The Leptin System in Patients with Classical Galactosaemia - Putative Role and Clinical Consequences

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Abstract

The hormone leptin is a polypeptide of 146 amino acids which is predominantly secreted by adipose tissue. Leptin has distinct effects on energy homeostasis, metabolism, neuroendocrine function and other systems through its interactive effects on the central nervous system (CNS) and peripheral tissues. It has a critical role on regulation of body weight, fat reserves and reproductive function. The leptin receptor (LepR, Ob-R) is expressed in different isoforms, with the main signaling carried out by its long glycosylated isoform, Ob-Rb. Soluble leptin receptor (sOb-R) represents the main binding protein for leptin in human blood and increases its bioavailability. There are numerous *N*-glycosylation sites which are of physiological relevance for receptor function. Galactose and other sugar moieties are physiologically relevant for glycosylation of complex molecules for a wide range of biological processes.

This review considers how leptin signaling pathways are dysregulated in the rare inherited disorder of carbohydrate metabolism, Classical Galactosaemia. This disease is caused by profound deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT). Classical Galactosaemia may cause significant morbidity and even mortality in neonates and long-term complications later in life, including pubertal delay and primary ovarian insufficiency (POI). The immediate removal of galactose from the diet is lifesaving in affected infants. The pathophysiology of its long-term complications is incompletely understood. Underlying mechanisms comprise intoxication with galactose and its metabolites together with altered glycosylation pathways and disruption of signaling pathways.

Here we discuss how interaction of leptin with the hypothalamic pituitary gonadal axis (HPG) and other circuits is potentially dysregulated in Classical Galactosaemia with clinical consequences for puberty and reproductive capacity, particularly in females. We have identified defective *N*-glycosylation as a major factor implicated in disrupted leptin-HPG signaling resulting from distorted receptor function. Finally, we speculate how leptin dysregulation may affect cognitive function and neuroprotection in patients with Classical Galactosaemia.

Keywords
Leptin; Leptin Receptor; Classical Galactosaemia; Glycosylation; Primary Ovarian Insufficiency

1. Introduction

Leptin has been a fascinating topic in regulation of energy balance since its discovery more than 20 years ago (1). It is a metabolic and neuroendocrine hormone which exerts a variety of central and peripheral effects which play an important role in the regulation of body fat stores and fertility. However, leptin is not only important in the regulation of appetite and food intake along with energy expenditure, but also for sexual maturation and reproductive function (2, 3), immune response (4) and bone formation (5). Furthermore, leptin exerts numerous effects on different endocrine axes, mainly on the hypothalamic-pituitary-gonadal axis (HPG)/hypothalamic-pituitary-ovarian (HPO) axis, hypothalamic-pituitary-adrenal axis, and also on insulin action (6).

Leptin is a non-glycosylated 146 amino acid polypeptide predominantly released by adipose tissue but also by the liver, kidneys and stomach (7). Under normal physiological function, circulating leptin is proportional to the amount of body fat present and acts as a vital signal to the brain regarding available energy for systematic processes (8). Individuals with hypoleptinaemia, who are suffering from reproductive complications, express phenotypes that are directly linked to leptin deficiency, such as hypothalamic amenorrhea or anorexia nervosa with delayed puberty and decreased bone density (9-11). Conversely, hyperleptinaemia is a commonly observed in obesity along with hyperinsulinaemia (12, 13).

Given the essential role of leptin in the modulation of the HPG axis, pubertal development and fertility (14, 15), it is a subject of interest and clinical relevance for the inherited metabolic disorder Classical Galactosaemia. Many patients with Classical Galactosaemia experience long-term complications such as pubertal delay and female infertility due to premature ovarian insufficiency (POI) (16).

The HPG is mainly driven by three leading sets of factors, the hypothalamic gonadotropin-releasing hormone (GnRH), pituitary gonadotropins luteinizing hormone (LH), follicle stimulating hormone (FSH) and steroidal/peptidergic gonadal hormones (17). These key elements are networked in a cross-talk web with internal and external cues which ensures that levels of hormone secretion and gonadal function are appropriate to environmental conditions and developmental stages (18). Normal physiological function of the HPG axis along with fertility is highly sensitive to body energy stores, particularly in females, as reproductive processes including pregnancy and lactation are highly energy demanding (19, 20). It is recognised that leptin has a crucial function in linking the magnitude of body fat stores to the reproductive axis with the influence of other neuroendocrine axes (19-21), and it has been well documented as an essential metabolic regulator of pubertal development and fertility via interaction with HPG components (19-23). It is suggested that leptin is a governing factor in the onset of puberty, although it is controversial whether leptin itself triggers puberty or attains a threshold blood concentration to allow puberty to occur (24-28).

Expression of LepR gene (Ob-R) occurs in the brain, particularly in the hypothalamus, choroid plexus and hippocampus, but also in other tissues, including the gonads (29). Structurally, Ob-R can be classified as a class I cytokine receptor. Although several isoforms have been identified, only the longest isoform, Ob-Rb, has full intracellular signaling capability, including activation of the JAK-STAT signal transduction pathway (30) and PI3K activation (31). In the blood stream, the soluble isoform, sOb-R, is thought to

regulate free leptin concentration by modulating its release (32).

