Obesity and the reproductive system disorders: epigenetics as a potential bridge

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Background: Obesity and overweight are significantly involved in several reproductive pathologies contributing to infertility in men and women. In addition, several cancers of the reproductive system, such as endometrial, ovarian, breast, testicular and prostate cancers, are strongly influenced by obesity. However, the molecular mechanisms involved in the association between obesity and reproductive disorders remain unclear. Our proposal is to review the current scientific evidence regarding the effect of obesity-related factors as the core of the collective mechanisms directly and indirectly involved in the relationship between obesity and reproductive disorders, with a special and original focus on the effect of the obesity state microenvironment on the epigenetic profile as a reversible mechanistic link between obesity and the reproductive disorders.

Methods: A PubMed search was performed using keywords related to obesity and adipose-related factors and epigenetics and associated with keywords related to reproduction. Full-text articles and abstracts in the English language published prior to 31 December 2013 were reviewed.

Results: The obesity state notably contributes to a reproductive dysfunction in both men and women, ranging from infertility to oncological outcomes. Several epidemiological and experimental studies demonstrate that factors secreted by the adipose tissue and gut in an obesity state can directly induce reproductive disturbances. Relevantly, these same factors are able to alter the epigenetic regulation of genes, a dynamic and reversible mechanism by which the organism responds to environmental pressures critical to the reproductive function.

Conclusion: This review outlines the evidence showing that the association between the reproductive pathologies and obesity is not inevitable but is potentially preventable and reversible. The epigenetic marks related to obesity could constitute a therapeutic target for the reproductive disorders associated with obesity.

Key words: adipose tissue / DNA methylation / cancer / infertility / obesity
Introduction

Currently, obesity is a major health problem for all countries due to its increasing prevalence and its substantial health implications related to chronic disease and mortality. The worldwide prevalence of obesity has nearly doubled in the last three decades. According to data from the National Health and Nutrition Examination Survey (NHANES), 35.7% of American adults are obese, and 17% of children and adolescents aged 2–19 years are obese. In Europe, over 50% of the population is overweight, and ~23% is obese. In Spain in 2006/07, 15.5% of men and 15.2% of women were obese. Despite being a country of Mediterranean diet, this prevalence in Spain rose to 22.9% in 2010 (Gutierrez-Fisac et al., 2011).

Obesity is a multifactorial chronic disease whose etiology is an imbalance between the energy ingested in food and the energy expended. This imbalance is promoted by complex interactions between inadequate dietary habits, diminished physical exercise and genetic background (Marti et al., 2008). Excess energy is stored in fat cells that enlarge and/or increase in number. This process results in an adipose tissue dysfunction and the pathological consequences of obesity that include the development of other diseases, such as diabetes mellitus, heart disease, neurological disease and some forms of cancer (Bray, 2004).

Obesity is instrumental in the development of type 2 diabetes mellitus (T2D), and abdominal obesity is strongly associated with cardiovascular disease and diabetes, even among patients with low BMI as demonstrated by data that was generated during the International Day for the Evaluation of Abdominal obesity (IDEA) study in Spain (Casanueva et al., 2010). In addition to the widely known relationships of obesity with cardiovascular disease and diabetes mellitus, obesity itself is thought to be a relevant risk factor for reproductive disorders.

Reproductive function is controlled by the hypothalamic–pituitary–gonadal axis, which is regulated by numerous endogenous and environmental factors (Sanchez-Garrido and Tena-Sempere, 2013). Several years ago, it was proposed that the metabolic conditions and nutritional status influence reproductive capacity. Thus, reproductive function is perturbed both by energy insufficiencies and energy excesses. Accordingly, several epidemiological and experimental studies have provided evidence that obesity and overweight can play relevant roles in the early onset of reproductive features which lead to decreased rates of conception and increases in infertility, such as puberty, menopause and andropause, ovulatory disorders and hypogonadism (Table I). Moreover, overweight and obesity have been demonstrated to be important risk factors for several types of cancers including those that involve the reproduction-related tissues. However, the molecular mechanisms that are involved in the association between obesity and reproductive disorders remain unclear.

Here we review the current scientific evidence regarding the effects of obesity-related factors that form the core of the collective mechanisms that are directly and indirectly involved in the relationship between obesity and reproductive disorders, with a special and original focus on the effects of obesity-state microenvironment on epigenetic profile as a reversible mechanistic link between obesity and the reproductive disorders.

Methods

The articles selected for this review were English-language, full-text articles and abstracts that were identified by a series of PubMed searches using keywords either alone or in combination and published before the 31 of December 2013. The data were extracted from the identified papers, and secondary data sources were identified within these papers. The keywords used included obesity, reproductive system, reproduction, reproductive pathologies, puberty, menopause, adipose tissue, adipokines, leptin, adiponectin, insulin, ghrelin, inflammation, oxidative stress, PCOS, estrogens, testosterone, ovary, tests, endometrium, breast, prostate, epigenetics and DNA methylation.

