PPARγ response element (Heintzman et al., 2009). Methylation patterns on active enhancers are distinct from the promoter marks discussed above. Histone H3 in active enhancers displays monomethylated and dimethylated histone, at lysine 4 (H3K4me1, H3K4me2) (Heintzman and Ren, 2009; Heintzman et al., 2007). Macrophages have activating H3K4me1 marks associated with the enhancers of cytokine and immunity genes. These are closely linked to binding of PPARγ and the ets-factor PU1 (Heinz et al., 2010; Lefterova et al., 2010). In contrast, the same enhancers are repressed in adipocytes, whereas those of highly induced genes are associated with adjacent PPARγ and C/EBPα binding (Siersbaek et al., 2010). An important, open question is whether the presence of these histone marks increases the likelihood of PPARγ binding to the DR1 consensus or if PPARγ itself directly recruits methyltransferases to the DNA. In the latter instance, obesogens such as tributyltin could directly act to alter the chromatin leading to preferential recruitment of PPARγ to the enhancers of its target genes in adipocyte progenitors.

ENDOCRINE DISRUPTION ACROSS MULTIPLE GENERATIONS

A profound and provocative possibility is that epigenetic changes caused by environmental exposures may be transmitted across generations. Although the data are currently scant, there are some indications that this might be the case. Vinclozolin exposure in the parental generation is linked to infertility, behavior, and mate preference for at least four subsequent generations (Anway et al., 2005). Many, but not all of the observed alterations in chromatin remodeling were stably inherited in subsequent generations (at least through F4) despite that vinclozolin exposure only occurred in the parental (F0) animals (Anway et al., 2008). This ''memory'' of an ancestral exposure is thought to be epigenetically maintained within the male germ cells (Anway and Skinner, 2006).

Epidemiological studies using a cohort of Swedish farmers from the Őverkalix region of Sweden demonstrated that food availability during the prepubescent period affected the longevity and mortality (from cardiovascular disease) of one's grandchildren. Remarkably, a single winter of overeating could lead to a 6-year decrease in longevity of a boy's grandsons, but not granddaughters, or a girl's granddaughters but not grandsons (Kaati et al., 2007). During the prepubescent period, the testes or ovaries are developing, and the primordial germ cells incorporate sex-specific imprinting patterns, making this time exquisitely sensitive to epigenetic changes that can be transmitted down the generations (Hajkova et al., 2002).

Although the Őverkalix example was a nutritional study, it serves to illustrate the possibility of environmental effects being epigenetically transmitted to one's descendents in humans. We propose that it is no less likely that exposure to EDCs during sensitive developmental windows could lead to similar transgenerational effects. There are currently no data regarding potential transgenerational effects of obesogens; however, it is reasonable to propose that obesogenic compounds that can directly alter gene expression (e.g., tributyltin, BPA, or phthalates) could also influence the propensity to be obese for some generations after the initial exposure. Whether or not the effects could be truly transgenerational (i.e., persist in F3 and beyond) compared with multigenerational (persist to F2) would likely depend on whether exposure occurred during a critical developmental window (Jirtle and Skinner, 2007).

CONCLUSIONS AND AREAS OF FUTURE RESEARCH

For a long time, obesity research has focused on what we call the ''central dogma'' of obesity—that all humans possess sufficient ''free will'' to manipulate their fat-laden somatotypes relatively simply via proper nutrition and physical activity. Much has been written about the behavioral origins of obesity and the fact that we live in a habitat that promotes a positive energy balance (Hill and Peters, 1998). Various governmental measures have been undertaken to improve this environment where fructose is more convenient than carrots, and sedentary choices champion over activities that require exercise. San Francisco banned toys from Happy Meals unless they were adjusted to meet nutritional guidelines (Martinez,

2010). Vermont Attorney General, Bill Sorrell, proposed legislature to tax soda pop (Kinzel, 2010). Yet, tap into any voice in the ''fatosphere'' (Rabin, 2008) and one quickly realizes that obesity may not solely be a matter of personal responsibility and discipline, or even the dearth of healthy food options and exercise opportunities. Many obese individuals passionately believe that an underlying, driving force keeps them fat. This phenomenon is commonly explained by genetics (Walley et al., 2009), an obesogenic environment (Gorin and Crane, 2009), and the unremitting biochemical and neurobiological forces that maintain the body in an obese state (Lustig, 2006; Friedman, 2009; Rosenbaum and Leibel, 2010).

