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THE ROLE OF LEPTIN IN THE PHYSIOLOGY AND PHYSIOPATHOLOGY OF THE REPRODUCTIVE SYSTEM

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ABSTRACT

The leptin plays an important role in the physiology of the reproductive system. This hormone acts as a critical link between the adipose tissue and the reproductive system, indicating mainly whether the level of energetic reserves is adequate for a normal reproductive function. Leptin presents complex interactions at all the levels of the hypothalamo-hypophyso-gonadal axis, exerting stimulating effects on the hypothalamus and hypophysis and inhibitory actions on the gonads. As marker of an adequate nutritional reserve, leptin acts as a permissive factor, consistently with gonadotropins and the somatotrope hormone within the complex process of puberty initiation . Regarding its action on gonads, it has been found that leptin inhibits ovulation, fact proved experimentally both in vivo and in vitro, and this even in the absence of certain modifications in the secretion of steroid hormones or in the number of preovulatory follicles.

KEYWORDS: *hypothalamo-hypophyso-gonadal axis, obesity, reproduction*

Obesity and weight control mechanisms represent important topics in current medical research given that in West European countries and in the United States of America the prevalence of obesity has become endemic. There have been identified numerous molecules involved in the regulation of energetic homeostasis, however the discovery of leptin was an important moment in the study of the etiopathogenesis of obesity. The pleiotropic role of this hormone has bee emphasized since its identification in 1994. Its main function is that of regulator of energetic homeostasis, but it also acts upon numerous tissues and systems of the body, leptin being involved in inflammation, angiogenesis, hematopoiesis, immunity and reproduction [1].

It has been found recently that leptin influences other target organs, such as the endometrium, the placenta and the mammary glands at the level of which there have been identified receptors for this hormone, probably involved in processes such as: menstruation, pregnancy (in zygote implantation and embryo development) or lactation [2- 6]. Observational studies have shown that both excessive and deficient secretion and resistance to leptin can be associated with anomalies of the reproductive function [7]. The conditions of

suboptimal nutritional status (nutrition disorders such as nervous anorexia or binge eating) are correlated with low serum levels of leptin, the dysfunction of the hypothalamo-hypophyso-gonadal axis being consecutive (partly, at least) to the leptin deficit; the administration of small doses of leptin has a permissive effect, surpassing the central threshold which regulates the secretion of gonadotropins at these low levels of leptinemia [8]. In the case of metabolic disorders associated with excess of the energy reserve like in obesity or in the virilizing polycystic ovary syndrome, there are often high concentrations of leptin in serum or in the follicular fluid, pointing to the possibility that the relative deficit of leptin or resistance to its action be at least partially responsible for the anomalies of the reproductive system described in these cases [3, 9]. On the other hand, secretion changes or action deficit of leptin may be involved in the etiopathogenesis of infertility or pathological pregnancy, an example being preeclampsia [10].

Leptin, peptide whose structure contains 167 amino acids, is codified by LEP gene (initially named ob gene), whose expression is preponderant at the level of the adipose tissue (major determiner of serum leptin level), but which can be detected at the level of many other tissues: epithelial cells from the gastric fornix, skeletal muscle, hypothalamus, gonadotrope secreting cells from the anterior hypophysis, granular, tecal and ovary interstitial cells, endometrium, epithelium of mammary glands and placenta [11]. Its name comes from the Greek “leptos” which means “weak”, thus emphasizing the main role of leptin, that of signaling to the central nervous system the level of the energy resources of the body, acting as a satiety factor on the hypothalamic centers in a feedback mechanism involved in the adipose mass control [12]. As far back as the 1950s, an animal model of genetic obesity was identified, the ob/ob mouse and a theory was formulated according to which the adipose tissue produces certain humoral factors which act by appetite inhibition (“lipostat theory”) [13]. This theory was to be confirmed by the discovery of leptin, whose administration to the ob/ob mice (in which the mutation of the ob gene was proved to be correlated with the absence of leptin by synthesis deficit and which present obesity secondary to marked hyperphagia) was found to cause a return to normal weight by hyperphagia removal and appetite normalization [14, 15]. Moreover, it was proved that the simple caloric restriction in these mice is followed by the redressing of body weight, but not of fertility as well, indicating thus for the first time that leptin might play a role in the physiology of reproduction. Thus, consecutively to leptin treatment, the anatomical changes found in the treated ob/ob mice (increase in the size of the uterus and ovaries as well as seminal vesicles and testicles), objectified on a functional level in the group treated with leptin as compared to the control group (which were subjected solely to diet) indicates that the resettlement of fertility in ob/ob mice cannot be achieved solely by weight normalization, but by normalizing serum leptin levels [16].