The overarching aim of our studies and the main focus of this review are to focus on the leptin system in the context of the largely enigmatic pathophysiology of long-term complications of Classical Galactosaemia which is a challenging hereditary metabolic disorder. This review attempts to correlate the activity of leptin and its binding protein sOb-R with clinical complications seen in Classical Galactosaemia, with a particular focus on its potential relationship with reproductive complications, such as POI which commonly occurs in these patients.

2. Classical Galactosaemia – A metabolic disorder with a spectrum of complications

Classical Galactosaemia is a rare inborn disorder of carbohydrate metabolism (OMIM 230400). It is caused by mutations in the GALT gene and inherited autosomal recessively. Affected newborns typically develop symptoms soon after feeding, i.e. galactose/lactose intake, including liver failure, sepsis, brain damage, cataracts or even death. Immediate restriction of dietary galactose/lactose rescues the neonatal phenotype. However, a majority of patients develop long-term complications despite early diagnosis on newborn screening and strict life-long galactose/lactose-restricted diet (33, 34). Among these complications are developmental and pubertal delay, speech difficulties, language delay, and cognitive and/or behavioral impairment, neurological symptoms such as ataxia or tremor, growth retardation, low bone mass, and POI in females (16, 35). The pathophysiology of these long-term complications is multifactorial and largely enigmatic (36). It comprises of toxic build-up of galactose and its metabolites galactose-1-phosphate and galactitol, altered glycoprotein and glycolipid production, gene dysregulation and associated disruption of cell signaling pathways (36, 37). Although it is well-known that females with Classical Galactosaemia have a high risk of hypergonadotropic hypogonadism along with infertility as a diet-independent complication of the disease, the underlying mechanisms for POI in affected patients remains incompletely understood (38, 39). We have recently described systemic *N*-glycan processing defects along with the potential use of IgG *N*-glycans as biomarker in this disease (37). As aetiology and timing of POI as well as other long-term complications are not yet clear, the need for more accurate diagnostic biomarkers in this cohort arises, including, for example, correlating findings on the leptin system and glycosylation profiles with hormonal status, pubertal development and fertility.

3. Leptin, its actions on the neuroendocrine reproductive axis and role in fertility

Leptin has emerged as unpredictable in the way it links body energy reserves to normal physiological functions. Studies have shown a steady increase of leptin in girls during puberty (3, 40) which is less pronounced in boys but also in male rhesus monkeys (40, 41). In fact, boys experience a rise in leptin serum concentrations during early puberty flowed by a decline of leptin levels in late puberty (3, 42). At the same time, physiological leptin concentrations appear to be required for the functions of the male reproductive system (43).

The hypothalamus is the primary location for leptin action in puberty and fertility (43, 44), and along these lines, leptin is involved in releasing GnRH along with LH and FSH secretion (44). GnRH neurons are essential players in the neuroendocrine network that mediates sex steroid feedback and controls the gonadotropic system. However, they apparently lack the functional receptors for dominant regulators such as the oestrogen

receptor (45). In mice studies, it appears that while leptin injection causes a surge of gonadotropins, GnRH neurons do not directly respond to leptin injection. Further, while LepR is necessary for puberty and fertility, ablation of LepR in GnRH neurons does not alter their reproductive function (46). Conversely, cell culture studies with immortalized GnRH neurons show that LepR is expressed in GnRH neurons and that direct response to leptin is followed by detectable release of GnRH (47). However, the physiological relevance of these findings in humans and in human pathology is discussed.

Among candidates for intermediate relay between leptin and GnRH neurons are neuronal populations expressing pro-opiomelanocortin (POMC), and agouti-related protein (AgRP)/neuropeptide Y (NPY) which are located in the hypothalamic arcuate nucleus (ARC). Both POMC and AgRP/NPY are key players in the modulation of energy balance and contain obvious populations of LepR expressing cells (30, 48). The product of POMC appears to electrically stimulate a subset of GnRH neurons and POMC neurons apparently form synapses with those expressing GnRH, with stimulatory effects on the gonadotropic axis (49, 50). Interestingly, deletion of LepR from POMC neurons in mice has no deleterious effect on reproduction (51). However, when both insulin and LepR were eliminated together in murine POMC neurons, females experience abnormal follicular tissue in the ovary along with fertility impairment and disruption of the oestrous cycle, while males also show subfertility (52).

Data in mice has suggested that ARC AgRP neurons are also important components in the permissive effects of leptin on fertility, however, evidence from leptin knockout mice (*ob/ob* mice) suggests that AgRP, unopposed by leptin, can propel a negative signal towards the HPG axis (53). Coupled to this, NPY, which also released from AgRP neurons, may similarly drive a negative effect on the HPG in a murine model (54).

