Genomic imprinting, growth and maternal–fetal interactions

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ABSTRACT
In the 1980s, mouse nuclear transplantation experiments revealed that both male and female parental genomes are required for successful development to term (McGrath and Solter, 1983; Surani and Barton, 1983). This non-equivalence of parental genomes is because imprinted genes are predominantly expressed from only one parental chromosome. Uniparental inheritance of these genomic regions causes paediatric growth disorders such as Beckwith–Wiedemann and Silver–Russell syndromes (reviewed in Peters, 2014). More than 100 imprinted genes have now been discovered and the functions of many of these genes have been assessed in murine models. The first such genes described were the fetal growth factor insulin-like growth factor 2 (Igf2) and its inhibitor Igf2 receptor (Igf2r) (DeChiara et al., 1991; Lau et al., 1994; Wang et al., 1994). Since then, it has emerged that most imprinted genes modulate fetal growth and resource acquisition in a variety of ways. First, imprinted genes are required for the development of a functional placenta, the organ that mediates the exchange of nutrients between mother and fetus. Second, these genes act in an embryo-autonomous manner to affect the growth rate and organogenesis. Finally, imprinted genes can signal the nutritional status between mother and fetus, and can modulate levels of maternal care. Importantly, many imprinted genes have been shown to affect postnatal growth and energy homeostasis. Given that abnormal birthweight correlates with adverse adult metabolic health, including obesity and cardiovascular disease, it is crucial to understand how the modulation of this dosage-sensitive, epigenetically regulated class of genes can contribute to fetal and postnatal growth, with implications for lifelong health and disease.

KEY WORDS: Adipose tissue, Leptin, Pregnancy

Introduction: genomic imprinting
The process of genomic imprinting results in the monoallelic expression of genes based on their parental origin. This is an epigenetic process because copies of identical deoxyribonucleic acid (DNA) sequence may be either expressed or silenced. This haploid expression is usually present in all tissues where the gene is expressed, although there are examples of tissue-specific imprinting. Following the discovery of the first imprinted genes in the 1990s, the work of multiple groups has established that the differential expression of imprinted genes is mediated by cis-regulatory regions where DNA is differentially methylated on cytosine residues (reviewed in Ferguson-Smith, 2011). This differential methylation at crucial sites (known as imprinting control regions or ICRs) is acquired during germline development and maintained during the epigenetic reprogramming of the early embryo and in somatic cells. Imprinted genes are often clustered in the genome, where neighbouring maternally and paternally expressed genes are co-ordinately controlled by a single ICR. The ICRs regulate monoallelic transcription by modulating further local epigenetic states, such as promoter methylation, expression of long non-coding ribonucleic acids (RNAs) and histone modifications (Ferguson-Smith, 2011).

Our knowledge of imprinting in humans has been informed by a number of paediatric growth disorders with over-lapping phenotypes and diverse aetiology, but which all alter the dosage of imprinted genes. Mutations or epimutations in an ICR, or a duplication of a section of chromosome from one parent (uniparental disomy or UPD), results in imprinted genes being either over-expressed or not expressed. Because many imprinted regions contain both paternally and maternally expressed genes, it is difficult to determine which genes are causal for their respective syndromes; in some cases, imprinting disorders are likely to be caused by polygenic gene dosage disruption. A common feature of imprinting disorders is developmental abnormality, resulting in altered growth and nutrient acquisition in early life (Table 1) and, hence, they highlight the key role of imprinted genes in these physiological processes.

Imprinting has been described across Therian mammals (including marsupials but not monotremes) and conservation of many imprinted genes [e.g. insulin-like growth factor 2 (Igf2) and its inhibitor Igf2 receptor (Igf2r), paternally expressed gene 1/mesodernally expressed transcript (PEG1/MEST) and insulin (INS)] within this group implies that imprinting was present in a common vertebrate ancestor that exhibited viviparity and had a placenta (Graves and Renfree, 2013). Coincident with their taxonomic correlation with the presence of a placenta, many imprinted genes are expressed in that organ and play a crucial role in the process of placentation. Moreover, genes involved in placentation are more likely to be imprinted (Graves and Renfree, 2013). In mammals, the level of dependence on the placenta correlates with both the known number of imprinted genes within each group (Renfree et al., 2009) and the complexity of their regulation (Graves and Renfree, 2013). The presence of parental-sex-dependent haploid expression has also been described in flowering plants, a subset of plants that utilise a nourishing external organ in their reproductive strategy. The endosperm in flowering plants provides nourishment from the parent’s energy stores, much like the placenta; similar selection pressures are likely to have led to the convergent evolution of imprinted genes in these distant groups (Pires and Grossniklaus, 2014).

