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Metabolic Control of Puberty Onset: New Players, New Mechanisms

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Abbreviation Footnote: GPR54, G protein-coupled receptor 54; GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; ARC, arcuate nucleus; AVPV, anteroventral periventricular nucleus.

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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 of the effects and mechanisms of action of two key metabolic hormones, leptin and ghrelin, in the control of puberty onset. In addition, the roles of the hypothalamic Kiss1 system in the metabolic gating of puberty will be reviewed, with special attention to its regulation by leptin and the recent identification of the putative roles of Crtc1 and mTOR signaling as molecular conduits for the metabolic control of Kiss1 expression. Elucidation of these novel players and regulatory mechanisms will help for a better understanding of the determinants of the timing of puberty, and its eventual alterations in adverse metabolic conditions.

1. Introduction

Puberty, as developmental period when full awakening of the gonadotropic axis and attainment of reproductive capacity take place, is a key maturational event, associated with important somatic and behavioral changes [1,2]. As such, the physiology of puberty onset and the potential mechanisms for its perturbation have been deeply scrutinized using different model species (from laboratory rodents to humans) and experimental/analytical approaches (from classical physiological studies to recent genome-wide association scanning) [1-3]. The consensus exists that the major determinants of the timing of puberty in healthy conditions are of genetic origin [1]. However, additional modifiers of the *tempo* of puberty have been recognized, with prominent roles of different environmental factors (from nutrient availability to different stressors). Indeed, puberty has been envisioned as 'sensor' for the dynamic interplay between genetic determinants and environmental cues throughout development. Such delicate balance between endogenous and exogenous regulators would be ultimately responsible for the proper timing of puberty, or its deviations [1]. Elucidating the pathways and molecular mechanisms whereby this continuous interaction is taking place remains as an appealing challenge for the future.

Among its different putative modifiers, it has been long recognized that the state of body energy reserves is a key determinant for the onset of puberty in mammals, including humans [1]. The functional coupling between energy sufficiency and puberty onset is especially, but probably not exclusively, present in the female, where threshold fat stores are needed in order to successfully cope with the considerable metabolic drainage of pregnancy and lactation [4]. The association between a critical fat mass and the occurrence of menarche was first proposed on a rigorous basis by Frisch and colleagues in early 1970's [5,6], in keeping with previous analogous observations from Kennedy and co-workers in laboratory rats [7]. This hypothesis provided the first scientific formulation for the ancient, intuitive knowledge of the existence of a close link between fatness and fertility. Recognition of such a link paved the way for the identification of the hormonal signals and neuroendocrine mechanisms involved in this phenomenon.

During the last two decades, considerable progress has been made towards the elucidation of the neurohormonal pathways responsible for the metabolic control of puberty onset and gonadotropic function. Without discussion, a major breakthrough in this field was the identification of the adipose hormone, *leptin*, as an essential neuroendocrine integrator responsible for the coupling of the state of body energy stores and different hormonal functions, including reproduction [4,8]. A number of additional peripheral hormones from key metabolic tissues, such as the gastrointestinal tract, the pancreas and the adipose, have been also proposed as putative regulators of the gonadotropic axis [9]; these likely include the gut –hormone, *ghrelin*, whose role as functional

antagonist of leptin in terms of food intake control may apply as well to the regulation of puberty [10,11]. Similarly, different central neuropeptides have been described as potential mediators in this phenomenon; *kisspeptins* being the most recently identified (and likely one of the most important) players in the central control of puberty onset by metabolic cues [12,13]. While systematic description of every molecule and mechanism reported so far in this context clearly exceeds the scope of this review, we aim to provide herein a succinct and up-dated summary of some recent developments in our knowledge of the signals and neuroendocrine pathways singled out above (leptin, ghrelin, kisspeptins), which will help to highlight and better define physiologically-relevant mechanisms for the modulation of the timing of puberty by metabolic factors in mammals.