Effects of obesity on male and female reproductive function

The increased global prevalence of obesity has occurred concomitantly with an apparent decline in fertility. Even though there is still insufficient evidence to claim that human fecundity was generally declining due to obesity, experimental studies of animal models and humans have shown direct relationships between obesity and infertility in both males and females.

Evidence for an association between high BMI and the periconceptual periods of both women and men exists. Obese women seeking to become pregnant experience longer times to conception, even if they are young and exhibit stable menstrual cycle patterns (Jensen et al., 1999; van der Steeg et al., 2008). In support of this observational evidence, it has been demonstrated that female mice with diet-induced obesity ovulate oocytes that exhibit developmental delays to the blastocyst stage and gave rise to embryos with altered cell type proportions (Minge et al., 2008). Moreover, the aging and function of the ovary in women appears to be influenced by obesity as observed in the general population (Gracia et al., 2005; Freeman et al., 2007) and, very recently, corroborated in a cohort of female survivors of childhood cancer (van Dorp et al., 2013). Thus, the levels of anti-Mullerian hormone (Freeman et al., 2007; van Dorp et al., 2013) and inhibin B (Gracia et al., 2005), which are markers of ovarian aging and ovarian function, respectively, are significantly lower in healthy obese women than in non-obese women in the menopausal transition period.

The aforementioned findings regarding the fertility of women are similar to those for males. High BMIs (>25 kg/m²) are associated with depletions in sperm concentrations and total sperm counts compared with these measures in subjects with BMIs between 20 and 25 kg/m² (Jensen et al., 2004). These alterations in spermato genesis have also been evidenced in animal models of genetic obesity; for example, obese Zucker rats exhibit decreased sperm production during puberty and increased sperm DNA fragmentation compared with their lean

| Table I  Summary of the consequences of overweight and obesity for male and female reproduction. |
|----------------------------------------|---------|--------|
| Reproductive landmarks | Men | Women |
| Increased time to conception | + | + |
| Early cessation of reproductive ability | + | ? |
| Hypogonadism | + | + |
| Spermatogenesis and ovulatory disorders | + | + |
| Gonadal tumorigenesis | + | + |
littermates (Vendramini et al., 2014). Additionally, a recent meta-analysis that included 21 relevant studies that were published before June 2012 (the total sample was 13 077 men) reinforced these conclusions and revealed that overweight and obesity are associated with increased prevalence of azoospermia and oligozoospermia (Sermonda et al., 2013).

With increasing age, men and women reach a period of cessation of reproductive ability, namely menopause in women and andropause or male midlife transition in men. This age-dependent cessation in reproductive function can be influenced by lifestyle factors such as obesity. Menopause is a natural transition in the life cycle of women that is caused by the depletion of ovarian function followed by the cessation of menstruation. This female transition occurs most frequently at the age of 50–51 years, but there is considerable variability across countries, and the age of menopause can be influenced by several factors. Regarding the effects of obesity, few studies have explored the associations between BMI and age at menopause, and those that have are controversial. Whereas some studies have observed an association between higher BMI and later age at menopause (Henderson et al., 2008), other studies, such as the large-cohort Fragingham heart study, have shown that age at natural menopause is not associated with measures of body composition, although post-menopausal women have increased risk of osteoporosis and cardiovascular disease. Moreover, a prospective evaluation of the EMAS survey revealed that weight gain is associated with suppressions of testosterone, free testosterone and sex hormone-binding globulin (SHBG). This potential effect of obesity on the hypothalamic–pituitary–testicular axis can be dissociated from those of age; whereas the reductions in testosterone that are observed with obesity are concomitant with unchanged or even reduced LH in obese older men, age is associated with a compensatory increase in the LH, which suggests that obesity impairs hypothalamic/pituitary function and that age affects testicular function (Wu et al., 2008).

In light of these findings, obesity appears to be a relevant determinant of the age-related decline in reproductive function and could accelerate the age-related infertility outcome. Therefore, even though declining fertility in ageing is a prevalent feature across the animal kingdom (Jones et al., 2014), and for men and women it will remain an inevitable fact of life, a significant part of the age-related reproductive dysfunction could potentially be slowed down by maintaining a healthy body weight. In agreement with this hypothesis, interventional weight loss regimes for obese diabetic patients result in increased testosterone (Grossmann, 2011). Moreover, a prospective evaluation of community-dwelling men in Europe (EMAS) observed that unsupervised weight loss over 4 years of follow-up was associated with increases in testosterone, free testosterone, SHBG and LH (Camacho et al., 2013).