Imprinted genes control maternal resource allocation during pregnancy and lactation
This selective pressure for the imprinting of a gene has acted almost exclusively at the maternal–offspring interface, giving imprinted genes a unique role in influencing maternal resource allocation. Maternal resource allocation is dependent on both the conceptus and the mother, and the role of imprinted genes reflects this, with imprinted gene action affecting both and involved in communication between the two. Imprinted genes that act in the conceptus to modulate maternal energy
supply can be further subdivided into those acting in the placenta and those acting in the fetus.

Fetal resource acquisition via the placenta
The rate at which nutrients can be supplied to the developing fetus is dependent upon the placenta. The primary substrate for fetal growth is glucose. Maternal–fetal glucose transfer is mediated by multiple factors, including the maternal–fetal blood glucose concentration gradient, the rate of blood flow and the placental glucose transporter density (Hay, 1991). Placental amino acid transport to the fetus from the maternal circulation operates against a concentration gradient and is an energy-dependent process requiring specialised transporter proteins (Hay, 1991).

Imprinting has been most extensively studied in humans and mice. These organisms share similar placental physiology: both use a hemochorial placenta where the maternal blood travels through sinusoidal spaces lined by trophoblast cells, rather than an endothelium, allowing for more efficient nutrient transfer (reviewed in Rai and Cross, 2014). The distinct populations of cells in the placenta, the trophoblast and the extraembryonic mesoderm, differentiate early in embryogenesis with the trophoblast cells invading the maternal uterine decidua following fertilisation. In mice, at mid-gestation the definitive placenta is formed by intercalation of the extraembryonic mesoderm (which will form the embryonic vasculature) with trophoblast cells that channel maternal blood flow through the placenta. The portion of the placenta where maternal and fetal circulation is closely opposed is known as the labyrinth in mice. The trophoblast further differentiates into multiple cell types that have both endocrine and energy-storage properties (Rai and Cross, 2014).

The action of imprinted genes on placentation development and nutrient transport capacity is well established and has been reviewed extensively (Fowden et al., 2011; Monk, 2015). Most imprinted genes are monoallelically expressed in the placenta, and have been shown to control early developmental processes that establish the organ (e.g. Peg10 (Ono et al., 2006)). In addition, imprinted gene products can modulate labyrinth size and the surface area for exchange [Igf2 (Sibley et al., 2004) and Growth factor bound protein 10 (Grb10); Charalambous et al., 2010] as well as vascular branching density [Aquaporin (Guo et al., 2016)]. Placental imprinted genes can also directly influence maternal physiology by controlling the differentiation of the trophoblast-derived endocrine cells (reviewed in John, 2017).

Imprinted genes involved in fetal resource acquisition
Nutrient uptake from maternal tissues and fetal growth rates are interconnected processes (Hay, 1991). Imprinted genes can act directly on fetal growth-promoting pathways and, thus, increase the demand for maternal resources. The IGF pathway is a major fetal growth-promoting pathway and includes two key components encoded by imprinted genes (Igf2 and Igf2r; DeChiara et al., 1991; Lau et al., 1994; Wang et al., 1994). Fetal growth is also restrained by imprinted genes, including the Cyclin-dependent kinase inhibitor 1C [Cdkn1c (Tunster et al., 2011)] and Grb10 (Charalambous et al., 2003). Grb10 encodes an adapter protein that acts downstream of tyrosine-kinase receptors to inhibit signalling, and is a crucial auto-regulatory component of the nutrient-sensing mechanistic target of rapamycin (mTOR) pathway (Hsu et al., 2011; Yu et al., 2011). Grb10 may act in the same signalling pathway as the fetal growth promoter Delta-like homologue 1 (Dll1; Madon-Simon et al., 2014). Dll1 is a paternally expressed gene encoding a single-pass transmembrane protein that can be cleaved to produce a soluble form that circulates in the blood (Smas et al., 1997). Dll1 expression levels in the embryo are positively correlated to embryonic mass in the second half of gestation (Cleaton et al., 2016). Dll1 deletion causes growth retardation (Cleaton et al., 2016; Moon et al., 2002) and overexpression causes overgrowth independently of the placenta (da Rocha et al., 2009). The signalling pathway by which Dll1 acts has yet to be elucidated. However, Dll1 from the fetus is secreted into the maternal circulation and acts to modify the pregnancy-specific response to nutrient restriction (Cleaton et al., 2016).

In summary, imprinted genes in the conceptus produce products that alter maternal resource allocation by: (i) altering the transport capacity of the placenta; (ii) increasing fetal demand for resources by their action on the intrinsic growth rate; and (iii) signalling to the mother by the production of fetal/placental hormones that modify maternal metabolism.