The reproductive system of mammals is extremely sensitive to the availability of food in the environment, acute changes in the energy status inducing immediate changes at the level of the hypothalamic-hypophyso-gonadal axis . In several species (mouse, sheep, monkey and man) food deprivation is followed by the suppression of the pulsatile secretion of LH [17- 20]. This can be normalized extremely fast (within several hours or even minutes) subsequent to re-feeding [21]. The suppression of the pulsatile secretion of LH, induced by food deprivation is considered to be the consequence of a deficient release of gonadoloberin (GnRH- Gonadotropin Releasing Hormone), finding that after administering exogenous GnRH to animals deprived of food, they present an LH secretion pattern similar in amplitude and frequency to the one of normally fed animals [22, 23].

Leptin has a permissive role in the release of GnRH, in vitro studies proving that the release of GnRH from the hypothalamic level is greater in the

environments which contain leptin [24]. The involvement of leptin in the alteration of the pulsatile secretion of LH secondary to food deprivation has also been proved by studies performed in vivo [25]. Ahima and Collab describe low levels of leptinemia and of the LH basal levels in the mice which were subject to caloric restriction, leptin administration normalizing the LH secretion and restoring the cyclicity of ovulation cycles [26]. Moreover, it was found that the administration of anti-leptin antibodies at the level of the lateral ventricle in mice with normal food share is followed by the alteration of the pulsatile secretion of LH and by the suppression of ovulating cycles. A study carried out on macacus rhesus monkeys showed that low levels of FSH and LH after a 48-hour fasting become normal subsequent to systematic leptin administration, confirming its role in opposing to the effect of food deprivation over the secretion of gonadotropines [27].

Leptin acts through the intermediary of a specific receptor which belongs to the family of cytokine receptors class I and presents two isoforms differentiated by the relative length of their cytoplasmatic region: long shape (LEPR L), which predominates at the hypothalamus level and the short form (LEPR S), which is found in several organs and tissues. LEPR L exhibits a sequence of amino acids involved in the connection of Janus Tyrosine kinase (JAT/STAT), while LEPR S has the capacity to bind to MAPK (Mitogen-Activated Protein Kinase). A soluble leptin receptor (solLEPR) is generated by proteolytic cleavage at the level of the membrane binding receptors [28].

The long form of the leptin receptor (the main form responsible for signal transduction) is synthesized mainly in the arcuate and ventromedial hypothalamic nuclei, important areas in the control of GnRH release and in the control of sexual behavior [27]. Leptin acts centrally in order to influence the reproductive function, but we cannot not know for sure whether these actions are exercised directly over the GnRh neurons or indirectly by means of the interneuronal circuits. In fact, there have not been identified any leptin receptors at the level of the GnRH neurons, making more likely the idea of neuronal intermediaries of leptin actions over the release of GnRH. If the action of leptin in regulating appetite is mediated by the inhibition of the hypothalamic NPY and by the stimulation of Pro-opiomelanocortin, the effects over the secretion of GnRH / LH seem to be mediated by NPY (also inhibited by leptin) and Kisspeptine [29]. An increased release of NPY at the hypothalamic level is described in various unfavorable metabolic conditions such as malnutrition or high energy consumption (obviously, in certain performance sports: gymnastic, marathon). The increase of the NPY activity whose synthesis and release are not efficiently inhibited by leptin – in low concentration in conditions of energy deficit, exerts an inhibitory effect over the hypothalamo-hypophyso-gonadal axis, representing a direct inhibition mechanism of the sexual maturation and of the reproductive function in conditions of food restriction or of energy consumption increase [30]. As a matter of fact, it was found experimentally that NPY mRNA levels in the arcuate nucleus are high both in the mice deprived from food and in those with deficient leptin secretion [31].

Leptin is secreted in a pulsatile way, a circadian variation being found at its serum levels with low values during the day and their progressive increase during the night, leptin secretion being synchronous with the pulsation of gonadotropines and estradiol [32, 33].

The expression of the leptin gene is influenced by many factors. Insulin stimulates leptin production, this partially accounting for the decrease in leptinemia found during food deprivation, as well as hyperleptinemia which accompanies insulin resistance conditions [34]. Glucocorticoids increase the expression of the leptin gene, independently of their effect on insulin resistance, but they can also induce a relative insulin resistance by inhibiting leptin actions [35]. Estrogens have a stimulating action [36], while androgens inhibit leptin production [37], thus explaining sexual dimorphism with

regard to serum leptin levels [38]. It was found that the amplitude of leptin secretions is two or even three times larger in females than in males, leptin production being 75% larger in females due to an increase expression of the leptin gene [39], moreover, leptin binding protein levels are lower, resulting in higher levels of free leptinemia in females [40]. Another possible explanation of this sexual dimorphism derives from the observation that both the total mass of adipose tissue and the adipose subcutaneous tissue / adipose visceral tissue are larger in females (the expression of mRNA for leptin being greater in the deposits of subcutaneous adipose tissue than in the deposits of visceral tissue) [41]. On the other hand, in females, the adipose tissue seems to be more sensitive to the actions of hormones (insulin, glucocorticoids) or of other substances which stimulate leptin production, as revealed by in vitro studies [42]. Thus, leptin level is higher in females ever since puberty (in its final stages), persisting then in adulthood even after adjustment, according to the amount of adipose tissue [43].