The association between excess body weight and polycystic ovary syndrome (PCOS) is particularly relevant. PCOS is the most common female endocrine disturbance, affects ~10% of the adult reproductive-aged population and is characterized by ovarian hyperandrogenism and chronic oligo-anovulation (Hart et al., 2004; Ehrmann, 2005). A pathogenic role of obesity in the subsequent development of this syndrome and the exacerbation of its phenotype has been suggested because excess body weight frequently precedes the onset of oligomenorrhea and hyperandrogenism and because 50% of PCOS women are overweight or obese (Gambineri et al., 2002; Lim et al., 2013).

**Obesity and oncologic outcomes of the reproductive system**

Excess body weight has been demonstrated to be a relevant preventable risk factor for several types of cancers (Calle et al., 2003; Renehan et al., 2008; Boeing, 2013). For example, the oncologic outcomes of the gonads can be influenced by increases in BMI. In women, obesity is a risk factor for endometrial, ovarian, uterine cervical and breast cancer malignancies (Reeves et al., 2007). In men, high body weight is associated with a greater incidence of testicular tumor and more aggressive prostate cancers (Fig.1).

A recent meta-analysis that included 25 157 women with ovarian cancer from 47 epidemiological studies (i.e. the majority of the available epidemiological information worldwide) indicated that highly significant increases in the risk of ovarian cancer accompany increases in weight and BMI (Beral, 2012). These increases in ovarian cancer risk do not vary with other factors that are relevant to the disease, and these effects do not differ across the common histological subtypes of ovarian cancer with the exception of serous tumors with borderline malignancy; for these tumors, the increase in the risk with increasing BMI is considerably greater than that for other tumor subtypes. However, the use of hormone therapy for menopause has a substantial effect on this association; the relative risk of ovarian cancer is increased among obese women who have never used menopausal hormone therapy (relative risk per every 5-unit increase in BMI of 1.10 [95% confidence interval (CI), 1.07–1.13]) but not among those who have used hormone therapy at some point (relative risk per every 5-unit increase in BMI of 0.95 [95% CI, 0.92–0.99]) (Beral, 2012).

The development of cervical cancer (the third most frequent cancer among women worldwide) is also influenced by obesity status. Obese and overweight women appear to be at a 2-fold (odds ratio (OR) 2.1 [95% CI, 1.1–3.8]) higher risk for cervical adenocarcinoma compared with women who are not overweight or obese (Lacey et al., 2003), and recent observations from the Metabolic Syndrome and Cancer Project (MeCan) cohort indicate that a 12% (hazard ratio 1.12; 95% CI, 1.01–1.25) increase in the risk of cervical cancer risk accompanies a 1 SD increase in BMI without differences among the histological types of cervical cancer (Ulmer et al., 2012).

Strikingly, the strongest influences of obesity on the development of gynecologic cancers have been observed for the risks of endometrial and breast cancers (Renehan et al., 2008). Obesity is a major risk
factor for endometrial cancer; the risk increases by 50–60% for every 5-unit increase in BMI, and it has been estimated that 30–34% of all endometrial cancers can be attributed to overweight and obesity (Webb, 2013). This association is strongest for low-grade endometrial cancers but is present, albeit modest, with more aggressive endometrial cancers (Yang et al., 2012).

Obesity is a known risk factor for breast cancer (Lorincz and Sukumar, 2006). For every 5-unit increase in BMI, the risk of post-menopausal breast cancer increases by 12% (relative risk (RR) 1.12 [95% CI, 1.08–1.16]), and elevated BMIs are associated with a protective effect against the development of breast cancer in premenopausal women (RR 0.92 [95% CI, 0.88–0.97]) (Renehan et al., 2008). This association has recently been corroborated in a study of homogeneous population from Spain that provided evidence that the prevalence of being overweight/obese is significantly higher among post-menopausal breast cancer patients (Crujeiras et al., 2012). However, although it is generally accepted that obese women are at an increased risk for postmenopausal breast cancer and decreased risk for premenopausal breast cancer, a recent study provided new and consistent data that suggests that there are 50 and 70% increases in the risks for premenopausal breast cancer among overweight and obese women, respectively (Cecchini et al., 2012). Indeed, post-menopausal obesity is more frequently associated with positive estrogen receptor (ER) breast cancers, and ages below 50 years and obesity are associated with a higher risk for ER-negative breast cancer (Anderson and Neuhausser, 2012). Therefore, these lines of evidence suggest that reducing obesity should be added to the cancer prevention toolbox for both pre- and the post-menopausal breast cancer.