Energy homeostasis and resource allocation during pregnancy
The energetic cost of pregnancy is distributed amongst three compartments, i.e. the energy invested in the products of conception, the energy deposited as adipose tissue in the mother and the energy required to maintain these new tissues (Thomson and Hytten, 1961). Well-nourished humans and rodents accumulate adipose tissue reserves during pregnancy in the first half of gestation (Herrera and Ortega-Senovilla, 2014; King, 2000). The total cost of pregnancy is correlated with pre-pregnancy fatness – individuals with a higher body mass index (BMI) gain more weight during pregnancy, both the adipose tissue reserve and conceptus mass (Prentice and Goldberg, 2000). This suggests that adipose reserves in the mother can produce a signal to the body that directs the level of nutritional allocation after conception (Prentice and Goldberg, 2000). Leptin and other adipokines are ideal candidates for such signalling molecules.
Leptin, a cytokine secreted exclusively by adipose tissue in proportion to adipose mass, is a major determinant involved in signalling the status of the energy reserve to central pathways controlling appetite, energy expenditure and reproducative behaviour (van Swieten et al., 2014). The action of leptin on the hypothalamic control of energy homeostasis is complex, and a detailed description is beyond the scope of this Review. Briefly, leptin signals to its receptors expressed on the surface of first-order neurons in the arcuate nucleus of the hypothalamus (ARH). Two populations of neurons in the ARH have been well defined – the orexigenic neuropeptide Y (NPY)/Agouti-related peptide (AGRP) neurons, which are inhibited by leptin, and the anorexigenic pro-opiomelanocortin (POMC) neurons, which are activated by leptin (reviewed in van Swieten et al., 2014). These neurons project to secondary hypothalamic sites, such as the paraventricular nucleus (PVN), which controls feeding behaviour, and the dorsomedial hypothalamic sites, such as the paraventricular nucleus (reviewed in van Swieten et al., 2014). These neurons project to secondary hypothalamic sites, such as the paraventricular nucleus (PVN), which controls feeding behaviour, and the dorsomedial hypothalamic sites, such as the paraventricular nucleus (ARH), which regulates the level of sympathetic neuronal activity. A crucial intermediate signalling molecule is the neuropeptide α-melanocyte-stimulating hormone (αMSH), which is secreted by POMC neurons and acts on melanocortin receptors to mediate downstream actions of leptin (van Swieten et al., 2014). AGRP antagonises αMSH. Simply, a high leptin level from adequate adipose stores suppresses appetite by deactivating orexigenic neuronal pathways, and increases energy expenditure by increasing activity and sympathetic neuronal activity. Increased sympathetic neuronal activity in turn increases body temperature by activating the expression of thermogenic genes in brown and beige adipose tissue (van Swieten et al., 2014). Low stores of adipose result in an opposite series of responses. Taken together, these mechanisms maintain a homeostatic ‘set point’ that maintains body weight within a narrow range.

Genetic and environmental modulations of leptin signalling pathway components can cause alterations to the value of these set points, or cause a failure to achieve homeostasis. For example, the pups of mice exposed to a high-fat maternal diet during the perinatal period fail to establish neuronal connectivity between the ARH and PVN, resulting in altered body composition and a predisposition to metabolic disease as adults (Vogt et al., 2014). Leptin-deficient (ob/ob) mice and people with mutations in components of the central melanocortin pathway are hyperphagic and obese – maintaining a higher body weight set point owing to leptin deficiency or resistance (reviewed by Farooqi and O’Rahilly, 2014).

Elegant studies utilising timed leptin-replacement therapy in ob/ob mice have demonstrated that this adipokine has a broad role in reproduction (Malik et al., 2001). Leptin signalling is required for fertility [by controlling gonadotrophin release (Padilla et al., 2017)], conception and implantation. Moreover, leptin is required during the perinatal period for lactation and for appropriate maternal behaviours following parturition (Malik et al., 2001).

Pregnancy is associated with leptin resistance. Circulating levels of leptin rise in the maternal blood in the second half of pregnancy; however, the expression of orexigenic peptides NPY and AGRP in the ARH remain stable. Moreover, injecting pregnant rats with leptin does not suppress food intake or activate downstream αMSH pathways (Ladyman et al., 2010). The leptin resistance of late pregnancy is thought to be mediated by placental secretion of hormones, specifically the prolactin and placental lactogen family of molecules. Intracerebroventricular injection of prolactin into female rats mimics the leptin resistance of pregnancy, and prolactin receptor expression is widespread in the hypothalamic areas associated with energy homeostasis (Ladyman et al., 2010). Consistently, global deletion of the prolactin receptor in mice causes changes to glucose homeostasis during pregnancy; however, the contribution of leptin signalling to this phenotype has yet to be explicitly tested (Rawn et al., 2015). Therefore, interactions between placental hormone secretion and central leptin sensitivity are crucial for an appropriate maternal response to pregnancy.