Another interesting candidate of metabolic interplay between leptin and GnRH neurons is Kisspeptin, a protein which is encoded by the *KISS1* gene. Kisspeptins have been described as key players in puberty, reproduction, and control of the HPG axis and have been documented as stimulators of GnRH in this regard (30, 55-58). Kiss1 neurons in the hypothalamic ARC express leptin mRNA, however, leptin signaling in Kiss1 neurons mainly occurs after completion of sexual maturation (59, 60). Interestingly, in the ewe model of low body weight on dietary restriction, leptin infusion partially restored the level of *KISS1* gene expression (61). Essentially, hypothalamic kisspeptin neurons respond to leptin, and gene expression of *KISS1* is affected by leptin status (61). Taken together, kisspeptin cells, NPY and POMC neurons substantially contribute to brain control of reproduction and metabolic homoeostasis.

In females with Classical Galactosaemia, hypogonadism along with decreased fertility is a frequent complication. In addition to POI, central dysregulation may also contribute to the underlying pathophysiology (33). The potential dysregulation of signaling pathways in Classical Galactosaemia is summarized in **Figure 1**.

It has been reported that some affected women with Classical Galactosaemia became pregnant spontaneously (62). Fluctuating POI can make predictions and fertility counseling of females with Classical Galactosaemia challenging (62). However, the majority of female patients, e.g., 91 % of female patients over 13 years in Ireland, suffer from hypergonadotropic hypogonadism (35). Hypogonadism due to

POI can be detected by ovarian imaging along with measuring FSH, LH, oestradiol and anti-Müllerian hormone (AMH) in the blood, however, given the inactivity of the HPO axis in childhood these markers may not be sensitive enough to identify ovarian dysfunction in a presymptomatic state. Therefore, there is a need for improved methods of monitoring of galactosaemic patients with regard to long-term outcome, including more informative biomarkers such as the leptin system, for example, along with translation into validated clinical practice.

4. Glycosylation biomarkers in Classical Galactosaemia

Glycosylation, the enzymatic attachment of glucose, galactose or other sugar moieties to specific protein residues, is a common post-translational modification of proteins. It has a great impact on protein structure and function, and carbohydrate–protein interactions are involved in many biological processes. Deregulation or altered glycosylation is associated with a wide range of diseases (33). We have hypothesized that long-term dietary over-restriction of galactose, an essential evolutionary conserved carbohydrate, may also have a role to play in the pathophysiology of long-term complications in Classical Galactosaemia. In this context, we have previously described glycosylation pattern given as galactose incorporation ratios as a novel method of studying the presence of *N*-glycan processing defects in children with Classical Galactosaemia (37).

We aimed at establishing if Classical Galactosaemia is a modifiable multi-systemic glycosylation defect in children on dietetic treatment. We, therefore, designed a pilot study in children with Classical Galactosaemia aged 5-12 years on a lactose-free diet (34). In the clinical setting, we provided temporary low-dose oral galactose supplements by using limited quantities of cow's milk. We assessed, firstly, tolerance and safety of temporary low-dose galactose supplementation, and, secondly, potential biochemical and endocrine markers as prognostic indices in these patients. We tested thirteen affected children on 300 mg of galactose/day which was then followed by 500 mg for 2 weeks each and compared their data with 13 matched patient controls. Galactose supplements were given with breakfast and well tolerated. We observed no clinical changes in the galactose supplementation group after 2 weeks of 300 mg of galactose intake and after 2 subsequent weeks of 500 mg of galactose and at the end of the study. In the galactose supplementation group, sOb-R concentrations in the blood at 500 mg of galactose supplementation were slightly higher than at 300 mg of galactose supplementation. As a trend, patients in the galactose supplementation group also had slightly higher sOb-R levels at the end of the study than patient controls without galactose supplements. Conversely, we found no significant changes in sOb-R serum levels for our patient controls in the course of the study. We then investigated individual glycosylation patterns, focusing on IgG *N*-glycans profiles in serum, and, in particular, the ratio of agalactosylated (G0) to digalactosylated (G2) *N*-glycans as previously described in detail (34). Within the galactose supplementation group (n=13) we identified 6 individuals (46%) as 'responders' with a decrease in the G0/G2 ratio, i.e. higher amounts of relative digalactosylated structures as a quantitative measure of galactose incorporation into glycoproteins. Conversely, in the patient control group there was only one patient (8%) with a spontaneous decrease in G0/G2 ratio indicative of an improved glycosylation pattern over the course of the study. Altogether, we found that temporary low-dose galactose supplementation in

children with Classical Galactosaemia over 5 years was well tolerated in the clinical setting. We observed changes in serum IgG *N*-glycosylation profile in the subgroup of 'responders'. There was a negative relationship between the IgG glycosylation ratio G0/G2 and serum sOb-R concentrations in the galactose supplementation group which may reflect an improved glycosylation pattern (34).

