



## Metabolic Control of Puberty Onset: New Players, New Mechanisms

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**ABSTRACT**

Puberty, as the end-point of a complex series of maturational events affecting the components of the hypothalamic-pituitary-gonadal (HPG) axis, is gated by the state of body energy reserves and sensitive to different metabolic cues; conditions of severe metabolic stress and energy unbalance (from anorexia to morbid obesity) being commonly linked to perturbation of the onset of puberty. In the last two decades, the neuroendocrine mechanisms responsible for the tight coupling between energy homeostasis and puberty onset have begun to be deciphered. These seemingly involve a plethora of metabolic hormones and neuropeptides, which impinge and integrate (mostly) at the hypothalamic centers governing reproduction. Yet, characterization of the mechanisms of action of such regulators (and even their nature and physiological relevance) still remains incomplete. In this review, we will summarize some recent developments in our knowledge











opening and first estrus [31]. In contrast, however, twice daily injection of 1 nmol ghrelin for 10 days was sufficient to delay vaginal opening and ovarian follicular development and ovulation in pubertal female rats (see **Figure 2**). Taken together, these data suggest that while both male and female rats are sensitive to the modulatory (inhibitory) actions of elevated levels of ghrelin on puberty onset, males appear to be more sensitive than females. This is in contrast to that reported previously for leptin, as females are more sensitive to its regulatory (permissive) effects in terms of puberty onset than males [9]. While these data have been obtained in laboratory rodents, their implications in terms of ghrelin influence on the timing of human puberty are worthy to explore. In fact, endocrine analyses have demonstrated a progressive decline in circulating ghrelin levels along puberty in humans [32]; a phenomenon which may play a permissive role on puberty onset, assuming a similar inhibitory action of ghrelin also in humans.

The mechanisms whereby ghrelin conducts the above modulatory actions on puberty onset remain to be characterized. Fragmentary evidence suggested that, in adult rats, ghrelin is able to inhibit hypothalamic GnRH secretion *ex vivo* [30], while systemic administration of ghrelin reduced GnRH pulse frequency *in vitro* [33]. A similar mechanism might be operative also in pubertal rodents, as a reduction in the frequency of GnRH pulses has been reported in immature rats following systemic administration of ghrelin [33]. Of note, the ability of ghrelin to delay puberty onset is mimicked by administration of UAG [29], thus suggesting the involvement of GHS-R1a independent mechanisms [29]. In contrast, we have been unable to demonstrate any discernible effect of obestatin in the timing of puberty onset in rats (*our unpublished observations*). Finally, even assuming a predominant action of ghrelin at the level of the GnRH pulse generator, whether this is conducted directly on GnRH neurons or through indirect regulatory pathways is yet to be determined. Circumstantial evidence, however, suggests an indirect mode of action, as ghrelin effects on LH secretion were inhibited by a non-selective antagonist of corticotrophin-releasing hormone (CRH) [34], and ghrelin was able to inhibit the effect of central elicitors of GnRH/gonadotropin release, such as kisspeptin [29]. In this sense, very recent evidence suggests that ghrelin is able to suppress *Kiss1* gene expression at discrete hypothalamic areas [35], thus providing the neuroendocrine basis for, at least part of, its inhibitory effects on puberty onset and gonadotropin secretion.

#### **4. Central Pathways for the Metabolic Regulation of Puberty: The Role of Kisspeptins**

Among the central signals responsible for the neuroendocrine control of the gonadotropic axis, kisspeptins, the products of *Kiss1* gene that operate via the G protein-coupled receptor, GPR54, have emerged very recently as essential gatekeepers of puberty onset and fertility, by virtue of its pivotal roles in mediating key reproductive phenomena, including sexual differentiation of the brain, the pubertal activation of the GnRH system, the feedback control of gonadotropin secretion and the pre-