A second adipokine, adiponectin, has important functions during pregnancy. Adiponectin is secreted from adipocytes according to their size and acts on multiple tissues, predominantly to increase insulin sensitivity (Stern et al., 2016). Adiponectin levels drop in both rodent and human pregnancy and remain low during lactation (Combs et al., 2003; Howell and Powell, 2017). Low adiponectin levels during the second half of gestation are thought to increase maternal insulin resistance, thus reducing maternal glucose uptake and increasing the maternal–fetal glucose concentration gradient in favour of fetal uptake. Consistent with this, obese pregnant women and rodents who are insulin resistant give birth to large babies and have further reduced adiponectin levels (Howell and Powell, 2017). Furthermore, supplementing obese mice with adiponectin during pregnancy can reverse both maternal insulin resistance and fetal overgrowth (Aye et al., 2015).

Experiments utilising deletion and overexpression models in mice have demonstrated that imprinted genes have important roles in energy homeostasis – resulting in altered steady-state levels of adiposity, adipokine production and sensitivity. However, many of these phenotypic alterations have not yet been tested directly for their contributions to the outcome of pregnancy, and adipokine levels during pregnancy have been measured for only very few imprinted gene manipulations (available data for murine models published to date are summarised in Table 2).

The actions of imprinted genes in energy homeostasis can be partitioned into those that modulate the central pathways, and those that act in peripheral tissues to alter adipose mass and type (Fig. 1).

Imprinted genes mediating the central control of energy homeostasis

The Gnas locus

The Gnas locus encodes maternally and paternally expressed products (Gsa and XLαs), as well as several regulatory RNAs (reviewed in Peters, 2014). Although Gsa is widely and predominantly biallelically expressed, it is imprinted in only a small number of tissues, including the kidney and neuroendocrine tissues, where it is maternally expressed. Gsa encodes the stimulatory G-protein alpha subunit that couples receptor-mediated signalling to intracellular cyclic adenosine monophosphate – causing the activation of protein kinase A (PKA). A second isoform from the Gnas locus encodes XLαs, containing a distinct amino-terminal of the protein from Gsa. This isoform is exclusively expressed from the paternally inherited chromosome, predominantly in the brain and endocrine tissues (reviewed by Peters, 2014).