2. Leptin Signaling and Puberty Onset: Recent Developments

As stated above, identification of leptin, as product of the *ob* gene, was a revolutionary finding in contemporary Endocrinology, as it allowed unveiling the mechanisms underlying quite diverse regulatory functions, from body weight control to metabolic gating of fertility [4,8]. For sake of concision, description of the fundamental features of leptin as adipose signal controlling reproductive maturation and function will be omitted, as these can be found elsewhere [4,8,14,15]. Sufficient to stress, a wealth of experimental evidence obtained in rodents and humans has conclusively demonstrated that leptin is a permissive signal for puberty to proceed (rather than a trigger itself), thus providing the neuroendocrine basis for the critical fat mass hypothesis postulated by Frisch several decades ago.

Despite the universal recognition of the essential roles of leptin in the control of puberty, some controversy has persisted on specific facets of such physiological action. Among those, the precise pathways and mechanisms whereby leptin modulates the function of the gonadotropic axis, and its activation at puberty, have been the subject of active investigation and debate. Notwithstanding, in spite of the demonstration of actions of leptin at other levels of HPG axis, such as the pituitary and the gonads [8], it has been long recognized that leptin conducts its stimulatory/permissive actions mainly by modulation of the gonadotropin-releasing hormone (GnRH) neuronal system at the hypothalamus [16]; a hypothesis that was substantiated, among other experimental data, by studies showing that intra-hypothalamic infusion of leptin elicits GnRH release *in vivo* [17], and that leptin treatment was sufficient to normalize pulsatile GnRH/LH secretion in fasted rats [18]. These findings, however, could not demonstrate whether leptin actions are conducted directly upon GnRH neurons or indirectly, through intermediate afferents; an issue that has remained contentious for years. In fact, initial RT-PCR analyses detected the expression of leptin receptor mRNA in GnRH-producing GT1-7 cells [19], which might be suggestive of direct actions on GnRH neurons. Similarly, leptin was shown to elicit GnRH secretion in enzymatically-dispersed hypothalamic tissue in a perfusion culture system

(i.e., after dispersion into single cells), which might be indicative, but not a conclusive proof, of direct actions at GnRH neurons [20]. However, despite thorough testing, localization analyses in rodents and primates (by means of *in situ* hybridization and immunohistochemistry) systematically failed to demonstrate the presence of leptin receptors in GnRH neurons [21,22]; findings that suggested that leptin acts (mainly) indirectly in the control of the GnRH system. Yet, there has been a conspicuous lack of functional data for such an indirect mode of action until very recently.

In the last months, however, Quennell and co-workers have presented conclusive experimental evidence to demonstrate that leptin regulates GnRH neuronal function via indirect mechanisms, through a series of elegant studies which combined powerful functional genomic approaches, immunohistochemical analyses and single-cell gene expression assays [23]. First, activation (phosphorylation) of STAT3, the primary mediator of leptin effects at the hypothalamus, following *i.c.v.* injection of leptin could not be detected in GnRH neurons, but rather in other hypothalamic areas which are known to send projections to the preoptic region, where most of GnRH neurons reside. Second, while neuron-specific leptin receptor knock-out prevented the onset of puberty and caused infertility in male and female mice, selective elimination of leptin receptors in GnRH neurons was fully compatible with (grossly) preserved puberty and normal fertility in mice. Finally, using single-cell RT-PCR techniques, GnRH neurons were shown to lack detectable leptin receptor mRNA expression. Altogether, these observations back-up previous data, and firmly demonstrate that, under physiological conditions, the actions of leptin in the control of GnRH function are indirect and likely conducted via the modulation of afferent pathways.

The nature of the neuronal afferents conveying leptin effects onto GnRH neurons is yet to be fully elucidated, and different neuropeptide pathways, such as neuropeptide Y (NPY) or galanin-like peptide (GALP), have been proposed in the last years [24,25]. Without any doubt, however, much of the efforts recently devoted in this front have focused in the characterization of the putative roles of kisspeptin neurons in transmitting the effects of leptin in the control of GnRH neuronal function. Importantly, not only the ability of leptin to modulate Kiss1 expression has been demonstrated, but also the potential molecular mechanisms whereby this modulatory action is brought about have begun to be characterized. Some key developments in this area will be summarized in sections 4 and 5 of this review.

