Obesity and the reproductive axis

F.P. Pralong (1), E. Castillo (1), P.D. Raposinho (2), M.L. Aubert (2), R.C. Gaillard (1)

(1) Division of Endocrinology, Diabetes and Metabolism, University of Lausanne Medical School, 1011 Lausanne, Switzerland.
(2) Division of Pediatric Endocrinology and Diabetology, University of Geneva Medical School, 1211 Geneva 14, Switzerland.

INTRODUCTION

Reproductive activity in mammals is crucially dependent upon sufficient levels of energy supply. The relationship existing between the onset of puberty and the level of adiposity, representing an index of the energy stores of the body, was first established in humans some 40 years ago by Frisch and McArthur [15]. In their milestone paper, these two authors proposed the so-called « critical fatness hypothesis », suggesting that puberty could not begin before a certain amount of energy was stored under the form of body fat. This hypothesis was later verified in animal models of puberty [14, 18], but most investigators now acknowledge that a minimum amount of body fat is a necessary but not sufficient pre-requisite for regular cycles to occur. In humans, it is also well established now that conditions of excessive weight loss like anorexia nervosa [35, 37], or of insufficient weight gain as observed in athletes [36], are accompanied by abnormalities of the menstrual cycle and infertility.

Adaptations of reproductive function to metabolism are mediated centrally at the level of the hypothalamic GnRH neurons [24]. Therefore, a considerable amount of work performed over the last two decades has focused upon the elucidation of the metabolic signal(s) responsible for transmitting information regarding body fat stores to the brain. This line of research culminated in 1994, with the cloning of leptin by Jeffrey Friedman and his colleagues [39]. Leptin, a secreted product of the adipocyte, is a satiety factor circulating in the periphery at levels proportionate to total body fat stores. Its main function is to signal the amount of body fat to the feeding centers of the hypothalamus [5]. Leptin deficiency, in rodent models as well as in humans, is accompanied by hyperphagic obesity [7, 26, 34, 39], and leptin replacement can correct this obese syndrome [12]. Remarkably, leptin deficiency also results in hypogonadotropic hypogonadism [7, 34] which can be rescued by exogenous leptin replacement [12]. These and many other observations (as reviewed in [13]) demonstrate that leptin indeed fulfills the criteria of a blood-borne hormone acting as a permissive factor upon pubertal development. Together, such data have helped clarify important mechanisms underlying the decrease in fertility observed in undernutrition. At the other end of the spectrum, obesity is also accompanied by irregularities of the menstrual cycle and/or infertility. Despite the existence of rare and very informative cases of monogenic human obesity, this condition in the vast majority of patients is not the result of leptin deficiency [8]. The relationship existing between human obesity and reproduction is discussed under the light of recent epidemiological data. Potential pathophysiological mechanisms implicating central leptin and insulin resistance are also reviewed.

OBESITY AND THE MENSTRUAL CYCLE: THE EPIDEMIOLOGICAL APPROACH

The relationship between obesity and irregularities of the menstrual cycle was first suggested in 1952 [30]. In that study, the authors found that the incidence of obesity was markedly increased among patients with a positive history of irregular cycles or amenorrhea compared to regularly cycling patients. Further suggesting the causal relationship with obesity, the same investigators were later able to demonstrate that normal menstrual cycles resumed after weight loss in affected patients [25]. Subsequent epidemiological studies mostly confirmed these original findings, although surprisingly little data are available.

In a very recent cohort study of 4900 pre-menopausal women aged 45 yrs or under, increased menstrual cycle length (>30 days) was associated with increased Body Mass Index (BMI), together with increasing age at menarche and parity [21]. On the other hand, decreased menstrual cycle length (<26 days) was associated with increasing age, cigarette smoking and ethnic origin (being non-white). In the final multivariate logistic re-
gression analysis of this cohort of American women living in New-York City, the likelihood of irregular cycles increased with BMI, increasing age and the number of cigarettes smoked per day [21]. These data probably represent the largest available human study demonstrating a relationship between obesity and cycle disorders. One of the very few existing prospective studies confirms the deleterious effects of obesity upon fertility [38]. In that study, the authors recruited 500 women attending a fertility clinic for artificial insemination with donor sperm. The main outcome measures were the probability of conception per cycle. This study showed that very lean or obese women were significantly less likely to conceive, as were women of increasing age [38]. Remarkably, a 0.1 unit increase in the waist/hip ratio lead to a 30 % decrease in the probability of conception per cycle, suggesting that body fat distribution could have more impact than obesity per se on fertility.

