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Does salt have a permissive role in the induction of puberty?

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Abstract

Puberty is starting earlier than ever before and there are serious physiological and sociological implications as a result of this development. Current research has focused on the potential role of high caloric, and commensurate high adiposity, contributions to early puberty. However, girls with normal BMI also appear to be initiating puberty earlier. Westernized diets, in addition to being high in fat and sugar, are also high in salt. To date, no research has investigated a link between elevated salt and the reproductive axis. We hypothesize that a high salt diet can result in an earlier onset of puberty through three mechanisms that are not mutually exclusive. (1) High salt activates neurokinin B, a hormone that is involved in both the reproductive axis and salt regulation, and this induces kisspeptin release and ultimate activation of the reproductive axis. (2) Vasopressin released in response to high salt acts on vasopressin receptors expressed on kisspeptin neurons in the anteroventral periventricular nucleus, thereby stimulating gonadotropin releasing hormone and subsequently luteinizing hormone secretion. (3) Salt induces metabolic changes that affect the reproductive axis. Specifically, salt acts indirectly to modulate adiposity, ties in with the obesity epidemic, and further compounds the pathologic effects of obesity. Our overall hypothesis offers an additional cause behind the induction of puberty and provides testable postulates to determine the mechanism of potential salt-mediated effects on puberty.

Introduction

There is general consensus that girls are now entering puberty at a younger age. In the US the age of puberty onset has decreased from approximately 11 years of age [1], to under 10 years of age in the most recent report [2]. Similarly, the Copenhagen Puberty Study reported that puberty in girls had advanced by a year between 1991 and 1998 [3]. These studies considered the start of breast development, *thelarche*, as the first marker of puberty. The age at menarche has not decreased [4]. Fewer studies have investigated the age of pubertal onset in boys but there is some evidence to suggest that puberty is also occurring earlier [5,6]. Adverse psychological and physical complications can result from a decreased age at pubertal onset, including depression, stress, increased alcohol use, reproductive cancers, and obesity [2,7–10].

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Conflict of interest

The authors report no conflicts of interest.

The timing of puberty is orchestrated by a series of endogenous and exogenous signals that coordinate the activation of the hypothalamic–pituitary–gonadal axis [11]. Puberty is a heritable trait and many genes associated with puberty are thought to affect its timing [12,13]. Several other factors are purported to affect puberty onset, such as xenosteroids, social cues, geographic location and metabolic status [4].

Reproduction, especially in mammals, is an energy-demanding task and it is crucial for puberty to commence after the deposition of adequate energy stores. The *critical mass hypothesis* postulated that a critical amount of body mass and fat was essential before puberty was initiated in an animal [14]. The discovery of leptin, an adipocyte hormone, provided the putative initiating messenger [15]. Indeed, several studies reported a correlation between the early onset of puberty and body mass index (BMI) [2,4,16]. However, leptin fails to induce early puberty in pre-pubescent humans [13,17] and rodents [18]. Thus leptin is no longer considered a trigger for the onset of puberty, but is thought to be one of several permissive factors, whose presence may be necessary, but alone is not sufficient, to initiate sexual maturation. Moreover, the obesity epidemic [19] does not fully explain the global pubertal advancement because girls with low BMI are also entering puberty earlier [3,4]. A recent study does, however, suggest that even brief changes in BMI, not BMI *per se*, can affect the onset of puberty [20].

Nevertheless it is clear that citizens, obese or otherwise, of developed countries are over consuming processed foods – the so-called “western diet”. This diet is high in fat, sugar, and salt [21–23]. To our knowledge, the effect, if any, of salt on puberty has not been investigated.

The Salt-Puberty hypothesis

The idea that salt is a sexual stimulant is not new [24]. Aristotle [25] recorded that “*sheep administered brine (salt water) copulated sooner*”. Similarly, Plutarch [26] wrote “*Those that breed dogs, when they find their bitches not prone to be hot, give them salt...to excite and arouse their sleeping lechery...*”. Aristotle [25] also noted “*Some people say, indeed stoutly maintain that, if they merely lick salt, mice become pregnant.*” Indeed, the word *salacious*, meaning lustful or lecherous, is derived from the Latin word for salt, *sal*. Nearly 2000 years later, extremely low dietary salt concentrations (0.01% NaCl) were shown to delay puberty compared to 0.05–0.09% NaCl [27]. Conversely, hypernatrophilic male rats displayed an earlier advance in testicular development than control animals [28]. In pigs, a high salt diet post-partum leads to earlier resumption of estrous cyclicity [29]. The onset of puberty has decreased within this century while daily salt consumption has increased [30]. To our knowledge, a scientific link between salt and stimulation of the reproductive axis has not been investigated. We hypothesize that a diet high in salt may change the timing of puberty through three potential mechanisms that are not mutually exclusive (Fig. 1).