3. Ghrelin and Puberty Onset: Modulatory Actions in the Male and Female

While the role of leptin, as signal of energy abundance, in the metabolic gating of puberty was recognized (and universally accepted) shortly after its cloning in 1994, identification of additional metabolic hormones with the potential to modulate puberty onset has remained elusive. Among other possible candidates, ghrelin, as circulating orexigenic factor that signals energy insufficiency,

has emerged in recent years as putative modifier of the timing of puberty [10,11]. Description of the major features of ghrelin in terms of structure and biological actions, as well as of its widespread distribution and signaling pathways, can be found elsewhere [26,27]. For the purpose of the present review, it is stressed that ghrelin exists in circulation in two major forms: acyl-ghrelin, which is able to activate the canonical receptor GHS-R1a and will be referred herein as ghrelin, and unacyl-ghrelin (UAG), which cannot activate GHS-R1a and signals through as yet unknown receptor pathways (see **Figure 1**). In addition, expression of the ghrelin gene in mammals is likely to give rise to another peptide, termed obestatin by virtue of its (apparent) ability to antagonize ghrelin effects in terms of body weight gain [28]. Yet, the nature of the biological actions and receptor(s) of obestatin has been the subject of intense controversy recently.

Although in this review special emphasis will be made on the experimental evidence supporting a potential role of ghrelin in the modulation of puberty, it is important to emphasize as background information that data available in the literature strongly suggest that ghrelin operates (predominantly) as an inhibitory signal for gonadotropin (mostly LH) secretion in different mammalian species, including rodents, sheep, non-human primates and humans [10,11]; a phenomenon that might contribute to the well-known suppression of gonadotropin levels in conditions of persistent negative energy balance, in which ghrelin levels are commonly elevated. Of note, rodent studies have demonstrated that the inhibitory actions of ghrelin on LH secretion *in vivo* can be mimicked by UAG [29], thus suggesting that this phenomenon is, at least partially, conducted via GHS-R1a independent pathways. In clear contrast, despite extensive testing, we have been unable to demonstrate any conclusive effect of obestatin on LH or FSH secretion in rats (*our unpublished observations*); the latter casts doubts on the potential role of this ghrelin-related peptide in the control of gonadotropic function, at least in this species.

The experimental evidence summarized above, and the proposed function of ghrelin as a signal of energy insufficiency, made it tempting to explore its potential roles in the control of puberty onset. Indeed, studies involving acute or repeated administration of ghrelin in pubertal rats do support a function of ghrelin as putative modulator of puberty, with a predominant inhibitory action in keeping with its reported effects in terms of gonadotropin secretion [10,11]. Thus, repeated injections of ghrelin to male rats, at two different dose-regimens (low dose: 0.5 nmol/every 12-h; high dose: 1.0 nmol/every 12-h), during the pubertal transition significantly decreased serum LH and testosterone levels, and partially delayed balanopreputial separation (as an external index of puberty onset) [29,30]. Of note, chronic treatment of peripubertal female rats with an analogous protocol of low doses of ghrelin (0.5 nmol/12-h for 10 days) did not cause major changes in serum levels of gonadotropins or estradiol, neither did it significantly change the timing of puberty, as estimated by the ages of vaginal

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-

ovulatory surge of gonadotropins, among others. While detailed description of the major features and key physiological roles of kisspeptins in reproductive physiology can be found elsewhere [36,37], including other chapters of this *Special Issue*, for the purpose of this mini-review, we will briefly summarize below the experimental data supporting an important role of central kisspeptin pathways in the metabolic control of puberty onset and reproductive function. Two major lines of evidence can be highlighted in this context: (i) the demonstration of changes in Kiss1 expression in conditions of metabolic stress, and the corresponding effects of kisspeptins in such adverse metabolic situations; and (ii) functional data concerning the effects of major metabolic regulators on hypothalamic Kiss1 expression.