Despite such results suggesting that obesity is deleterious for female reproductive function, it should also be stressed that other well-executed studies failed to identify a clear-cut association between obesity and fertility [2, 23]. In a European, population-based study involving over 4000 pregnant women from 5 different countries, a strong association between obesity (BMI > 30 kg/m²) and delayed conception was only identified in the sub-group of women who smoked [2]. The same analysis performed in the non-smoker women disclosed no association [2]. These apparent inconsistencies of the literature can partially result from the difficulties to adjust for sociodemographic, biologic and lifestyle-related factors in large epidemiologic studies. However, they are also likely to reflect the fact that many obese patients will remain normally fertile. This observation suggests that obesity per se is not the sole explanation, and may relate to the very complex physiopathological basis of infertility problems in obesity. Another confounding factor may reside in the high prevalence of polycystic ovarian disease (PCOD) patients among overweight females. These patients are likely to experience fertility problems that are not directly or solely related to their obese phenotype. A complete discussion of the gonadotrope axis abnormalities specific to polycystic ovarian disease is beyond the scope of the present chapter, and the reader is referred to several reviews addressing this subject [1, 17, 20].

Finally, a population-based study published in 1978 and investigating the relationship between fertility and nutrition deserves mention here [9]. The authors used the gross birth rates of 61 different countries as the dependent variable. The independent variables (13 in total) introduced in the statistical analysis included socioeconomic criteria such as per capita income, percent urban population and newspaper circulation per 1000 persons. Nutritional data included daily caloric intake, protein intake, as well as index of nutrition. The values of all these variables were obtained from United Nations and UNESCO publications. In their analysis, the authors could show that when levels of nutrition increase from insufficient to sufficient, and then to excessive, fertility first increases, and then decreases again, suggesting for the first time that the relationship between fertility and nutrition has the shape of an inverted U [9].

**OBESITY AND THE MENSTRUAL CYCLE: THE PATHOPHYSIOLOGICAL APPROACH**

Different pathophysiological mechanisms participate to the disturbances of gonadotrope axis activity reported in obesity [29]. Until recently, insulin resistance was thought to be at the center of the dysregulations observed in obese syndromes. It is now increasingly recognized that leptin resistance, which has become another hallmark of obesity, probably plays an important role in the central dysregulation of reproductive function. Both mechanisms occur to some degree in simple obesity syndromes, as well as in PCOD. The two clinical entities are therefore extremely difficult to distinguish, since obese patients as well as PCOD patients share many metabolic and endocrine dysfunctions.

Obese patients have an altered balance between circulating androgens and estrogens, with higher concentrations of estrone and estradiol [40]. This is believed to result from the increased peripheral conversion of androgens to estrogens occurring in adipose tissue. As obese patients also experience an increased production of adrenal and gonadal androgens, this higher exposure of adipose tissue to androgens further increases net estrogen output. However, one should stress that this particular mechanism has been particularly well established in PCOD, rather than in simple obese women [1].

Overall, increased peripheral production of estrone and estriol results in somewhat acyclic production of estrogens, inducing changes in pituitary secretion of gonadotropins secondary to abnormal sex steroid feedback at the central level. Estrogens tend to inhibit FSH release and stimulate LH secretion, thus resulting in the characteristic augmented LH:FSH ratio. Then, these changes enter a sort of vicious cycle, with LH stimulating production androgens by the ovary, which in turn get aromatized in peripheral tissues with the resulting increase in circulating estrogens. Hyperinsulinemia also worsens the hyperandrogenemia by two mechanisms: it increases ovarian thecal production androgens, and decreases circulating levels of sex-hormone binding globulin. This results in a further increase in bioactive androgens circulating in the periphery. The exact role...
played by adrenal androgen overproduction in these phenomena is not yet completely understood.

**OBESITY AND THE MENSTRUAL CYCLE: THE HYPOTHALAMIC INVOLVEMENT**

**Sensing of insulin and leptin by the hypothalamus**

In addition to these various mechanisms occurring at the ovarian and possibly the adrenal levels, insulin and leptin resistance in obesity may also have significant and direct relevance for hypothalamic function. The hypothalamus is remarkable in that it integrates metabolic signals conveyed by these two peripheral factors, and consequently adapts food intake and energy expenditure [32]. This role is assumed by highly specialized neuronal sub-populations expressing an array of neurotransmitters involved in these regulations. In addition, the hypothalamus represents the center of activation of the different neuroendocrine axes, including reproductive function. Therefore, the hypothalamus is an anatomical and functional center of convergence of metabolic and neuroendocrine regulations. It could thus easily be hypothesized that metabolic signals like leptin or insulin, which influence the control of food intake [32], may also modulate the reproductive function at the level of the hypothalamus. This hypothesis is supported by the phenotype of the *Lep<sup>ob</sup>* mouse, an animal bearing an inactivating mutation of the leptin gene [39]. This model, which represents a state of absolute leptin resistance, exhibits profound hyperphagic obesity associated with hypothalamic hypogonadism [16]. Moreover, both defects can be corrected by exogenous leptin replacement [5, 6]. Therefore, leptin originating from the periphery can be viewed as a metabolic signal to the GnRH neurons: adequate circulating leptin levels provide a necessary facilitating input to these neurons. Whether leptin regulates directly the function of GnRH neurons, or rather acts via a modulation of intermediate neurotransmitters like Neuropeptide Y (NPY [33]) remains an open question.