1. Arcuate nucleus kisspeptin-neurokinin B-dynorphin (KNDy) neurons link the reproductive system and the salt-regulatory system.
2. Salt-induced vasopressin affects anteroventral periventricular (AVPV) nucleus kisspeptin neurons.

3. Salt affects metabolism and consequently modulates activation of the reproductive system.

KNDy neurons

Kisspeptin is a potent activator of gonadotropin-releasing hormone (GnRH) neurons [31] and is required for progression through puberty. At puberty, there is an increase in the number of kisspeptin neurons, overall kisspeptin tone, and an increase in kisspeptin projections onto GnRH neurons [32–34]. Mutations in either the *Kiss1* gene, or *Kiss1R* the receptor gene, compromise pubertal maturation, and induce additional deficits in reproductive function [35,36]. The two main populations of kisspeptin neurons are located in the AVPV and the arcuate nucleus [37,38]. Arcuate kisspeptin neurons co-localize with neurokinin B (NKB) and dynorphin (DYN) and are termed KNDy neurons [39]. They play a major role in the activation of the reproductive axis at puberty [39]. Neurokinin B is also necessary for humans to progress normally through puberty. Humans with a loss-of-function mutation of TAC3, the gene for NKB, or TACR3, the gene for the NKB receptor, NK₃R, present with hypogonadotropic hypogonadism [40,41]. However, the precise role of NKB in the reproductive axis remains unclear as there is evidence of both stimulatory and inhibitory effects. Specifically, NKB has a primarily stimulatory effect on luteinizing hormone (LH) [42,43]. However, some reports indicate that NKB can be inhibitory to LH secretion [44]. The effect of NKB on LH release is likely determined by the precise hormonal environment [45].

NKB acts on NK₃ receptors expressed on vasopressin neurons. NK₃ receptors are activated in hypertonic conditions, causing vasopressin release [46,47]. High salt conditions increase NK₃ receptor activation [47,48], which may affect arcuate nucleus KNDy neurons in two ways. First, NKB in KNDy neurons may be stimulated and feed back on itself to increase kisspeptin release. Second, high salt may stimulate NKB release from cells other than KNDy neurons, but still activate NK₃ receptors on KNDy neurons and stimulate kisspeptin release. If these conjectures were upheld, increased kisspeptin, NKB and LH may be evident after a salt challenge.

The medial amygdala may also provide a connection between the salt-regulatory system and the KNDy neurons of the reproductive system. The medial amygdala is implicated in reproductive behaviors, the regulation of reproductive cycles, and sex steroid receptors are abundant [49–52]. The kisspeptin receptor is also expressed within the rodent amygdala and kisspeptin expression in the medial amygdala responds to sex-steroids [53]. Injections of kisspeptin into this region stimulates LH secretion [54]. The medial amygdala is currently thought to provide a reproductive “brake” on the reproductive axis. The medial amygdala contains an established network of GABA neurons and connects to the AVPV [55,56] and, when lesioned, results in an increase in circulating LH. Injections of tachykinin agonists for the NK₃ receptor into the medial amygdala inhibit salt appetite, a motivational state that drives salt consumption [57].

Vasopressin and the reproductive system

Vasopressin is also a well-known regulator of water balance and blood pressure. In response to hypertonic conditions, vasopressin is secreted from neurons in the paraventricular and supraoptic nuclei with axonal projections to the neurohypophysis. Chronic hyperosmolarity or salt loading increases not only vasopressin secretion, but also vasopressin gene expression in the paraventricular and supraoptic nuclei [58]. Vasopressin is also released into the interstitial fluid of the brain [59] and vasopressin concentrations in cerebrospinal fluid may even be higher than in the peripheral circulation [60]. This widespread release of vasopressin has the potential to reach kisspeptin neurons in either the AVPV or arcuate nucleus, especially as these regions are adjacent to the 3rd cerebral ventricle. It is known in rodents that vasopressin neurons from the SCN project to kisspeptin neurons in the AVPV and are involved in the circadian timing of the preovulatory LH surge in these species [61–64]. Vasopressin is sufficient to alter LH levels within plasma. Previous studies suggest that the amount of vasopressin infused icv affects the resulting LH levels [65–67]: low doses stimulate LH release, whereas higher doses suppress it. Thus, at least in rodents, there is compelling evidence that vasopressin has an established reproductive role. We hypothesize that higher levels of vasopressin induced by a high salt diet during the pre-pubertal period could impact the timing of puberty.