On the first point, data obtained in laboratory rodents have demonstrated that conditions of negative energy balance due to acute food deprivation, which are associated to variable degrees of hypogonadotropism, evoke a detectable suppression of Kiss1 mRNA levels at the hypothalamus [13]. Of important note for this chapter, this phenomenon has been also detected in pubertal male and female rats [38]. While the precise location for such changes awaits further clarification, studies in female rats subjected to chronic calorie restriction along puberty (20% for 7 days) demonstrated a detectable suppression of Kiss1 expression at the arcuate nucleus (ARC) [39], where a prominent population of kisspeptin neurons resides [37]. Notwithstanding, additional changes in Kiss1 expression at the anteroventral periventricular nucleus (AVPV) cannot be excluded in pubertal females under more extreme conditions of food deprivation, in keeping with analogous observations in adult female rats subjected to 48-h fasting [40]. From a functional standpoint, such a decrease in Kiss1 expression seems causative for the perturbation (delay) in the timing of puberty at conditions of persistent energy balance, as suggested by our studies in a model of chronic subnutrition during the pubertal transition in female rats, as pharmacological ‘replacement’ with kisspeptin at the expected time of puberty in this model, without any other metabolic intervention, was sufficient to rescue vaginal opening (in a subset of animals) and to induce potent gonadotropic and estrogenic responses, despite the prevailing suppression of circulating levels of gonadotropins due to chronic subnutrition [38]. These findings are in good agreement with data from adult models of metabolic stress (such as uncontrolled diabetes) [41,42], suggesting a conserved role of kisspeptin pathways in the metabolic regulation of the gonadotropic axis along the lifespan.

Despite the accepted role of kisspeptin neurons as conduits for transmitting metabolic information onto the centers governing the reproductive axis, the signals that are actually responsible for the metabolic regulation of the Kiss1 system remain to be fully elucidated. In this front, most of the efforts conducted so far have been directed towards characterization of the potential roles of leptin in this phenomenon. Solid evidence supports a crucial role of leptin in the

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 Crtc1 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 (Crtc1) 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 Crtc1 were not only obese and hyperphagic, but also infertile [48]. Interestingly, in that study, leptin was also demonstrated to dephosphorylate (and activate) Crtc1, which in turn stimulated the recruitment of Crtc1 to Kiss1 gene promoter and the expression of Kiss1 mRNA at the hypothalamus [48].

Altogether, these findings unveil a putative leptin-Crtc1-kisspeptin pathway whereby nutritional cues and body energy stores would modulate puberty onset and fertility. As call of caution, a very recent report has been unable to replicate the consequences of functional inactivation of Crtc1 on mouse fertility [49], suggesting that, under some specific conditions or genetic backgrounds, the function of Crtc1 in the control of Kiss1 expression and reproductive function may be compensated by redundant regulatory pathways. Generation of conditional and/or tissue-specific knock outs of Crtc1 will help to solve this issue.

In a parallel line, we have recently obtained evidence that hypothalamic signaling through mTOR (*mammalian target of rapamycin*) is also involved in the control of puberty onset, at least partially, via modulation of Kiss1 expression [39]. Of note, mTOR is a serine/threonine kinase that operates as sensor of the cellular energy status and effector for its coupling to cell growth and proliferation [50,51]. At the ARC, mTOR signaling had been proposed as transducer for leptin effects on energy homeostasis and food intake [52]; yet, its potential involvement in the metabolic regulation of puberty onset and fertility remained unexplored. Our recent data demonstrate that blockade of central mTOR disrupts the normal timing of puberty in female rats. More importantly, inhibition of mTOR signaling prevented the permissive effects of leptin in terms of puberty onset, and resulted in the suppression of Kiss1 mRNA levels, mainly at the ARC [39]. Altogether, these findings provide evidence for a leptin-mTOR-kisspeptin pathway in the metabolic control of puberty. Whether other central fuel-sensing mechanisms are also involved in the metabolic regulation of Kiss1 expression at the hypothalamus is yet to be explored. In this context, AMPK has been suggested to reciprocally cooperate with mTOR in the central control of energy homeostasis; AMPK being an appetite-stimulating factor [53,54]. In keeping with this view, our preliminary data suggest that activation of AMPK induced a partial delay of puberty onset and modestly decreased Kiss1 mRNA levels at the ARC in female rats (*unpublished observations*), which may be caused, at least partially, by its ability to inhibit mTOR signaling [55,56].