In a separate project from this group focusing on a fibroblast model (33), LepR gene expression was increased in samples from patients with Classical Galactosaemia patients compared with unaffected control samples. In more detail, we measured the expression of LepR using a time-course assay in Classical Galactosaemia human dermal fibroblasts (HDF) versus Normal Human Dermal Fibroblasts (NHDF) ((33) and unpublished data). All cells were grown in galactose-free media, and gene expression was measured at 30 minutes, 1 hour, 2 hours and 4 hours of incubation with galactose. We observed a statistically significant change in a Galactosaemia cell-line at 30 minutes, where expression was increased compared with baseline levels. As a trend expression of LepR increased at 1 and 2 hours of incubation and subsequently decreased. In addition to *in vitro* gene expression data, it is of interest to see that the leptin receptor is heavily glycosylated so that altered glycosylation could have further impact on leptin signaling in patients with Classical Galactosaemia.

Essentially, other glycosylated proteins could be also affected in Classical Galactosaemia, including G-protein coupled receptors (GPCRs) which are extensively glycosylated. An aberration of *N*-glycosylation in Classical Galactosaemia patients may contribute to disruption of receptor function. GnRH receptors are GPCRs (63), and in the pituitary, pulsatory doses of GnRH activate these GPCRs which stimulate downstream LH and FSH. It may be that Galactosaemia patients with defunct GnRH GPCRs from irregular *N*-glycosylation experience pubertal delay and reproductive complications from inadequately stimulated LH and FSH release despite varied leptin signaling.

Furthermore, glycosylated receptor Ob-R is, among others, expressed in the hippocampus, an area in the brain which is essential for learning and memory and LepR gene expression may related to learning performance and memory processing (64, 65). In this context we propose that aberrant glycosylation of Ob-R, as well as other glycoproteins/receptors could be of particular relevance in patients with Classical Galactosaemia on a strict diet who experience significant long-term complications, such as learning difficulties, developmental delay, cognitive dysfunction, failure to thrive and growth faltering.

5. Leptin in children and adults with Classical Galactosaemia

We recently described a potential systemic dysregulation of the serum leptin system in children and adults with Classical Galactosaemia with a trend towards hypoleptinaemia (66). In more detail, we investigated 10 girls (age 0.6–17.9 years, mean 7.7 years) and 18 boys (age 0.5–16.7 years, mean 7.6 years) with Classical Galactosaemia on a strict galactose-restricted diet. We calculated standard deviation scores (SDS) for leptin serum concentrations to compare our paediatric cohort to the reference population. We found lower leptin levels and leptin-SDS in affected children compared to age-matched healthy controls (i.e., mean leptin-SDS -0.71 for girls; -0.97 for boys compared with SDS 0 for controls). As expected, girls had significantly higher serum leptin concentrations than boys in both children with Classical Galactosaemia and controls

(4.4 ± 4.1 ng/ml for affected girls versus 0.9 ± 0.5 for affected boys; and 8.1 ± 3.2 for female controls versus 2.1 ± 1.4 for male controls (66)). In age-related analysis, serum leptin levels did not correlate with age in the entire paediatric Classical Galactosaemia group or for gender comparisons. We also found no significant correlation between leptin and age when data of prepubertal boys with Classical Galactosaemia was tested separately to exclude effects of rising testosterone levels. Conversely, leptin was found positively correlated to age in our paediatric control group, i.e in our female paediatric controls and unaffected prepubertal boys. We then focused on the relationship between serum leptin and body mass index (BMI), a measure of body fat based on height and weight. In both paediatric cohorts, there was a significant linear relationship between log-leptin and BMI. In the same study (66), we also compiled data for adult patients with Classical Galactosaemia, 10 females (age 19–37 years, mean 25.4 years) and 12 males (age 18–28 years, mean 22.4 years). Higher leptin levels, along with higher BMI, were found in women compared to girls with Galactosaemia, while lower leptin levels were found despite higher BMI in men compared to boys with Galactosaemia. Serum leptin concentrations and log-leptin in male adults were lower than in female adults in the Galactosaemia and controls (66). In female adults with Galactosaemia, BMI was strongly associated with log-leptin values and this was also found in healthy controls of both sexes. Although serum leptin concentrations were within normal limits for women and men with Galactosaemia when adjusted for gender and BMI the association between log-leptin and BMI was no longer detectable in male patients and in the entire group of Galactosaemia adults.

Potential dysregulation of serum leptin system in patients with Classical Galactosaemia may indicate a putative role for leptin/leptin receptor in the pathophysiology of long-term complications of this disease, including subfertility. It should be noted, however, that female subjects in this study (66) have been placed on hormone replacement therapy (HRT) as treatment for POI, obscuring any potential dysregulation of leptin system. Treatment with oestrogen in non-Galactosaemia individuals who had underwent bilateral ovariectomy for benign reasons showed a protective role of this hormone in preventing decrease in leptin concentrations (67). It is of interest to see that leptin deficiency in mice is associated with impaired folliculogenesis and also increased follicular atresia (68). These findings are reminiscent of those seen in ovarian tissue from females with Classical Galactosaemia (39). Childhood and early puberty might be a particular vulnerable period in individuals at risk of decreased reproductive capacity, particularly in females with Classical Galactosaemia (38, 39). However, reduced leptin levels were also found in adolescent boys with constitutional delay of growth and puberty (69). Alterations in leptin system and its impact on the reproductive axis may help to explain delayed pubertal development in adolescent boys with Classical Galactosaemia and perhaps also cryptorchidism which has been described in some cases (70).