Maternally inherited Gsa mutations cause pseudohypoparathyroidism type A, a syndrome of multiple hormone resistance, reduced sympathetic tone, early severe obesity and insulin resistance (reviewed in Weinstein, 2014). This is phenocopied in mice that inherit a deletion in the first exon of Gnas from their mother. Central melanocortin signalling limits food intake and stimulates sympathetic tone and energy expenditure, and the melanocortin receptor 4 (MC4R) is known to couple to Gsa (Weinstein, 2014). Maternally expressed Gsa is required for melanocortin signalling in the DMH. Deletion of Gsa in this tissue causes obesity and insulin resistance by reducing energy expenditure, both by reducing physical activity and the expression of uncoupling protein 1 (UCP1) in brown adipose tissue (BAT). Food intake is not affected. The mice are resistant to the effect of melanocortin agonists, and these phenotypes
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<td>Gnas</td>
<td>Global maternal deletion of Gnas exon 1 – specific to Gsα</td>
<td>Increased adipose deposition, reduced glucose tolerance and insulin sensitivity. Metabolic rate reduced owing to reduced SNS activity</td>
<td>NR</td>
<td>Leptin high Adiponectin unchanged</td>
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<td></td>
<td>DMH deletion of Gnas exon 1</td>
<td>Increased adipose deposition, reduced glucose tolerance and insulin sensitivity. Reduced energy expenditure owing to reduced activity. Food intake unchanged</td>
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<td>GnasXL</td>
<td>Global deletion of GnasXL exon 1 – specific to XLα</td>
<td>Reduced adiposity, increased metabolic rate, increased glucose tolerance and insulin sensitivity. Increased SNS activity</td>
<td>NR</td>
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<td>NR</td>
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<td>Paternal</td>
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<td></td>
<td>Deletion</td>
<td>Increased body fat, reduced lean mass. Reduced food intake and body temperature. Central leptin resistance</td>
<td>Poor maternal care Reduced maternal investment in pregnancy</td>
<td>NR</td>
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<td>Asb4</td>
<td>Overexpression in hypothalamic POMC neurons</td>
<td>Reduced body fat, increased food intake, increased energy expenditure by thermogenesis and activity</td>
<td>NR</td>
<td>Leptin low</td>
<td>Maternal</td>
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<td>Snord116</td>
<td>Global deletion</td>
<td>In mature animals, reduced body fat, increased food intake, increased energy expenditure by activity</td>
<td>NR</td>
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<td>NR</td>
<td>NR</td>
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<td>Magel 2</td>
<td>Global deletion</td>
<td>Maturity-onset insensitivity to leptin causes increased adipose mass and reduced energy expenditure</td>
<td>NR</td>
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<td>Bischof et al., 2007; Mercer et al., 2013; Pravdivyi et al., 2015 Fujiwara, 2012</td>
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<td>Necdin</td>
<td>Global deletion</td>
<td>Increased adipose mass on HFD Food intake unchanged</td>
<td>NR</td>
<td>NR</td>
<td>Paternal</td>
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<td>Cdkn1c</td>
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<td>Reduced white adipose mass, increased BAT activity Food intake reduced</td>
<td>NR</td>
<td>NR</td>
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<td>Grb10</td>
<td>Global deletion of somatic expression (maternal transmission of deletion)</td>
<td>Reduced white adipose mass, increased lean mass, improved glucose tolerance and insulin sensitivity Food intake unchanged</td>
<td>Reduced resource allocation to litter</td>
<td>Leptin low</td>
<td>Maternal</td>
<td>Smith et al., 2007; Cowley et al., 2014</td>
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<td>Deletion in adipocytes (adiponectin promoter-Cre)</td>
<td>Increased WAT mass, impaired glucose and insulin sensitivity. Thermogenic gene expression is reduced in BAT, and animals do not ‘brown’ their WAT on cold challenge and have impaired ability to defend BAT</td>
<td>NR</td>
<td>NR</td>
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<td>Liu et al., 2014</td>
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<td>Dlk1</td>
<td>Global deletion</td>
<td>Increased WAT mass, impaired glucose and insulin sensitivity Food intake unchanged</td>
<td>Increased maternal allocation to litter</td>
<td>Leptin high</td>
<td>Paternal</td>
<td>Moon et al., 2002; Cleaton et al., 2016</td>
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<td></td>
<td>Tissue-appropriate overexpression (BAC transgenic)</td>
<td>Reduced WAT mass, improved glucose tolerance and insulin sensitivity Food intake unchanged</td>
<td>NR</td>
<td>Leptin low Adiponectin unchanged</td>
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<td>Charalambous, 2012</td>
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Abbreviations: BAC, bacterial artificial chromosome; BAT, brown adipose tissue; BT, body temperature; DMH, dorsomedial hypothalamus; HFD, high-fat diet; mRNA, messenger ribonucleic acid; NPY, neuropeptide Y; NR, not reported; POMC, pro-opiomelanocortin; SNS, sympathetic nervous system; WAT, white adipose tissue.
that caused by maternal $G_s$ (Relaix et al., 1996). Mice that inherit a protein is expressed in the placenta and ovaries as well as in some neuroendocrine tissues (Hiby et al., 2001; Kuroiwa et al., 1996; Paternally expressed gene 3 ($\text{Peg3}$).

Deaths due to maternal $G_s$ or MC4R mutant mice, and both were able to mount a transcriptional response to cold challenge in both BAT and by altering the balance of thermogenic brown adipose tissue (BAT) to white adipose tissue (WAT). Imprinted genes may act on additional pathways to modify body composition for brevity these mechanisms are omitted from this Review. In the hypothalamus, imprinted gene products are expressed in the first order onexigrionic (neuropeptide Y/Aguardi-related peptide, NPY/AGRP) and anorexigienic (pro-opiomelanocortin, POMC) arcuate nucleus (arcuate nucleus of the hypothalamus, ARH) neurons and modulate their sensitivity to leptin. These neurons interact with other hypothalamic structures such as the dorsomedial hypothalamus (DMH) and paraventricular nucleus (PVN) to mediate the physiological responses to leptin, such as suppression of appetite and increased energy expenditure. Gene products that promote leptin production or the activity of leptin signalling are shown in orange, those that suppress leptin production or its actions are shown in green. References are listed in Table 2. Abbreviations: ASB4, Ankyrin repeat, SOCS box-containing 4; CDKN1C, cyclin-dependent kinase inhibitor 1C; DLK1, Delta-like homologue 1; GRB10, growth factor bound protein 10; PEG1/MEST, paternally expressed gene 1/mesodermally expressed transcript.