6. Obesity and Puberty Onset

Most of the experimental evidence summarized in previous sections originates from studies in models of negative energy balance. These are enormously instrumental to unveil putative metabolic regulators of puberty onset, and very helpful to predict the patho-physiological mechanisms and consequences of puberty disruption due to (e.g.) strenuous exercise or anorexia nervosa. Admittedly, however, these may not provide a proper insight into pubertal alterations linked to conditions of energy excess, such as morbid obesity, which are much more common among adolescents across developed countries. Indeed, epidemiological studies in humans demonstrate that obesity is frequently linked to changes in the timing of puberty and decreased reproductive performance later

in life; the former manifesting usually as earlier onset of puberty in obese girls [57-59]. The mechanisms for such association, and even its causative relationship, remain unclear and have been the matter of debate, as excess of estrogen –caused by precocious puberty- could contribute to fat deposition and may predispose to obesity, but excess of adiposity –due to prevailing obesity- may as well induce inappropriately high estrogen levels that may cause earlier breast development (as conventional marker of initiation of puberty).

Several studies in human populations point out, however, that earlier puberty may be caused, or at least facilitated, by a prevailing excess of fat mass; increased body fatness or a rapid elevation in BMI being predictors of earlier onset of puberty [57]. A potential explanation for such a phenomenon would be that excessive production of leptin at early stages of development may accelerate the timing of puberty within a certain temporal range. This has been suggested on the basis of longitudinal studies, where elevated leptin levels clearly preceded the pubertal rise of LH and estradiol in girls, and the age at menarche was partially related to circulating leptin concentrations. This is not incompatible with the proposed permissive role of leptin in the control of puberty; while leptin *per se* does not operate as trigger of puberty, threshold leptin levels are mandatory for puberty to proceed. If this threshold is achieved precociously, in the presence of other positive modulators, puberty may take place earlier. The central mechanisms whereby elevated leptin may transmit the signal for earlier puberty in humans is yet to be elucidated, but data from rodent studies would suggest a role of hypothalamic kisspeptin neurons (see section 4). In fact, using a model of postnatal overfeeding in female rats, which results in higher body weight and earlier puberty onset, we have recently obtained evidence for a close correlation between the age of vaginal opening, the circulating levels of leptin, and the hypothalamic content of Kiss1 mRNA and kisspeptin-positive neurons at the time of puberty; all parameters being advanced/elevated in overweighed animals (Castellano & Tena-Sempere, *manuscript in preparation*). Admittedly, however, the causative nature of such association is yet to be fully demonstrated.

In addition to the excess of leptin, other mechanisms may contribute to alterations in the timing of puberty in obese individuals. These may include the state of hyperinsulinemia and insulin resistance, frequently linked to obesity, and the excess of estrogen production due to enlargement of the adipose tissue –which is an active source of aromatase activity [58,59]. In addition, recent studies in female rodents have suggested that the elevation in androgen levels, seen in models of diet-induced obesity, could be also responsible for earlier onset of puberty, as this was blocked by the anti-androgen flutamide [60]. According to this model, excess of androgens in the obese female would cause a decrease in the negative feedback restraint of sex steroids on the central elements of the HPG axis, thereby making the system more prone to its precocious activation. In any event, the

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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Legends to Figures

Figure 1: Scheme of the major peptide products of the ghrelin gene. This gene encodes a precursor protein of 117 amino acids, termed prepro-ghrelin, which after excision of the signal peptide (SP) gives rise, through differential proteolytic processing, to ghrelin (28 amino acids) and obestatin (23 amino acids). Ghrelin exists in two major forms: acyl-ghrelin, generated by octanoyl incorporation at Ser3 -a process mediated by ghrelin O-acyltransferase (GOAT), and unacylated ghrelin (UAG). Taken from ref [11], with minor modifications.