Two recent studies came as the demonstration that indeed, insulin sensing by the hypothalamus also plays an essential role in the metabolic adaptations of reproductive functions [3, 4]. The first one deals with the physiological role of insulin signaling in the brain [3]. Indeed, the insulin receptor is specifically expressed in the hypothalamus and some other brain regions, but very little was known until recently about the functional significance of this expression. To address this issue, investigators succeeded in creating a mouse bearing a neuron-specific inactivation of the insulin receptor gene (NIRKO mice) [3].

**Figure 1:** Potential effects of insulin and leptin to modulate reproductive function. Hypothalamic neurons involved in the neuroendocrine control of reproduction can sense variations in circulating insulin and leptin levels. In addition, insulin affects several steps of ovarian steroidogenesis, a process that can also be directly modulated by leptin. In states of insulin and/or leptin resistance such as human obesity, this fine tuning of the gonadotrope axis can therefore be hindered both at the central level and directly in the ovary.

Figure 1 : Effets potentiels de l’insuline et de la leptine dans la régulation de la fonction reproductive. Les neurones hypothalamiques participant à la régulation de la reproduction sont sensibles aux variations des taux de l’insuline et de la leptine. L’insuline agit sur les différentes étapes de la stéroïdogenèse ovarienne, également modulée par la leptine. Une résistance à l’insuline ou à la leptine, comme dans l’obésité, perturbe le contrôle fin de l’axe gonadotrope au niveau central et directement au niveau de l’ovaire.

Despite the well accepted role of insulin to stimulate growth of neurons in culture, inactivating the insulin receptor had no impact on brain development of these animals. However, the authors noticed a consistent 10-15% increase in the body weight of NIRKO females over the initial six months of life. Further characterization of their genetic model showed that both male and female NIRKO mice placed on a regular diet had a nearly two-fold increase in perigonadal white adipose tissue at 6 months compared to their wild type littermates. This increase was also paralleled by an increase in circulating leptin levels in animals of both sexes. Despite this rise of leptin, female NIRKO mice exhibit increased food intake at baseline, suggesting leptin resistance. Re-
markably, neuronal disruption of the insulin receptor also lead to moderate peripheral insulin resistance as well as hypertriglyceridemia. Thus, brain-specific disruption of the insulin-receptor gene resulted in hyperphagia in females, and in obesity, hyperleptinemia, hypertriglyceridemia and insulin resistance in both sexes.

In addition, male and female NIRKO animals turned out to have impaired reproductive function secondary to hypothalamic hypogonadism. Indeed, circulating levels of LH were low in both sexes at 6 months compared to wild type littersmates, and could be stimulated appropriately by a GnRH agonist. These studies are remarkable in two ways. First, they indicate that pure central insulin resistance can modify and aggravate changes characteristic of the metabolic syndrome including leptin resistance. Moreover, they provide for the first time a functional link between insulin signaling and the central nervous system control of reproduction.

The other study was performed in mice bearing an inactivation of the insulin-receptor substrate 2 (IRS-2) [4]. IRS-2 is a member of a family of proteins which undergo rapid tyrosine phosphorylation in response to insulin and insulin-like growth factor binding to their receptor. It is particularly important for peripheral carbohydrate metabolism and β-cell function. During the course of the study of these mice, the authors noticed that no single pregnancy could be induced between IRS-2−/− male and IRS-2−/− female animals: male IRS-2−/− mice could be adequate breeders if mated before the onset of severe diabetes, but even relatively euglycemic young IRS-2−/− females remained infertile due to anovulation. Therefore, the profound disturbance of fertility observed is probably not the consequence of abnormal glucose metabolism [4]. Further characterization of female IRS-2−/− mice showed surprisingly low levels of LH and sex steroid hormones, demonstrating the central origin of the defect. As the insulin/IGF-1/IRS pathway has been implicated in the maintenance of fuel homeostasis as well, food intake was then monitored in IRS-2−/− mice. These studies showed that IRS-2−/− mice exhibit a hyperphagic obese syndrome, accumulating twice as much fat as their age-matched wild type controls. As observed in the NIRKO animals described above [3], hyperphagia in IRS-2−/− mice occurred despite increased circulating levels of leptin, suggesting the existence of central leptin resistance associated with IRS-2 inactivation.