Leptin dysregulation may play a role in the fertility issues associated with Classical Galactosaemia, particularly in females. Along these lines, we propose that leptin serum levels are valuable diagnostic parameters with regard to patients' individual degree of altered hormonal status along with abnormal metabolism and clinical consequences, such as pubertal delay or infertility. We speculate that some

patients with Classical Galactosaemia can tolerate varying degrees of limited galactose intakes later in childhood as they may have the ability to utilize auxiliary pathways of galactose metabolism, and that sOb-R might be among the modifiable glycoproteins. However, it is important to emphasise that long-term elimination of dietary galactose is the only available treatment for patients with Galactosaemia at present and that it is life-saving, especially in the neonate.

6. Leptin and neuroprotection

In addition to its key role in neuroendocrine pathways, leptin appears to exert further effects on the brain, including neuroplasticity and neuroprotection. Potential implications have been studied in number of clinical studies which support the concept of a pro-cognitive effect of leptin (71-72), potentially via activating AMP-dependent kinase at a cellular level (73). In more detail, a study of leptin levels in the elderly, it was found that higher levels of serum leptin appear to protect individuals from cognitive decline, indicating a potential neuroprotective role for leptin in cognition during ageing (71). In a morbidly obese boy with primary leptin deficiency due to a leptin gene mutation (OMIM #614962), replacement with recombinant leptin improved his delayed cognitive development by age 7 years (72). Leptin replacement therapy is now well established in the treatment of genetic leptin deficiency and has demonstrated an excellent safety profile, even after prolonged treatments (72). In genetic leptin deficiency, treatment with recombinant leptin leads to marked weight loss, resolution of hypertension, dyslipidaemia and hyperinsulinaemia as well as improvement of neurocognitive profile in many neurocognitive domains (72). These findings indicate that leptin may have a cognitive enhancing role in the developing brain in humans. Furthermore, *in vitro* studies on primary neurons and neuronal cell lines (73) support these observational studies. In more detail, incubation with leptin reduces the phosphorylation of tau protein and apparently ameliorates both amyloid beta and tau-related pathologies of Alzheimer's disease (73). This *in vitro* data support the use of leptin as a novel therapeutic approach for Alzheimer's disease.

As summarized earlier (66), we described lower serum leptin levels in children with Classical Galactosaemia compared with age-matched controls. Given that leptin has peripheral, hypothalamic and extra-hypothalamic effects (14, 74), we can only speculate whether decreased levels at a neuroendocrine could have potential clinical consequences for, e.g., pubertal maturation and cognitive development in patients with Classical Galactosaemia. Although it is not possible to directly compare subtle alterations in the leptin system with severe genetic leptin deficiency, it is of particular relevance to see that leptin replacement therapy in ultra-rare primary leptin deficiency has been shown to improve cognitive development in childhood (72) and later in life (75).

In this context, it is of interest to discuss preliminary results from our *in vitro* pilot study focussing on leptin expression in fibroblasts of Classical Galactosaemia patients. There was a trend towards lower leptin gene expression in a subgroup of Classical Galactosaemia patients with significant neurological morbidity compared with patients with good neurological outcome, i.e., a 3.7 fold decrease in poor versus good outcome patients (unpublished data, 2015). The significance of these *in vitro* findings needs to be established; however, given that disruption of leptin signalling may have a role in cognitive defects we can only speculate that the decreased expression of leptin, e.g. in

fibroblasts, may indicate a potential systemic role for this pathway in Classical Galactosaemia patients. Leptin has a cognitive enhancing role in the CNS where it affects synaptic plasticity and neuroendocrine circuits (72). In this context, larger-scale studies are needed to examine the underlying mechanisms together with potential diagnostic and clinical implications in the inherited metabolic disease Classical Galactosaemia.

Summary

We propose that an altered leptin system may contribute to dysregulation of reproductive pathways, along with other long-term complications, in patients with Classical Galactosaemia at a neuroendocrine level from an early age. It seems possible that leptin dysregulation may also have a potential role in cognitive complications which can be associated with Classical Galactosaemia. Endogenous intoxication and long-term dietary over-restriction of galactose along with altered glycosylation may have a role to play in the pathophysiology of this inborn metabolic disease. In this review, we focus on dysregulation of glycosylation processes and key pathways including leptin and its binding sites (Ob-R, sOb-R), highlighting the need for further research into these pathways in the metabolic disease context. There is growing body of evidence that metabolic hormones including the leptin system are promising biomarkers to monitor patients with Classical Galactosaemia. However, larger-scale clinical studies are needed along with translation into validated clinical practice.