A newly recognized contributing factor to the seemingly intractable problem of obesity is exposure to EDCs, during the prenatal period or early life. Although EDCs are hypothesized to interfere with broad metabolic processes to encourage adipogenesis, fat storage, and feeding (Grun and Blumberg, 2009a; b), the strongest evidence for obesogen action is at the level of stem cell programming. Prenatal exposure to tributyltin alters the adipose vascular network of MSC-pericytes, pushing them down the adipocyte lineage (Kirchner et al., 2010). We showed that obesogens encourage MSC-pericytes to give rise to a higher proportion of committed WAT precursors at the expense of bone precursors, and that tributyltin exposure gives rise to larger adipose depots than those of unexposed animals (Grun et al., 2006). Whether these changes in adipogenesis create an adipose mass that defies reduction in size remains to be determined. However, there are data to suggest that adipocytes ''crave'' to be filled (although this point remains controversial), and we propose that having a larger adipocyte progenitor population will result in a larger steady state adipocyte population that may interfere with the success of subsequent weight loss attempts.

An alarming recent trend is the increasing rate of obesity in very young children, even infants (Taveras et al., 2009; Koebnick et al., 2010; McCormick et al., 2010). Unless one wants to argue that the typical infant is now consuming far more calories than in the past and refraining from exercise that previous generations embraced, the most reasonable conclusion is that the infant was born with more fat, and/or that something about the early postnatal environment is vastly different than in the past. Remarkably, a recent study showed that animals (pets, cats and dogs; laboratory animals, rats, mice, and four species of primates; and feral rats) living in proximity to humans in industrialized societies exhibited pronounced increases in obesity over the past several decades (Klimentidis et al., 2010). Notably, these populations included laboratory animals living in strictly controlled environments, as well as feral animals living in cities (Klimentidis et al., 2010). The likelihood of 24 animal populations from eight different species all showing a positive trend in weight over the past few decades by chance was estimated at 1.2×10^{-7} (Klimentidis et al., 2010).

These increases in weight over time in humans, and in animal populations associated with humans, argue for alternative explanations than simply diet and exercise; obesogens have a role to play here. What remains unknown is the extent to which obesogens influence obesity in humans compared with other recognized factors such as the timing, amount and nature of calories consumed, physical activity, other lifestyle factors such as stress, amount of sleep, virus exposure, gut microbes, and genetic factors such as single polynucleotide polymorphisms in a variety of genes. The obesogen hypothesis fits well with the DOHaD model to provide molecular explanations for how obesity might begin in the womb. Epigenetics, a strong component of DOHaD, is predicted to drive early programming events in the MSC-pericyte compartment, where cells receive cues from their local environment that limit potential for future differentiation. Since critical events in the development of the adipocyte compartment occur early in life, this is when obesogenic chemicals likely act to alter epigenetic programming events that predispose a stem or progenitor cell toward a particular lineage. Evidence to support an epigenetic basis for obesogen action is only now emerging (Kirchner et al., 2010) as is evidence supporting epigenetic effects of EDC exposure on fertility, behavior, stress and other endpoints (Jirtle and Skinner, 2007; Skinner and Guerrero-Bosagna, 2009). The field of adipose development, beginning at the stem cell stage, is still in its infancy, and future research should endeavor to understand whether and how this process can be misregulated by EDCs in obesity.

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