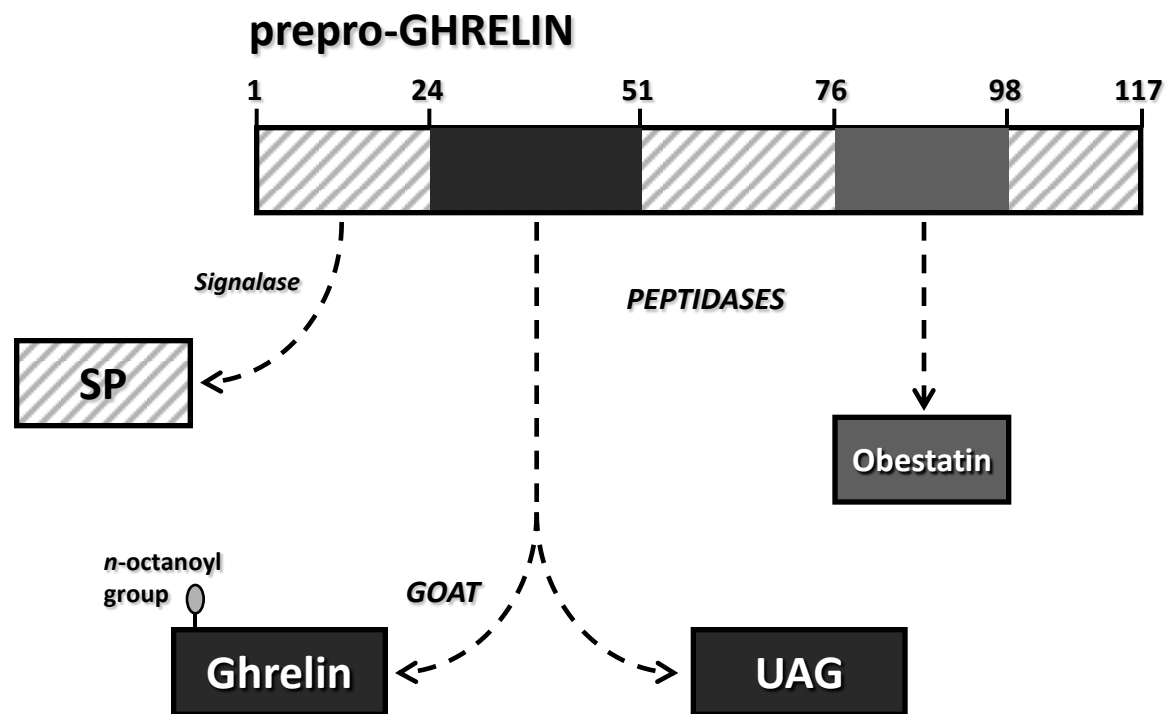
Figure 2: Summary of the effects of chronic treatments of peripubertal female rats with of repeated injections of (i) ghrelin (1.0 nmol/12-h for 10 days), (ii) UAG (1.0 nmol/12-h) or (iii) a combination of both. Treatments were initiated on day 25 postpartum. Experimental procedures were as described in detail in ref [29] for male rats . In panel **A**, evolution of body weights (BW) in the different treatment groups is depicted. In panel **B**, accumulated percentage of vaginal opening (V.O.) in the different experimental groups, during the last three days of treatment, are shown. Finally, in panel **C**, different indices of follicular/ovarian maturation are provided, as described in detail elsewhere [39]. Thus, the percentages of animals of each group not showing signs of ovulation are indicated. In addition, the most advanced stage of follicular/corpus luteum (CL)development for each group is indicated; for this calculation, ovarian sections from each animal were scored from -5 to -1 when showing follicle classes 1-5 as the most advanced stage, respectively, or from +1 to +4 when showing 1- to 4-day-old CL, respectively. Finally, ovarian weights from each group at the end of treatments are presented (** $P < 0.01$; ANOVA followed by Student-Newman-Keuls multiple range test). Overall, these data demonstrate that persistently elevated ghrelin levels across puberty has an inhibitory impact on puberty onset in the female rat, as evidenced by delayed V.O., delayed follicular maturation and ovulation and decreased ovarian weight.

Figure 3: Tentative integral model for the metabolic control of puberty onset by the dynamic interplay between peripheral hormones and central circuits that ultimately impinge onto GnRH neurons, as final output pathway for the pubertal activation of the reproductive axis. According to the focus of this chapter, the putative roles of leptin (as prominent signal for *energy abundance*; permissive/stimulatory factor) and ghrelin (as putative signal of *energy insufficiency*; inhibitory factor) are highlighted. In addition, the major nodal role of kisspeptin neurons as integrators for a wide diversity of peripheral and central signals transmitting metabolic information onto reproductive centers is also represented. Of note, Kiss1 neurons have been proposed as targets for the regulatory actions of leptin (and ghrelin), and their role as mediators

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.