Regarding the effect of excess body weight on oncologic outcomes in men, the literature has reported heterogeneous findings. Testicular cancer is a relatively rare malignancy in the general population, but it is the most commonly occurring cancer among young men in many countries (Chia et al., 2010). One hypothesis suggests that a high-calorie diet and the resultant increase in body size are among the risk factors for testicular cancer (Dieckmann et al., 2009). However, several epidemiological studies have found positive associations between the risk of testicular cancer and height, and inverse or null associations of testicular cancer with BMI and body weight (OR per every unit increase in BMI 0.99 [95% CI, 0.97–1.00]). A recent systematic review and meta-analysis of body size in relation to testicular cancer corroborated these conclusions (Lerro et al., 2010).

Inconsistent results regarding the association between obesity and prostate cancer have also been published. While some studies of adult BMI and the incidences of all prostate cancers have yielded null results, other studies have reported that adult BMI is associated with a decrease in prostate cancer risk among men who are diagnosed before 60 years of age and those with family histories of prostate cancer (Rodriguez et al., 2007). However, when the association between obesity and prostate cancer is evaluated while accounting for the stage or grade at diagnosis, adult BMI has been positively associated with the risk of more aggressive tumors (RR BMI ≥ 30 kg/m² 1.23 [95% CI, 1.00–1.55]) and inversely associated with the risk of less-aggressive tumors (RR BMI > 30 kg/m² 0.86 [95% CI, 0.77–1.06]) (Rodriguez et al., 2007).

The epigenetic impact of obesity on reproductive function

The biological regulatory system through which the organism responds to environmental pressures is mediated by epigenetic modifications to the genome (Herceg and Vaissiere, 2011). These modifications refer to mitotically and/or meiotically heritable changes in gene expression that occur without altering DNA sequences (Holliday, 1987; Berger et al., 2009). Such mechanisms play important roles in many biological processes that occur over a person’s lifetime, including embryogenesis and the phenotypic variations of genetically identical individuals (Herceg and Vaissiere, 2011). The epigenetic machinery involves several levels of regulation including DNA methylation, post-translational
histone modifications, nucleosome positioning and non-coding RNAs (Portela and Esteller, 2010; Taby and Issa, 2010; Rodriguez-Paredes and Esteller, 2011). Among these mechanisms, the best-known epigenetic marker is DNA methylation, which occurs in certain areas of the genome with high concentrations of CpG dinucleotides (‘CpG islands’) and leads to the silencing of both coding and non-coding genes. In addition, the DNA methylation also occurs in areas of low CpG density (<10 CpG/100 bp) named ‘CpG deserts’. Although the previous dogma is that epigenetic modifications in CpG islands or shores (regions up to 2 kb away from CpG islands) with highest CpG density are critical, these ‘CpG deserts’ may be especially important for gene regulation as it is reflected by the maintenance of small CpG clusters in these deserts, despite the high mutation rates observed in CpG sites (Guerrero-Bosagna et al., 2013; Skinner and Guerrero-Bosagna, 2014).

All levels of epigenetic regulation appear to have wide-ranging effects on development and health and can be reversible. Aberrant epigenetic regulation has been described in many human diseases including cancer (Heyn and Esteller, 2012), obesity (Milagro et al., 2013), T2D (Andersen et al., 2014), atherosclerosis (Zaina et al., 2014) and cardiovascular disease (Ordoval and Smith, 2010), neurodegenerative and neurological diseases (Urdinguio et al., 2009; Sanchez-Mut et al., 2013) as well as several inflammatory processes such as inflammatory bowel disease, autoimmune diseases and rheumatoid arthritis (Glossop et al., 2014). Therefore, epigenetic marks might explain the link between lifestyle and the risk for disease and have been proposed to be sensitive biomarkers of disease and potential therapeutic targets for disease management (Hamilton, 2011).

**Epigenetics and reproductive function**

Reproductive function is highly sensitive to environmental conditions including season, diet and exposure to chemical contaminants. In this regards, recent reports have described epigenetic differences that seem to be critical to reproductive function (Kurian and Terasawa, 2013). The development of the GnRH neuronal circuit is essential for reproductive function (Kurian and Terasawa, 2013). Moreover, recent reports have described epigenetic differences that seem to be critical to reproductive function (Kurian and Terasawa, 2013). The development of the GnRH neuronal circuit is essential for reproductive function (Kurian and Terasawa, 2013). Further analysis demonstrated that EED associates with the Kiss1 promoter to reduce its expression and function of a large array of genes and lead to tumorigenesis, tumor progression and metastasis (Dobosy et al., 2007; Li, 2007).

Frequent epigenetic aberrations, such as DNA hypo- and hypermethylation and altered histone acetylation and methylation, have also been observed in prostate cancer, and these aberrations affect the expression and function of a large array of genes and lead to tumorigenesis, tumor progression and metastasis (Dobosy et al., 2007; Li, 2007).