were recapitulated when the MC4R was ablated in the DMH, suggesting that they are a direct result of aberrant signalling through this pathway. Surprisingly, cold tolerance was not affected in either the mother or offspring having a Peg3 mutation are strikingly similar, with pups having reduced protection from the cold and reduced growth pre- and postnatally. Despite the functional similarity, Peg3 is expressed primarily in the paraventricular nucleus of the hypothalamus in the adult, an area known to regulate maternal behaviours and milk letdown, whereas the dysfunction due to mutation in the pups is most likely to be because of the actions of Peg3 in the placenta. As noted earlier, the placenta regulates maternal metabolic adaptation and maternal behaviours through endocrine signalling, and the loss of Peg3 in the offspring is associated with the altered expression of prolactin-like genes (Broad and Keverne, 2011; John, 2013). The placenta and hypothalamus may interact through endocrine signalling, leading to a cumulative effect seen when both mother and offspring lack Peg3 (Curley et al., 2004).

Asb4
Ankyrin repeat, SOCS box-containing 4 (Asb4) is a ubiquitin ligase that is maternally expressed in mice but not imprinted in humans. It is expressed in the first-order POMC and NPY/AGRP neurons of the hypothalamus and its transcription is regulated by fasting, leptin and insulin injection. Overexpression of Asb4 in POMC neurons causes a hypermetabolic phenotype with increased energy expenditure, reduced adipose mass and resistance to weight gain on a high-fat diet. Therefore, ASB4 dosage mediates the sensitivity of POMC neurons to leptin given that increased gene dosage promotes leptin signalling (Li et al., 2010). In mice, Asb4 also plays a role in the early vascular differentiation of the placenta (Townley-Tilson et al., 2014).

Prader–Willi syndrome cluster
$\text{Snord116}$ maps to the critical region for Prader–Willi syndrome (PWS) (characterised by early hypertonia and a failure to thrive followed by hyperphagia at 2–3 years and obesity). It encodes a C/D box small nuclear RNA (SnRNA). This family of catalytic RNAs regulate RNA processing; however, the targets of $\text{Snord116}$ are unknown. In the mouse, $\text{Snord116}$ is exclusively expressed in the brain, and localised to the appetite centres (Cavaillé et al., 2000). Deletion of $\text{Snord116}$ in mice causes postnatal growth retardation

father are growth retarded at birth and remain small throughout life. After weaning, males show delayed adipose accumulation and females enter puberty late. In adulthood, the animals have increased white adipose deposition and very high levels of circulating leptin. The animals show reduced energy expenditure, reduced resting body temperature and are less able to defend body temperature following cold challenge (Curley et al., 2005). Peg3 is expressed in the developing hypothalamus (Li et al., 1999). In young mice with deleted Peg3, the transcriptional regulation of neuropeptides in the feeding centres of the hypothalamus is disrupted. Although this is resolved in adulthood, the animals remain less sensitive to leptin challenge, suggesting a permanent change in the set points of central energy homeostasis (Curley et al., 2005).

Females with an ablated Peg3 gene are poor mothers (Li et al., 1999). During pregnancy, Peg3 mutant mothers fail to increase food intake in early gestation and, consequently, have a reduced post-partum adipose reserve. In addition, wild-type pups born to Peg3 mutant mothers do not gain weight appropriately and enter puberty late. Mutant mothers fail to express maternal-specific behaviours such as pup-retrieval and nest building, and litters from such mothers are much less likely to survive to weaning (Curley et al., 2004; Li et al., 1999).

Interestingly, the consequences of either the mother or offspring having a Peg3 mutation are strikingly similar, with pups having reduced protection from the cold and reduced growth pre- and postnatally. Despite the functional similarity, Peg3 is expressed primarily in the paraventricular nucleus of the hypothalamus in the adult, an area known to regulate maternal behaviours and milk letdown, whereas the dysfunction due to mutation in the pups is most likely to be because of the actions of Peg3 in the placenta. As noted earlier, the placenta regulates maternal metabolic adaptation and maternal behaviours through endocrine signalling, and the loss of Peg3 in the offspring is associated with the altered expression of prolactin-like genes (Broad and Keverne, 2011; John, 2013). The placenta and hypothalamus may interact through endocrine signalling, leading to a cumulative effect seen when both mother and offspring lack Peg3 (Curley et al., 2004).
C57BL6 mice are fed a high-fat diet they show a variable adipose hydrolase fold family (Kaneko-Ishino et al., 1995). When inbred Cdkn1c increasing cluster (Chibuk et al., 2001). Deletion of Cdkn1c from the paternally inherited allele in mice phenocopies some aspects of PWS: the animals fail to thrive in the early postnatal period, but later experience catch-up growth and as adults have increased adiposity (Bischof et al., 2007). However, this energy imbalance is not caused by hyperphagia in the mutant mice because although they eat less, they have reduced energy expenditure. Magel2 deletion causes multiple defects in hypothalamic function, including the insensitivity of first order POMC neurons to leptin (Mercer et al., 2013). Leptin insensitivity in Magel2 knock-out animals is associated with an impairment in the developmental accumulation of neuronal connections between arcuate nucleus neurons and their targets, which occurs in the early postnatal period (Maillard et al., 2016; Pravdivyi et al., 2015).