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Figure-1



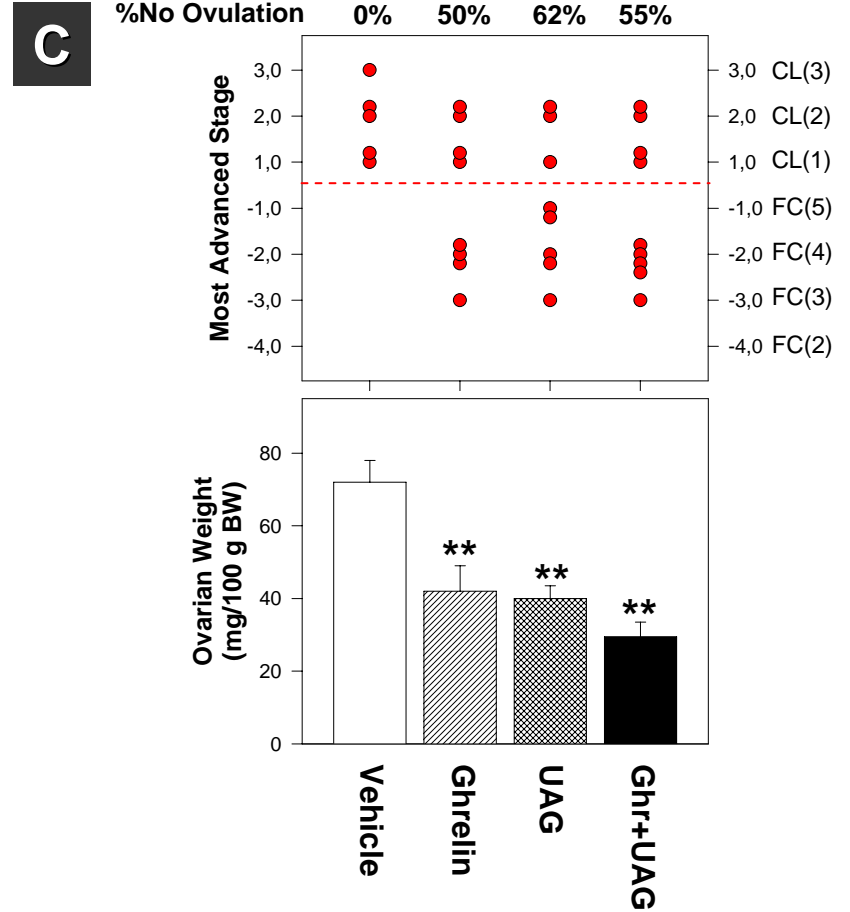
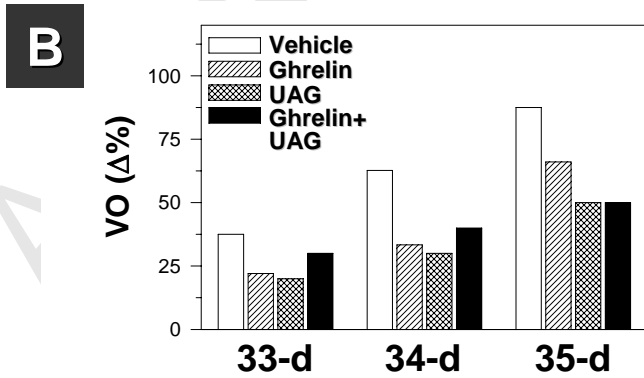