However, despite the extensive evidence regarding the relationship between epigenetic aberrations and reproductive dysfunction, the
concrete mechanisms responsible for the dysregulation of the epigenetic machinery remain to be elucidated.

All of the aforementioned epigenetic states of different reproductive disorders can be induced by combinations of environmental and nutritional factors because epigenetic regulation may potentially be altered by diet, age, BMI, inflammation and oxidative stress (Milagro et al., 2013). Therefore, we hypothesize that the disturbances in reproductive function that result from obesity may be mediated, at least in part, by the effect of obesity-related factors on epigenetic regulation of reproductive function, or an environmental-related epigenetic regulation can induce an obesity state that leads to dysregulation of adipokines that then causes reproductive disorders (Fig. 2).

**Obesity-related factors, epigenetic alterations and reproductive dysfunction**

Here we review the evidence for the hypothesis that obesity induces epigenetic alterations, which in turn may increase susceptibility to reproductive dysfunction.

Together with a dysbalance in the energy homeostasis, obesity is characterized by a state of chronic low-grade inflammation that promotes oxidative stress due to the dysfunction of adipose tissue and the alterations of adipocyte-derived hormone secretion and cytokine synthesis (Zou and Shao, 2008).

In addition to its primary role as a fuel reservoir, adipose tissue is a highly active metabolic and endocrine organ that secretes factors that include leptin, adiponectin, and other cytokines (Catalan et al., 2009) and other new signals that have been identified with the proteomics approach (Roca-Rivada et al., 2011). Moreover, adipose tissue is a major site of the metabolism of sex steroids and glucocorticoids (Kershaw and Flier, 2004). Obesity is strongly associated with changes in the physiological function of adipose tissue that lead to adipose tissue dysfunction, which in turn results in increased systemic levels of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin 6 (IL-6), C-reactive protein and matrix metalloproteinases (Dizdar and Alyamac, 2004). The chronic inflammation that is induced by adipocyte dysfunction induces increases in the release and accumulation of reactive oxygen species (ROS). Additionally, obesity alone induces an excessive generation of ROS due to inefficient energy metabolism (Crujeiras et al., 2008). Such obesity-related inflammatory and oxidative stress has been hypothesized to be a link between obesity and its comorbidities (Vincent and Taylor, 2006).

Several enzymes involved in the epigenetic modifications utilize co-factors or substrates that are crucial metabolites in core pathways of intermediary metabolism such as acetyl-CoA, glucose, α-ketoglutarate (α-KG), nicotinamide adenine dinucleotide (NAD+), flavin adenine dinucleotide (FAD), ATP or S-adenosylmethionine (SAM) (Gut and Verdin, 2013). Histone acetylation primarily depends on glucose-derived, cytosolic pools of acetyl-CoA. This chromatin modification allows a feed-forward control mechanism for the selective expression of genes that regulates cellular function (Gut and Verdin, 2013). Therefore, disturbances in energy metabolism such as occur in obesity, might...
also lead to stable epigenetic changes that are maintained through the germline or they can occur in adult tissues and may affect the health of the organism, as was recently reviewed (Gut and Verdin, 2013).

Oxidative stress induces DNA damage (e.g. base modifications, deletions, strand breaks and chromosomal rearrangements) that reduces the ability of DNA to be methylated by DNA methyltransferases (DNMTs) and results in global hypomethylation (Wachsman, 1997; Franco et al., 2008). Additionally, ROS can induce the hypermethylation of certain tumor suppressor genes and thus promote carcinogenesis (Govindarajan et al., 2002; Lim et al., 2008). Moreover, oxidative damage has also been implicated in the regulation of histone modifications and microRNA expression (Simone et al., 2009; Mateescu et al., 2011; Rajendran et al., 2011). Inflammation also induces epigenetic alterations in tissues that are associated with disease manifestations, as revealed by recent therapeutic interventions utilizing histone deacetylase and DNMT inhibitors, the effects of certain anti-inflammatory dietary elements on DNA methylation and chromatin remodeling, and the actions of several inflammatory-related transcription factors such as nuclear factor kappa B (NFkB) (Milagro et al., 2013).

This epigenetic impact of obesity on reproductive function may be broadly divided into two areas; somatic effects and germline effects. The obesity-related factors can induce epigenetic alterations in adult target tissues and cells but these factors can induce an epigenetic phenotype in germline cells as well, that not only impede gamete function and contribute to infertility but also that are transmittable to the next generation.