regulation of Kiss1 expression at the hypothalamus. In brief, (i) Kiss1 mRNA levels at the ARC are decreased in ob/ob mice, but increase following treatment with leptin [43]; (ii) diabetic rats –with severe hypoleptinemia- show decreased levels of Kiss1 mRNA at the hypothalamus, which are normalized by intracerebral infusion of leptin, but not of insulin [41,42]; and (iii) leptin can increase Kiss1 mRNA expression in the murine hypothalamic cell line, N6, and human GnRH-secreting neuroblasts [44,45]. In good agreement, intracerebral administration of leptin to adult female rats induced phosphorylation of STAT3 at the AVPV [23], where a prominent population of Kiss1 neurons is detected in the female. Admittedly, however, the studies summarized above have not been specifically conducted in pubertal animals/models, and thus may not be representative of physiological mechanisms during puberty. Anyhow, our preliminary evidence in models of postnatal undernutrition in female rats suggests that leptin is also an important regulator of Kiss1/kisspeptin expression at the time of puberty, as in animals subjected to restricted feeding during lactation, which are leaner and display delayed vaginal opening at the time of puberty, there is a tight correlation between (low) circulating leptin and the hypothalamic levels of Kiss1 mRNA and number of kisspeptin-positive neurons (Castellano & Tena-Sempere, *manuscript in preparation*). The latter is of interest, as important developmental/plastic changes have been reported for Kiss1 neurons along pubertal maturation [46]. This opens the possibility that, together with other factors –such as estrogens- early changes in leptin levels may contribute to the ‘shaping’ of kisspeptin projections during puberty.

Finally, the involvement of other neuropeptides in the metabolic control of the Kiss1 system remains virtually unexplored. Nonetheless, fragmentary evidence suggests that NPY, IGF1 and ghrelin may operate as putative regulators of Kiss1 expression [35,44,47]. Again, the (scarce) data so far available in this front do not come from models of puberty, which warrants specific analyses on the putative roles of these (and other) metabolic signals in the regulation of the Kiss1 system at the time of puberty.

## 5. Novel Players in the Metabolic Regulation of Kiss1: The roles of Crct1 and mTOR

While the ability of leptin to regulate the hypothalamic expression of Kiss1 was demonstrated by initial studies, the intracellular mechanisms for such an action have remained elusive for years. Recent findings, however, suggest the involvement of the Creb1-regulated transcription coactivator-1 (Crct1) in mediating leptin effects on the Kiss1 system at the hypothalamus. Thus, in a recent elegant study, Altarejos and co-workers demonstrated that mice engineered to lack functional Crct1 were not only obese and hyperphagic, but also infertile [48]. Interestingly, in that study, leptin was also demonstrated to dephosphorylate (and activate) Crct1, which in turn stimulated the recruitment of Crct1 to Kiss1 gene promoter and the expression of Kiss1 mRNA at the hypothalamus [48].





ultimate neuroendocrine mechanisms responsible for the acceleration in the onset of puberty in the obese female are yet to be fully deciphered. Similarly, whether obesity may also have an impact on male puberty warrants further investigation [58].

## 7. Conclusions

While the contention that puberty onset critically depends on adequate body energy stores has been known for Ages, the neuroendocrine substrate for such a metabolic regulation of puberty and fertility has begun to be revealed only recently. Nevertheless, in the last decades, significant progress has been achieved in this area, and several peripheral hormones, with key roles in the control of metabolism, have been demonstrated to influence the timing of puberty, through the modulation of diverse central pathways that converge, mainly, at the level of the GnRH system. Among those, we have summarized herein some of the recent developments in our knowledge on the mode of action of leptin and ghrelin in the control of puberty, with emphasis being made on the indirect mechanism whereby leptin influences the function of GnRH neurons and the detailed description of the effects of ghrelin on male and female puberty. In addition, we have provided a succinct overview of the putative roles of kisspeptin neurons at the hypothalamus as conduits for the metabolic control of puberty and reproduction, with special attention to the role of leptin in the regulation of the Kiss1 system, and the recently identified roles of *Crtc1* and mTOR in such a phenomenon. A tentative integral model for such a network of peripheral signals and central pathways transmitting metabolic information to the centers (GnRH neurons) driving puberty onset is provided in **Figure 3**. We are confident that further research efforts along these lines will help to better define the physiological basis for the regulation of the timing of puberty by metabolic factors, and to identify the pathophysiological mechanisms for its eventual perturbations in unfavorable metabolic conditions, ranging from anorexia to morbid obesity.

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for leptin effects is reinforced by the fact that leptin receptors are not expressed in GnRH neurons, which excludes the possibility of direct effects of leptin at this site. Notwithstanding, it remains plausible that part of leptin actions on Kiss1 neurons may be indirectly conducted via intermediary neuronal pathways, whose nature and physiological relevance is yet to be elucidated. In addition, the potential involvement of mTOR and Crtc1 in transmitting metabolic (leptin) information onto Kiss1 neurons is also indicated. For sake of simplicity, discrimination between different populations of Kiss1 neurons (i.e., ARC vs. AVPV) is not provided in the scheme; yet, it remains possible that not all Kiss1 neurons are similarly regulated by different metabolic cues along puberty. For further details, see the text.

Figure-1