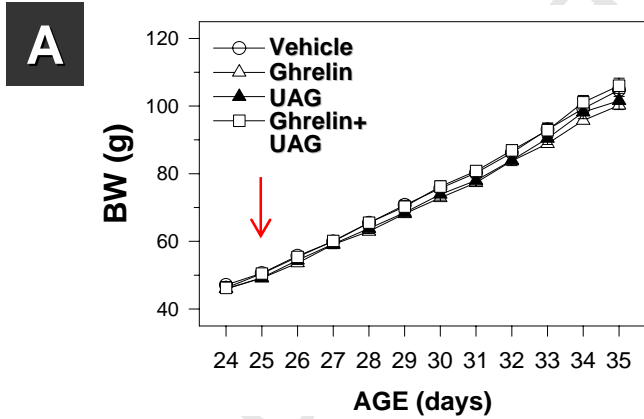
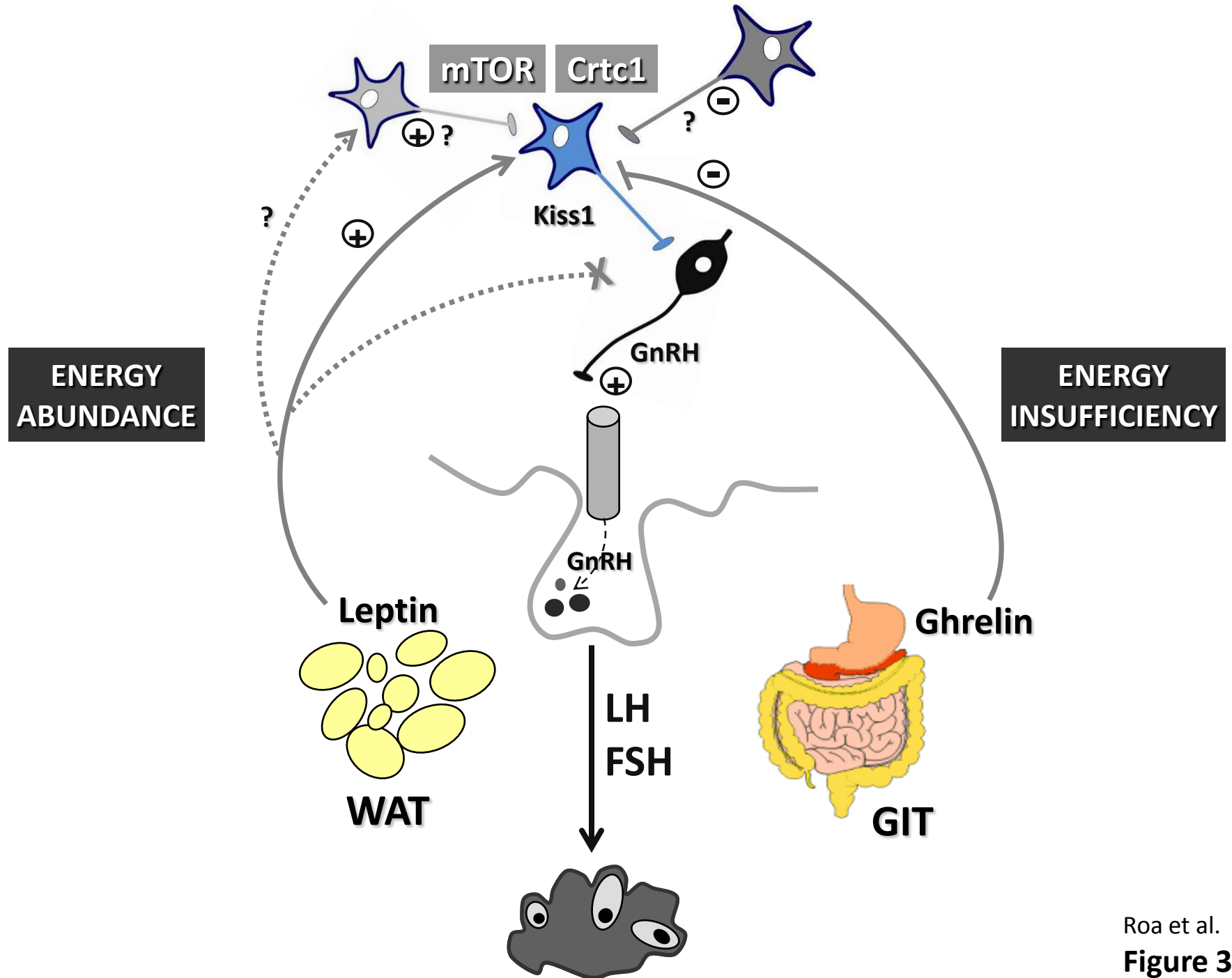


Figure-3



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Figure 3