Even though the notion that epigenetic marks are transmitted across generations is still controversial because it is uncertain whether and to what extent such epigenetic inheritance exists (Heard and Martienssen, 2014), over the past few years, evidence has been accumulating that epigenetic transgenerational inheritance occurs in mammals (Skinner et al., 2010; Guerrero-Bosagna et al., 2013; Pembrey et al., 2014; Skinner and Guerrero-Bosagna, 2014). In this regard, obesity status can exert a germ-line epigenetic impact since a parental obesity effect on the reproductive health of subsequent generations was demonstrated (Fullston et al., 2012). Relatedly, a recent study observed that diet-induced paternal obesity modulates sperm microRNA content and germ cell methylation status (which are potential signals that program the health of the offspring) and impairs the metabolic health of future generations (Fullston et al., 2013). Similarly, maternal obesity adversely affects oocyte quality, embryo development and the health of the offspring. The DNA methylation status of several imprinted genes and metabolism-related genes appears to be the underlying mechanism responsible for the adverse effects of maternal obesity on oocyte quality and the embryo development of the offspring. These findings have recently been corroborated in oocytes from an obesity mouse model and in oocytes and liver from their offspring (Ge et al., 2014). The results of this study revealed that the DNA methylation patterns of several metabolism-related genes are not only altered in the oocytes of the obese mice but also in the oocytes and liver of their offspring (Ge et al., 2014).

Epigenetic alterations, obesity-related factors and reproductive dysfunction

Here we review the evidence for the hypothesis that epigenetic changes can induce obesity, which in turn leads to reproductive dysfunction.

Specific patterns of epigenetic factors, including DNA methylation, have also been found to be associated with obesity itself in a genome-wide DNA methylation analysis in leucocytes and adipose tissue (Carless et al., 2013; Xu et al., 2013; Dick et al., 2014) and in analyses of specific genes, such as the circadian clock genes (e.g. CLOCK; clock circadian regulator, BMAL1; aryl hydrocarbon receptor nuclear translocator-like and PER2; period circadian 2), whose methylation status in human leucocytes is associated with obesity (Milagro et al., 2012). This specific epigenetic profile associated with obesity can be induced by environmental factors on somatic cells but also a number of studies demonstrate that ancestral environmental exposure can promote the epigenetic transgenerational inheritance of obesity through germline epimutations, i.e. the germline (sperm or egg) transgenerational transmission of epigenetic marks that influence physiological parameters and disease, in the absence of direct environmental exposures (Skinner et al., 2010). For example, a recent study demonstrated the presence of differentially methylated regions in the sperm of the F3 generation of male rodents with ancestral exposure to the insecticide dichlorodiphenyldichloroethane (DDT) and a number of previously identified obesity-associated genes correlated with the epimutations identified (Skinner et al., 2013). This germline transmission of epimutations associated with obesity in rodents was also observed after the exposure to plastics-derived endocrine disruptors (bisfenol-A, Diethylhexyl phthalate and dibutyl phthalate) (Manikkam et al., 2013) or jet fuel hydrocarbons (Tracey et al., 2013).

Strikingly, therapeutic strategies for counteracting excess body weight are able to remodel DNA methylation profiles concomitant with the reduction of body weight. The DNA methylation and expression levels of several genes, which are related to metabolic processes and mitochondrial functions (e.g. peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; PGC-1α and pyruvate dehydrogenase kinase, isozyme 4; PDK4), are altered in the skeletal muscle of obese people and after Roux-en-Y gastric bypass (RYGB), a type of weight-loss surgery, and were normalized to levels observed in normal-weight, healthy controls (Barres et al., 2013). A 6-month intervention of exercise can induce changes in the genome-wide DNA methylation patterns of human adipose tissue that potentially affect adipocyte metabolism (Ronn et al., 2013). Similar results have been observed in the muscle of patients with T2D; in these patients, exercise altered the DNA methylation of genes involved in retinol metabolism and calcium signaling pathways that have known functions in the muscle and T2D (Nitert et al., 2012). The DNA methylation status of specific genes in human leucocytes (Milagro et al., 2011) and adipose tissue can be altered by caloric restriction interventions (Bouchard et al., 2010). Additionally, it has been demonstrated that responses to weight loss treatments can be influenced and predicted by DNA methylation status prior to beginning treatment; i.e. differences in the DNA methylation patterns of specific genes have been found between low and high responders to weight loss therapy (Bouchard et al., 2010; Milagro et al., 2011) and between patients who are prone to regain lost weight and those who are able to maintain weight loss during a free-living period after dieting (Crujeiras et al., 2013).

Once obesity is established, factors secreted by the dysfunctional obese adipose tissue can directly influence reproductive function (Budak et al., 2006; Cominos et al., 2014) and have carcinogenic properties in several tissues (Doyle et al., 2012) (Fig. 3).