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References

[1] Zhang Y, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994; 372(6505):425-32.
[2] Wauters M, Considine RV, Chagnon M, et al. Leptin levels, leptin receptor gene polymorphisms, and energy metabolism in women. Obesity research. 2002; 10(5):394-400.
[3] Kratzsch J, Lammert A, Bottner A, et al. Circulating soluble leptin receptor and free leptin index during childhood, puberty, and adolescence. The Journal of clinical endocrinology and metabolism. 2002; 87(10):4587-94.
[4] Otero M, Lago R, Lago F, et al. Leptin, from fat to inflammation: old questions and new insights. FEBS letters. 2005; 579(2):295-301.
[5] Karsenty G. Convergence between bone and energy homeostases: leptin regulation of bone mass. Cell Metab. 2006; 4(5):341-8.
[6] Young EA, Midgley AR, Carlson NE, et al. Alteration in the hypothalamic-pituitary-ovarian axis in depressed women. Archives of general psychiatry. 2000; 57(12):1157-62.
[7] Meissner U, Hanisch C, Ostreicher I, et al. Differential regulation of leptin synthesis

in rats during short-term hypoxia and short-term carbon monoxide inhalation. Endocrinology. 2005; 146(1):215-20.
[8] Maffei M, Halaas J, Ravussin E, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nature medicine. 1995; 1(11):1155-61.
[9] Chou SH, Chamberland JP, Liu X, et al. Leptin is an effective treatment for hypothalamic amenorrhea. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(16):6585-90.
[10] Chan JL, Mantzoros CS. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. Lancet (London, England). 2005; 366(9479):74-85.
[11] Welt CK, Chan JL, Bullen J, et al. Recombinant human leptin in women with hypothalamic amenorrhea. The New England journal of medicine. 2004; 351(10):987-97.
[12] Knerr I, Herzog D, Rauh M, et al. Leptin and ghrelin expression in adipose tissues and serum levels in gastric banding patients. European journal of clinical investigation. 2006; 36(6):389-94.
[13] Nobili V, Manco M, Ciampalini P, et al. Leptin, free leptin index, insulin resistance and liver fibrosis in children with non-alcoholic fatty liver disease. European journal of endocrinology / European Federation of Endocrine Societies. 2006; 155(5):735-43.
[14] Goumenou AG, Matalliotakis IM, Koumantakis GE, et al. The role of leptin in fertility. European journal of obstetrics, gynecology, and reproductive biology. 2003; 106(2):118-24.
[15] Myers MG, Jr. Leptin receptor signaling and the regulation of mammalian physiology. Recent progress in hormone research. 2004; 59:287-304.
[16] Hughes J, Ryan S, Lambert D, et al. Outcomes of siblings with classical galactosemia. J Pediatr. 2009; 154(5):721-6.
[17] Schwartz N. Neuroendocrine Regulation of Reproductive Cyclicity. In: Conn PM, Freeman M, eds. Neuroendocrinology in Physiology and Medicine: Humana Press; 2000. p. 135-45.
[18] Fink G. Neuroendocrine Regulation of Pituitary Function. In: Conn PM, Freeman M, eds. Neuroendocrinology in Physiology and Medicine: Humana Press; 2000. p. 107-33.
[19] Fernandez-Fernandez R, Martini AC, Navarro VM, et al. Novel signals for the integration of energy balance and reproduction. Molecular and Cellular Endocrinology. 2006; 254–255:127-32.
[20] Casanueva FF, Dieguez C. Neuroendocrine Regulation and Actions of Leptin. Frontiers in Neuroendocrinology. 1999; 20(4):317-63.
[21] Mantzoros CS, Magkos F, Brinkoetter M, et al. Leptin in human physiology and pathophysiology. American journal of physiology Endocrinology and metabolism. 2011; 301(4):E567-84.
[22] Tena-Sempere M. Roles of ghrelin and leptin in the control of reproductive function. Neuroendocrinology. 2007; 86(3):229-41.
[23] Cheung CC, Thornton JE, Kuijper JL, et al. Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinology. 1997; 138(2):855-8.
[24] Ahima RS, Dushay J, Flier SN, et al. Leptin accelerates the onset of puberty in normal female mice. The Journal of clinical investigation. 1997; 99(3):391-5.
[25] Chehab FF, Mounzih K, Lu R, et al. Early onset of reproductive function in normal female mice treated with leptin. Science (New York, NY). 1997; 275(5296):88-90.
[26] Farooqi IS, Jebb SA, Langmack G, et al. Effects of recombinant leptin therapy in a