Imprinted gene actions in peripheral tissues

Peg1/Mest

Peg1/Mest is paternally expressed and encodes a member of the α/β-hydrolase fold family (Kaneko-Ishino et al., 1995). When inbred C57BL6 mice are fed a high-fat diet they show a variable adipose tissue gain response. Peg1/Mest messenger RNA levels are induced in WAT following only 2 days of high-fat feeding, and expression positively correlates with weight gain (Koza et al., 2006). Peg1/ Mest levels have been shown to correlate with adipocyte size in a variety of genetic and dietary models of obesity (Nikonova et al., 2008; Takahashi et al., 2005), and overexpression of Peg1/Mest in adipocytes causes increased cell size. Mice with deleted Peg1/Mest have reduced adipose stores and reduced adipokine production. It has been proposed that Peg1/Mest induction during positive energy balance controls the initial phase of adipose tissue expansion by adipocyte hypertrophy (Nikonova et al., 2008).

Peg1/Mest mutant mothers exhibit abnormal maternal behaviour – they fail to clean new-born pups and ingest extraembryonic tissues, they do not retrieve pups to the nest, and perform poorly at nest building. Consequently, pup survival is severely compromised (Lefebvre et al., 1998).

Necdin

Necdin function in multiple contexts has been associated with the restriction of cell proliferation, at least in part through interactions with p53 (Hasegawa and Yoshikawa, 2008). Necdin is paternally expressed in mice and humans, where it was mapped to the PWS cluster (Chibuk et al., 2001). Deletion of Necdin in mice causes hyperproliferation of the WAT compartment without affecting central energy balance (Fujiwara, 2012). In mutant mice, the WAT depot size increased without a concurrent increase in fat cell size, indicating that expansion occurred by a mechanism of preadipocyte hyperplasia (Fujiiwara, 2012).

Cdkn1c

Increasing Cdkn1c expression in tissues where it is normally expressed results in a phenotype similar to Silver–Russell syndrome, pre- and postnatal growth retardation with failure to gain adipose mass. Cdkn1c is expressed in multiple adipose tissue depots in early perinatal life, and the level of expression reflects the propensity of that depot to ‘brown’ – i.e. to express thermogenic genes following stimulus. Mice with an increased Cdkn1c dosage showed increased thermogenic gene activity in WAT and BAT and increased body temperature. Deletion of Cdkn1c results in perinatal lethality and, therefore, the postnatal phenotype could not be assessed. However, ablation of Cdkn1c causes impaired BAT development during embryogenesis (Van De Pette, 2016).

Grb10

Unusually for an imprinted gene, Grb10 is both maternally and paternally expressed, but in different tissues. Maternal expression is seen in the peripheral tissues whereas expression in the central nervous system is solely paternal, with maternally expressed Grb10 controlling growth and energy homeostasis, and paternal Grb10 regulating social dominance behaviours (Garfield et al., 2011). Postnatally, maternally inherited deletion of Grb10 results in increased lean mass, reduced adipose mass (with low leptin) and improved glucose homeostasis (Smith et al., 2007). Food intake is not grossly affected. Grb10 expression is low in mature fat, but can be induced in brown adipocytes by cold or adrenergic stimulation (Liu et al., 2014). The deletion of Grb10 in mature fat cells causes the failure of BAT to respond to stimuli and, consequently, thermogenesis is impaired (Liu et al., 2014).

Cross-fostering studies have been used to disentangle the effects of Grb10 loss in either the mother or the offspring on offspring growth. These studies showed that Grb10 plays complementary roles in the offspring and the mother. Wild-type pups cross-fostered onto Grb10-deleted mothers (inheriting a deleted allele from their mother) show reduced growth in early life compared with those reared by wild-type mothers, suggesting that the normal role for Grb10 in the mother is to increase resource allocation to the offspring (Cowley et al., 2014). Interestingly, Grb10 mutant pups fostered onto wild-type mothers gain more weight than wild-type pups; however, this effect is negated if they are raised by mutant mothers – indicating that the balance of Grb10 dosage in the mother and pup is a crucial determinant of nutrient flow from the mother to her offspring (Cowley et al., 2014). Maternal care provision and milk let-down were unaltered by Grb10 deletion, but in response to increased demand and corresponding increased prolactin levels, Grb10 mutant mothers could not increase milk production, suggesting resistance to elevated prolactin. Grb10 is expressed in the mammary epithelium during pregnancy; however, its role here is unclear because no defect was found in the tissue morphology or the constituents of the milk produced by Grb10 mutant females (Cowley et al., 2014).