Leptin, which is mainly secreted by adipose tissue and is related to body fat, has been demonstrated to play a central role in the metabolic control of the reproductive system (Carro et al., 1997;
At the central level, endogenous leptin is required for the activation of hypothalamic GnRH secretion and physiological GnRH pulse generation, which are mediated by the intermediate signaling factors of kisspeptin or glutamate (Comninos et al., 2014). At high concentrations, such as those that occur in states of obesity, leptin can directly inhibit some gonadal functions, including sex steroid secretion; thus these inhibitory actions of leptin may explain part of the hypogonadal states frequently observed in obese patients (Sanchez-Garrido and Tena-Sempere, 2013).

Adiponectin is another protein that is abundantly secreted by white adipose tissue and whose serum concentrations are inversely correlated with body fat mass. In addition to its well-known antidiabetic, antiatherogenic and anti-inflammatory properties (Campos et al., 2008), adiponectin has been suggested to play a beneficial role in reproductive tissues. Adiponectin has been suggested to play roles in oocyte maturation, granulosa cell proliferation and death, and as a modulator of estradiol and progesterone secretion (Lagaly et al., 2008; Maillard et al., 2010). The deletion of functional adiponectin in mice results in infertility in female but not male mice (Combs et al., 2004). Moreover, knockdown of the genes for the adiponectin receptors Adipo R1 and Adipo R2 is associated with increased androstenedione secretion by bovine theca cells, which suggest that the hyperandrogenism observed in the obese and overweight can be explained by reductions in adiponectin in obesity (Comim et al., 2013).

The roles of other adipose-related proteins, such as resistin and omentin, remain to be established (Comninos et al., 2014).

Gut hormones, such as ghrelin, may also operate as modifiers of male and female reproduction. Ghrelin is primarily secreted by the oxyntic cells of the gastric mucosa, although it is also produced in the intestine, pancreas, hypothalamus and pituitary (Gualillo et al., 2003). Additionally, further evidence has demonstrated that ghrelin and its functional receptor are expressed in rat and human gonads, suggesting paracrine/autoocrine effects of this peptide in different tissues (Barreiro and Tena-Sempere, 2004). Thus, in addition to its roles in the regulation of feeding behavior and energy metabolism as an orexigenic signal that increases food intake and adiposity, ghrelin has been demonstrated to play a relevant role in the regulation of reproductive function via its inhibitory effect, which has previously been reviewed elsewhere (Barreiro and Tena-Sempere, 2004; Comninos et al., 2014). Ghrelin inhibits the proliferative activity of immature Leydig cells (Barreiro et al., 2004), testosterone secretion in the testes (Tena-Sempere et al., 2002), and prevents spermatogenesis (Kheradmand et al., 2012). In the female reproductive tract, ghrelin exerts inhibitory effects on luteal function (Tropea et al., 2007) and the biosynthesis of estradiol and progesterone in granulosa-lutein cells (Viani et al., 2008).

Energy metabolism and reproductive function are also connected by insulin, which is a hormone that is produced by pancreatic β-cells. In addition to its function in the regulation of glucose homeostasis, this hormone can stimulate reproductive function at the hypothalamic and
gonadal levels (Comninos et al., 2014). Hyperinsulinemia is associated with increased LH secretion due to increased expression and secretion of hypothalamic GnRH, particularly in females (Burcelin et al., 2003; Kim et al., 2005). Moreover, insulin can stimulate ovarian (Nestler and Jakubowicz, 1996; Tosi et al., 2012) and testicular (Ahn et al., 2013) steroidogenesis.

As previously mentioned, accumulated body fat is also associated with increased inflammation and oxidative stress (Vincent et al., 2007). The complex interplay between cytokines and oxidative stress plays a role in the etiologies of female and male reproductive disorders that has been highlighted in a recent review (Agarwal et al., 2012). The oxidant status of the cell modulates angiogenesis, which is critical for follicular growth, corpus luteum formation, endometrial differentiation and embryonic growth as highlighted in the aforementioned review. Chronic inflammation has been proposed to promote delays in oocyte development and thus the poor reproductive outcomes that are typically observed in obese women (Robker et al., 2009). Oxidative stress also appears to be a powerful mechanism that can lead to sperm damage, deformity and eventually, male infertility, as oxidative stress is toxic to human spermatozoa (Agarwal et al., 2009; Makker et al., 2009). The inflammatory factors secreted by adipose tissue also appear to play an inhibitory role at the central level of the hypothalamic–pituitary–gonadal axis. For example, cytokines, such as TNF-alpha and IL-6, have been observed to dose-dependently suppress GnRH-stimulated LH release in in vitro pituitary glands isolated from female and male rats (Russell et al., 2001). Moreover, in rabbits with high-fat diet (HFD)-induced metabolic syndrome (MetS), it has been observed that hypogonadism-associated MetS is related to an inflammatory state that is concomitant with a decrease in gonadotrophin levels; this finding suggests that HFD-induced metabolic derangements negatively affect GnRH neuron function via inflammatory injuries at the hypothalamic level (Morelli et al., 2014).