child with congenital leptin deficiency. The New England journal of medicine. 1999; 341(12):879-84.
[27] Barash IA, Cheung CC, Weigle DS, et al. Leptin is a metabolic signal to the reproductive system. Endocrinology. 1996; 137(7):3144-7.
[28] Castellano JM, Roa J, Luque RM, et al. KiSS-1/kisspeptins and the metabolic control of reproduction: physiologic roles and putative physiopathological implications. Peptides. 2009; 30(1):139-45.
[29] Donato J, Jr., Cravo RM, Frazao R, et al. Hypothalamic sites of leptin action linking metabolism and reproduction. Neuroendocrinology. 2011; 93(1):9-18.
[30] Elias CF, Purohit D. Leptin signaling and circuits in puberty and fertility. Cellular and molecular life sciences : CMLS. 2013; 70(5):841-62.
[31] Trinko R, Gan G, Gao X-B, et al. Erk1/2 Mediates Leptin Receptor Signaling in the Ventral Tegmental Area. PloS one. 2011; 6(11):e27180.
[32] Lavens D, Piessevaux J, Tavernier J. Review: Negative regulation of leptin receptor signalling. European cytokine network. 2006; 17(3):211-9.
[33] Coss KP, Treacy EP, Cotter EJ, et al. Systemic gene dysregulation in classical Galactosaemia: Is there a central mechanism? Molecular genetics and metabolism. 2014; 113(3):177-87.
[34] Knerr I, Coss KP, Kratzsch J, et al. Effects of temporary low-dose galactose supplements in children aged 5-12 y with classical galactosemia: a pilot study. Pediatr Res. 2015; 78(3):272-9.
[35] Coss KP, Doran PP, Owoeye C, et al. Classical Galactosaemia in Ireland: incidence, complications and outcomes of treatment. J Inherit Metab Dis. 2013; 36(1):21-7.
[36] Coman DJ, Murray DW, Byrne JC, et al. Galactosemia, a single gene disorder with epigenetic consequences. Pediatr Res. 2010; 67 286-92.
[37] Coss KP, Byrne JC, Coman DJ, et al. IgG N-glycans as potential biomarkers for determining galactose tolerance in Classical Galactosaemia. Mol Genet Metab. 2012; 105(2):212-20.
[38] Fridovich-Keil JL, Walter JH. Galactosaemia Chapter 72. The Online Metabolic and Molecular Bases of Inherited Disease, OMMBID. New York: McGraw Hill; 2008: New York: McGraw Hill; 2008
[39] Rubio-Gozalbo ME, Gubbels CS, Bakker JA, et al. Gonadal function in male and female patients with classic galactosemia. Human reproduction update. 2010; 16(2):177-88.
[40] Garcia-Mayor RV, Andrade MA, Rios M, et al. Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. The Journal of clinical endocrinology and metabolism. 1997; 82(9):2849-55.
[41] Plant TM, Durrant AR. Circulating leptin does not appear to provide a signal for triggering the initiation of puberty in the male rhesus monkey (Macaca mulatta). Endocrinology. 1997; 138(10):4505-8.
[42] Mantzoros CS, Flier JS, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. The Journal of clinical endocrinology and metabolism. 1997; 82(4):1066-70.
[43] Vazquez MJ, Romero-Ruiz A, Tena-Sempere M. Roles of leptin in reproduction, pregnancy and polycystic ovary syndrome: consensus knowledge and recent developments. Metabolism. 2015; 64(1):79-91.
[44] Watanobe H. Leptin directly acts within the hypothalamus to stimulate gonadotropin-releasing hormone secretion in vivo in rats.