Overall, these results implicate the insulin receptor and IRS-2 in a central pathway integrating reproduction and energy homeostasis. Further research should focus upon the identification of such IRS-2 dependent pathways, which could provide potential targets for the development of novel therapeutic approaches towards the treatment of obesity and/or reproductive disorders. It should be stressed however that IRS-2−/− female mice also have defects at the level of the ovary, suggesting the combined participation of peripheral and central insulin resistance to the infertility (fig. 1).

**Neuropeptide Y and melanocortin 4 receptor subtype in the hypothalamic control of feeding and reproduction**

NPY is one of the most abundant neuropeptides present in the hypothalamus, and remains among the most potent orexigenic (food intake promoting) factors [10]. This neuropeptide is particularly expressed in the arcuate hypothalamic nucleus, where it is an important target of the effects of leptin: high concentrations of circulating leptin act to reduce hypothalamic NPY expression and secretion [31], thus inhibiting feeding behavior. Hypothalamic NPY is also involved in the neuroendocrine regulation of the growth, stress and reproductive axes. In particular, chronic intra-cerebroventricular NPY administration exerts a strong inhibition on the reproductive function of intact animals [27]. Since food deprivation stimulates hypothalamic NPY synthesis and release, it has been postulated that this peptide could be implicated in the metabolic and neuroendocrine responses to poor metabolic conditions.

α-MSH is produced by post-translational processing of the precursor peptide Pro-Opio-Melanocortin (POMC) in specialized neurons of the arcuate nucleus. In contrast to NPY, α-MSH inhibits feeding behavior by interacting with melanocortin 4 receptors (MC4-R) located in the lateral hypothalamic area [32]. Disruption of the MC4-R gene [19], as well as chronic antagonism of the binding of α-MSH at this receptor, result in a marked hyperphagic obese syndrome. Such antagonism is observed after pharmacological blockade of MC4-R [11] as well as in the agouti (A/y/a) mouse [22]. A y/a mice are hyperphagic obese animals due to the ectopic overexpression of the protein agouti, a naturally occurring antagonist at melanocortin receptors, in the hypothalamus. However, unlike in Lepob mice, reproductive function is preserved in MC4 receptor-dependent obesity, suggesting a dissociation of the pathways controlling reproduction from those controlling feeding. We recently conducted a series of studies to further elucidate the hypothalamic mechanisms responsible for this dissociation [28].

Hyperphagia was induced in adult male rats by the central (icv) infusion of either porcine NPY, or of a pharmacologic antagonist of the MC4-R (SHU9119). As expected, the two treatments induced a strong stimulation of food intake, resulting in an obese syndrome of a similar magnitude in both groups [28]. However, animals were markedly different with respect to the endocrine alterations associated. NPY-dependent hyperphagia was accompanied by insufficiencies of both the
The discovery of leptin has allowed significant advancements in the understanding of the pathophysiology of obesity and related disorders. It now appears that normal levels of circulating leptin represent a necessary, but probably not sufficient signal to allow pubertal development to start and normal cycles to occur. In addition, it has become increasingly recognized over recent years that the two hormones leptin and insulin interact at several levels to modulate metabolic as well as endocrine functions. In states of obesity, leptin and insulin resistance develops both peripherally and at the level of the hypothalamus. Recent data suggest that this resistance is a key phenomenon in the pathophysiology of disorders of the gonadotrope axis observed in obesity. A major challenge of future research will be to better understand and dissect the role of specific hypothalamic neurotransmitters and amino acids involved in the regulation of metabolic functions.

CONCLUSIONS

The discovery of leptin has allowed significant advancements in the understanding of the pathophysiology of obesity and related disorders. It now appears that normal levels of circulating leptin represent a necessary, but probably not sufficient signal to allow pubertal development to start and normal cycles to occur. In addition, it has become increasingly recognized over recent years that the two hormones leptin and insulin interact at several levels to modulate metabolic as well as endocrine functions. In states of obesity, leptin and insulin resistance develops both peripherally and at the level of the hypothalamus. Recent data suggest that this resistance is a key phenomenon in the pathophysiology of disorders of the gonadotrope axis observed in obesity. A major challenge of future research will be to better understand and dissect the role of specific hypothalamic neurotransmitters and amino acids involved in the regulation of metabolic functions.

REFERENCES


© 2018 Elsevier Masson SAS. Tous droits réservés. - Document téléchargé le 30/12/2018 Il est interdit et illégal de diffuser ce document.


27. Pierroz DD, Gruaz NM, D’Allèves V, Aubert ML. Chronic administration of neuropeptide Y into the lateral ventricle starting at 30 days of life delays sexual maturation in the female rat. Neuroendocrinol 1995; 61: 293-300.

28. Raposinho PD, Castillo E, d’Alleves V, Broqua P, Pralong FP, Aubert ML. Chronic blockade of the melanocortin 4 receptor subtype leads to obesity independently of neuropeptide Y action, with no adverse effects on the gonadotropic and somatotropic axes. Endocrinology 2000; 141: 4419-27.