Salt and metabolism: an indirect effect of salt on puberty?

The role of high dietary salt on adiposity is unclear. Fonseca-Alaniz et al. [68] reported that male rats fed a high salt (7.8% NaCl) diet from weaning laid down significantly more fat, especially periepididymal fat, than controls. This effect was notable after 6 weeks but had disappeared by 9 weeks. Leptin was also elevated after 6 weeks and, assuming that this fat deposition was progressive, would encompass the pubertal transition. Coêlho et al. [69] recorded increased food intake and overall energy gain in male rats on similar high salt diets. Intriguingly this diet increased GLUT4 in white adipose tissue [70], which may explain the increased insulin-stimulated glucose uptake in adipocytes of high salt fed rats [71]. Other adipokines, such as FGF21 and/or adiponectin, may also play a central role. FGF21 has been implicated in puberty onset [72].

A relationship between salt intake and weight has also been found in humans [73,74]. Salt intake has also been strongly associated with obesity and the metabolic syndrome [75]. Moreover, Yi et al. [76] recently reported a predictive relationship between salt intake and body fatness: for each 1 g of salt consumed per day there was a 1.2% increase in body fatness after adjustment for energy intake. This relationship was most strongly evident in women. Thus, it is reasonable to hypothesize that the effect of salt on puberty may occur indirectly, through an effect on fat and consequently leptin or other adipokines, thereby creating a permissive environment for pubertal activation.

Integration of pubertal signals

A general question that pertains to all putative signals that are driving puberty earlier is, how do these signals work? In our salt hypothesis, it is likely that individuals will have repeated

or continuous exposure to a high salt dietary regimen. Nevertheless it is possible that sporadic or only exposure during a critical window of development will impact pubertal onset. One hypothesis is that kisspeptin and/or NKB concentrations are increasing in response to salt, eventually breaching a threshold of exposure to initiate puberty earlier. An analogy of this is how estrogen concentrations rise above a certain threshold to trigger the preovulatory GnRH surge in the brain [77]. This hypothesis will be difficult to test directly because it has not yet been possible to measure dynamic changes in kisspeptin or NKB secretion *in vivo* [78,79]. Another hypothesis is that maturation of the neuronal systems responsible for initiating puberty may be altered in response to a high salt diet. A third conjecture is that puberty permissive signals are elevated earlier due to the high salt diet and, as soon as maturation of the neuronal systems are complete, then puberty commences. An analogous situation appears to occur in the seasonal onset of reproduction in sheep, which has been termed “seasonal puberty” [80], where melatonin concentrations must be elevated for several weeks before the reproductive system is activated [81]. This last hypothesis would also suggest that there will be a minimum age of possible onset, unless maturation itself can be driven earlier. Clearly, further research is warranted to address the unknown mechanisms underlying puberty induction as well as the means by which these mechanisms become functional.

Conclusion and perspective

The high salt content of processed foods so prevalent in developed nations has the potential to negatively impact reproductive health. Our Salt-Puberty hypothesis is intended to further our knowledge on diet influences on reproduction. If upheld, this hypothesis will have significant implications on the amount of salt consumed. Salt activation of the NKB system at the arcuate nucleus and/or the medial amygdala may induce puberty. Alternatively, vasopressin receptors on kisspeptin neurons in the AVPV or arcuate nucleus can modulate reproductive activity. Lastly, we cannot discount a more indirect pathway of salt, its effects being mediated through adipocytes and their accompanying endocrine output.

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Fig. 1. Schematic of the Salt-Puberty hypothesis illustrating 3 different, but not mutually exclusive, physiological pathways through which salt may affect puberty. (1) Salt activates KNDy neurons by stimulating release of NKB, either from KNDy neurons or local NKB neurons. NKB binds to NK₃R leading to kisspeptin release which activates GPR54 receptors on GnRH neurons. (2) Salt stimulates release of vasopressin into 3rd ventricle, which activates its receptor, V1aR, on kisspeptin neurons in the AVPV that activate GnRH neurons. (3) Adipocyte released adipokines (e.g. leptin) which activate adipokine receptors. Adipokine receptor expressing neurons either directly or indirectly contact KNDy neurons to drive the effects of salt on puberty.