The Journal of physiology. 2002; 545(Pt 1):255-68.
[45] Herbison AE, Pape JR. New evidence for estrogen receptors in gonadotropin-releasing hormone neurons. Frontiers in neuroendocrinology. 2001; 22(4):292-308.
[46] Quennell JH, Mulligan AC, Tups A, et al. Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. Endocrinology. 2009; 150(6):2805-12.
[47] Magni P, Vettor R, Pagano C, et al. Expression of a leptin receptor in immortalized gonadotropin-releasing hormone-secreting neurons. Endocrinology. 1999; 140(4):1581-5.
[48] Williams KW, Elmquist JK. From neuroanatomy to behavior: central integration of peripheral signals regulating feeding behavior. Nature neuroscience. 2012; 15(10):1350-5.
[49] Xu Y, Faulkner LD, Hill JW. Cross-Talk between Metabolism and Reproduction: The Role of POMC and SF1 Neurons. Frontiers in endocrinology. 2011; 2:98.
[50] Roa J, Herbison AE. Direct regulation of GnRH neuron excitability by arcuate nucleus POMC and NPY neuron neuropeptides in female mice. Endocrinology. 2012; 153(11):5587-99.
[51] Balthasar N, Coppari R, McMinn J, et al. Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. Neuron. 2004; 42(6):983-91.
[52] Hill JW, Elias CF, Fukuda M, et al. Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. Cell metabolism. 2010; 11(4):286-97.
[53] Wu Q, Whiddon BB, Palmiter RD. Ablation of neurons expressing agouti-related protein, but not melanin concentrating hormone, in leptin-deficient mice restores metabolic functions and fertility. Proc Natl Acad Sci U S A. 2012; 109(8):3155-60.
[54] Erickson JC, Hollopeter G, Palmiter RD. Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. Science (New York, NY). 1996; 274(5293):1704-7.
[55] Elias CF. Leptin action in pubertal development: recent advances and unanswered questions. Trends in endocrinology and metabolism: TEM. 2012; 23(1):9-15.
[56] Navarro VM, Tena-Sempere M. Neuroendocrine control by kisspeptins: role in metabolic regulation of fertility. Nature reviews Endocrinology. 2012; 8(1):40-53.
[57] Oakley AE, Clifton DK, Steiner RA. Kisspeptin signaling in the brain. Endocrine reviews. 2009; 30(6):713-43.
[58] d'Anglemont de Tassigny X, Colledge WH. The role of kisspeptin signaling in reproduction. Physiology (Bethesda, Md). 2010; 25(4):207-17.
[59] Cravo RM, Margatho LO, Osborne-Lawrence S, et al. Characterization of Kiss1 neurons using transgenic mouse models. Neuroscience. 2011; 173:37-56.
[60] Smith JT, Acohido BV, Clifton DK, et al. KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. Journal of neuroendocrinology. 2006; 18(4):298-303.
[61] Backholer K, Smith JT, Rao A, et al. Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide Y and proopiomelanocortin cells. Endocrinology. 2010; 151(5):2233-43.
[62] Gubbels CS, Kuppens SMI, Bakker JA, et al. Pregnancy in classic galactosemia despite undetectable anti-Müllerian hormone. Fertility and Sterility. 2009; 91(4):1293.e13-.e16.
[63] Millar RP, Lu ZL, Pawson AJ, et al. Gonadotropin-releasing hormone receptors. Endocrine reviews. 2004; 25(2):235-75.
[64] Moult PR, Harvey J. Hormonal regulation of hippocampal dendritic morphology and synaptic plasticity. Cell adhesion & migration. 2008; 2(4):269-75.

[65] Schlichting ML, Mumford JA, Preston AR. Learning-related representational changes reveal dissociable integration and separation signatures in the hippocampus and prefrontal cortex. Nature communications. 2015; 6:8151.
[66] Knerr I, Coss KP, Doran PP, et al. Leptin levels in children and adults with classic Galactosaemia. JIMD reports. 2013; 9:125-31.
[67] Messinis IE, Kariotis I, Milingos S, et al. Treatment of normal women with oestradiol plus progesterone prevents the decrease of leptin concentrations induced by ovariectomy. Hum Reprod. 2000; 15(11):2383-7.
[68] Hamm ML, Bhat GK, Thompson WE, et al. Folliculogenesis is impaired and granulosa cell apoptosis is increased in leptin-deficient mice. Biol Reprod. 2004; 71(1):66-72.
[69] El-Eshmawy MM, Abdel Aal IA, El Hawary AK. Association of ghrelin and leptin with reproductive hormones in constitutional delay of growth and puberty. Reprod Biol Endocrinol. 2010; 8:153.
[70] Gubbels CS, Welt CK, Dumoulin JC, et al. The male reproductive system in classic galactosemia: cryptorchidism and low semen volume. J Inherit Metab Dis. 2012.
[71] Holden KF, Lindquist K, Tylavsky FA, et al. Serum leptin level and cognition in the elderly: Findings from the Health ABC Study. Neurobiology of aging. 2009; 30(9):1483-9.
[72] Paz-Filho GJ, Babikian T, Asarnow R, et al. Leptin replacement improves cognitive development. PloS one. 2008; 3(8):e3098.
[73] Greco SJ, Sarkar S, Johnston JM, et al. Leptin reduces Alzheimer's disease-related tau phosphorylation in neuronal cells. Biochemical and biophysical research communications. 2008; 376(3):536-41.
[74] Paz-Filho G, Wong ML, Licinio J. Ten years of leptin replacement therapy. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2011; 12(5):e315-23.
[75] Matochik JA, London ED, Yildiz BO, et al. Effect of leptin replacement on brain structure in genetically leptin-deficient adults. The Journal of clinical endocrinology and metabolism. 2005; 90(5):2851-4.

Legend to figure

Figure 1
Potential dysregulation of signalling pathways in Classical Galactosaemia

1. Cross talk of leptin (from adipose tissue) and insulin (from the pancreas) signaling pathways; altered response in the CNS due to altered sOb-R in the bloodstream and Ob-R in the brain as a result of *N*-glycosylation abnormalities [15, 31-33, 53].
2. Leptin differentially engages hypothalamic NPY and POMC neurons; potential dysregulation and altered neuroprotective capabilities of leptin, e.g. due to alterations in glycosylation affecting Ob-R-expressing neurons in hypothalamic nuclei [29, 52, 69, 71-72].
3. Effect on hippocampal function due to distorted Ob-R [64-65, 72-74].
4. Altered response to GnRH in the anterior pituitary due to contorted GnRH GPCRs resulting from altered *N*-glycosylation [33, 44, 46, 63].
5. Altered expression of LH and FSH from the anterior pituitary as a result of pathway **4**.