The above mentioned obesity-related factors are also associated with carcinogenesis in the reproductive system (Calle and Kaaks, 2004; Catalan et al., 2009; Khandekar et al., 2011; Howe et al., 2013). For example, leptin induces the growth, migration and anti-apoptotic functions of several types of cancer cells (Li et al., 2012; Weichenhan and Plass, 2013), and elevated circulating leptin levels in patients are correlated with the risk for and progression of several cancers, such as endometrial and ovarian cancers (Luhn et al., 2013), but no changes have been observed in patients with breast cancer (Aliustaoglu et al., 2010). Insulin has been demonstrated to be a potent mitogen that stimulates DNA synthesis and appears to have a role in tumor initiation and progression (Reneman et al., 2006). In vitro studies have demonstrated that insulin increases the neoplastic proliferation of cell lines at both physiological and pharmacological doses (Osborne et al., 1976; Pollak, 2008). Moreover, insulin increases the bioactivity of insulin-like growth factor I (IGF-I) by enhancing hepatic IGF-I synthesis and by reducing the hepatic production of IGF binding protein 1 (IGFBP-1) and 2 (IGFBP-2); IGF-I has mitotic properties, and low levels of the expression of IGFBPs are associated with malignant transformation (Clemmons, 2007). Therefore, although insulin can directly induce tumor growth, many of its mitogenic and anti-apoptotic effects operate through the IGF-I system, through increasing the bioavailabilities of hormones, through increasing the levels of pro-inflammatory cytokines, and through oxidative stress (Arcidiacono et al., 2012). In contrast, adiponectin has been found to exhibit anticarcinogenic effects in vitro (Dalamaga et al., 2012), and epidemiologic studies have shown that low levels of adiponectin are inversely associated with the risks of developing of multiple cancers and the advanced progresses of these diseases (Barb et al., 2007). Moreover, adiponectin inhibits leptin-induced oncogenic signaling in vitro (Beales et al., 2014).

Therefore, the systemic alterations induced by obesity, such as hyperleptinemia and hyperinsulinemia, that are concomitant with low levels of adiponectin, chronic inflammation and oxidative stress determine the specific microenvironments in the reproductive system that are favorable to poor reproductive outcomes and determine the onset of the reproductive-related cancers that are typically observed in the obese. These obesity-related factors not only can act directly to dys-regulate reproduction, but also may affect the epigenome, and these epigenetic effects further lead to reproductive disorders (Fig. 2).

Conclusions

Several epidemiological and observational studies provide evidence for the relationships between obesity and reproductive pathologies, ranging from early in the onset of puberty to menopause/andropause, and several types of tumors in the reproductive tissues. Attempts to reduce obesity-related factors could improve reproductive function and reduce the incidence and poor prognoses of cancers in reproductive tissues. Although the molecular mechanisms involved in these associations require further elucidation, the molecular etiology of reproductive disease associated with obesity could be the epigenetic changes that could be either the result of, or cause, obesity. Obesity could contribute to infertility and malignancies of the reproductive system by an altered microenvironment that favors epigenetic changes that increase the susceptibility for inducing and cementing a disease state. In addition, epigenetic changes induced by environmental stressors can increase the susceptibility to obesity with the consequent dys-regulation of the obesity-related factors that obstruct the reproductive function. Therefore, metabolic environment, chronic inflammation, oxidative stress and deregulation of adipokine secretion that are induced by the obesity state may play direct roles in the regulation of the hypothalamic–pituitary–gonadal axis or indirect roles in inducing epigenetic modifications of specific genes in the reproductive pathways. Taken together, this review highlights the need for further scientific research to elucidate the role of epigenetic regulation as a potential mechanistic link between obesity and disturbances in reproductive function. Because epigenetic modifications are dynamic and reversible and change in response to dietary patterns, physical activity and weight loss, the epigenetic markers related to obesity may constitute therapeutic targets for the prevention of obesity-related disorders including reproductive pathologies. Future efforts will therefore be needed to carry out genome-wide analysis that provides information on reproductive dysfunction-specific epigenetic markers and longitudinal studies will be required to evaluate the effect of dietary- or surgery-induced weight loss on reprogramming these disease-specific epigenetic modifications. Understanding the influence of the obesity-related microenvironment on epigenetic regulation of the reproductive function machinery will provide new tools to improve the management as well as the prevention of reproductive disorders.

Authors’ roles

A.B.C. designed the review, performed the literature search, extracted the data, wrote the manuscript and provided final approval of the
manuscript. F.F.C. designed the review, supervised the writing, critically reviewed the complete manuscript and provided final approval of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References