Dlk1

Dlk1 has been widely reported as an inhibitor of preadipocyte differentiation because the soluble form of the protein can inhibit in vitro differentiation of 3T3-L1 cells or mesenchymal cells into adipocytes (Smas and Sul, 1997). Moreover, ectopic expression of Dlk1 in adult WAT causes lipodystrophy (Lee et al., 2003). However, lineage-appropriate overexpression of Dlk1 from a bacterial artificial chromosome transgene (Dlk1-TG) does not cause a failure of adipose expansion in adults, even when animals are challenged with the hyperphagic leptin-deficient background (Charalambous et al., 2014). Instead, Dlk1-TG mice have a larger proportion of small adipocytes with increased insulin sensitivity compared with wild-type mice. Conversely, Dlk1 null mice have
lager adipocytes than wild-type mice (Moon et al., 2002). Manipulation of Dlk1 dosage has the expected effect on circulating leptin levels: Dlk1 null mice have elevated leptin and Dlk1-TG have reduced levels. However, despite this, food intake is not altered by either genetic manipulation (Charalambous et al., 2014; Cleaton et al., 2016), suggesting impaired leptin sensitivity. Dlk1 is likely to be acting during early life to increase adipocyte number given that a lineage tracing study of cells expressing GFP from a minimal Dlk1 promoter labelled adipose tissue in all depots of the adult mouse. Moreover, ablation of this embryonic DLK1-positive cell population caused a reduction in adipose depot size (Hudak et al., 2014).

Dlk1 null mothers give birth to larger litters than wild-type mothers (approximately one extra pup per litter), and overall litter mass is increased. Moreover, the conceptus mass of like-genotype offspring in litters from Dlk1 null mothers is not different from that in litters from mothers with an intact Dlk1 gene. Therefore, Dlk1 null mothers increase their investment in each litter, rather than reducing offspring size to offset offspring number. In addition, although Dlk1 null mothers enter pregnancy with increased adipose stores, they gain less adipose mass during pregnancy than wild-type mothers. Altogether, females without a functional copy of Dlk1 invest more resources in pregnancy, suggesting that the normal role for Dlk1 in female reproduction is to decrease nutrient allocation (Cleaton et al., 2016).

Summary
Imprinted gene products act at multiple levels in the adipose–hypothalamic axis to modulate set points of energy homeostasis. Experiments from murine models with ablation or overexpression of imprinted genes demonstrate that they are required in the hypothalamus to modulate the sensitivity of neuroendocrine pathways to leptin. Moreover, the dosage of imprinted genes in developing and mature adipose tissue can modulate leptin secretion (summarised in Fig. 1).

The degree of maternal investment in pregnancy is positively correlated with the pre-pregnancy adipose reserve. Mothers with high BMI invest more in their own adipose reserves, and in the products of conception (Prentice and Goldberg, 2000). Leptin has been proposed as a potential mediator of this effect. Given that imprinted gene products can modulate both leptin production and central sensitivity to leptin, we predict that an impaired imprinted gene dosage in females may influence their resource allocation as mothers. To date, few imprinted genes have been tested explicitly for their role in maternal physiology. However, where data exist, the prediction that low leptin/leptin-resistant mothers should invest less in a reproductive cycle appears consistent. Peg3, Peg1/Mest and Grb10 deletions in the mother all reduce leptin signalling, and maternal investment is reduced; conversely Dlk1 deletion increases leptin production and maternal investment in pregnancy. Further work is required to establish whether other models of imprinted gene misregulation cause defects in the maternal phenotype, and if other adipokines (such as adiponectin) are involved.

Conclusions
Since their discovery nearly 30 years ago, imprinted genes have been a paradigm for exploring the epigenetic control of gene expression. Moreover, their roles in early life growth and placentation are undisputed. However, it is becoming increasingly clear that imprinted gene function has a wider role in maternal physiology during reproduction – both by modulating fetal and placental endocrine products that signal to alter maternal energy homeostasis, and by altering maternal energetic set points, thus producing downstream actions on nutrient provisioning. Uncovering the molecular nature of these pathways has a broad application in terms of understanding natural reproductive strategies and provides a basis for preventing complications to pregnancy in human populations.

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