The time of puberty settlement in mammals depends on several factors, the most important among the individual factors being body weight / nutrition condition and life expectancy, and among the environmental factors, season and temperature [44]. The role of leptin in the process of pubertal development was initially emphasized by studies carried on mice, being found that leptin administration to sexually immature animals accelerates the time of puberty settlement [45, 46]. In rats, the level of leptinemia increases progressively in the prepubertal period (and during puberty in females), a concentration of approximately 700 pg / ml being required for the beginning of the sexual maturation process [47, 48]. On various models of delayed sexual maturity induced by severe food restriction it was found that the levels of leptinemia are very low. Leptin injection (at the level of the lateral ventricle) in food-deprived rats was followed by an increase in energy consumption, with additional weight loss, but with the induction of the sexual maturation process, even when maintaining severe caloric restrictions [49].

The hypothesis that leptin acts as a metabolic trigger signal in puberty initiation was initially advanced by Chehab and collab., who found that healthy mouse females injected with leptin in the prepubertal period present a slower weight gain, but a more precocious sexual maturation as compared to a group of females which received a similar amount of food but which did not receive leptin (the reference group). In a study carried out by Cheung and collab., leptin (in 6.3 µg/g dose administered twice a day) was administered to healthy rat females during their prepubertal period. The rate of sexual maturation in this group was compared to two reference groups: a lot of females fed with the same amount of food as the leptin-treated group (the "pair-feed" group) and a lot of ad libitum fed females. The share of food in the leptin-treated group was of 80% approximately from the share of ad libitum fed rats, determining a delay in development with regard to body weight both in the leptin-treated group and in the "pair-feed" group. All the determined sexual maturation parameters revealed a development delay in the "pair-feed" group but there were no differences between the leptin-treated group and the ad libitum fed group. Similar results were found in the experiment carried by Ahima and collab. on mice which received leptin in small doses (2 µg/g) which do not influence body weight, being found that sexual maturation is accelerated in the group of leptin-treated healthy females as compared to the reference group. In a subsequent study carried out by Keung, the animals were subject to severe food restriction, the ratio of food being of 75% approximately from the one received by ad libitum fed rats. It was found that leptin administration to these underfed animals only partially reestablished the moment of pubertal sexual maturation. Recent studies involving leptin administration for 20 days to sexually immature rat females, with normal food share, did not reveal any acceleration in the sexual maturation process. The

authors concluded that leptin is neither the only, nor the main signal of puberty beginning, but rather a permissive factor which acts as a threshold allowing sexual maturation to take place only in proper metabolic conditions.

In primates, as revealed by studies carried out on rhesus monkeys the level of serum leptin decreases during fasting and a concomitant decrease is outlined in GnRH pulsatility, having as consequence the decrease in LH levels [19, 27]. Recombined leptin administration in adult male monkeys did not determine however the correction induced by starvation on LH, FSH or testosterone levels, even though the concentration of serum leptin was ten times greater than in the reference group of ad libitum fed monkeys (10 vs 1 ng / ml) [50,51]. In another study, higher doses of human recombined leptin were used, revealing the partial reversibility of (inhibitory) effects, induced by food restriction, on the secretion of gonadotropines in pubertal male Rhesus monkeys, the authors supporting the hypothesis according to which leptin seems to play an important part in puberty initiation, a certain level of leptinemia being required by puberty beginning in Rhesus monkeys [27]. Other studies did not succeed however to prove this, neither the systematic administration, nor intra-cerebral leptin infusion in male unsexualised (in the prepubertal stage) monkeys being followed by changes in GnRh pulsatility [52]. Moreover, in other three studies no significant changes were found in the level of leptinemia, correlated with the critical events which occur during the prepubertal period in male Rhesus monkeys [53-55].

The observation that food restriction can lead to puberty settlement in humans or can alter the reproductive function in adults is not a recent discovery, as is the case with the hypothesis that it is necessary to reach a “critical” weight or a certain amount of adipose tissue (“critical body fat” [56], which can be named today “critical levels of metabolic signals” [57]) for puberty to begin. Puberty triggering is correlated rather with the size reached at some point, than with age, partly explaining the observation that in West-European countries menarche occurs at lower ages than in the last century, weight in the onset not being however modified (given the better nutrition, increase and development are accelerated, the young girls reaching faster the “critical” weight necessary for puberty initiation, the figure mentioned by most of the studies being 47.5 kg.

The role of leptin in pubertal sexualization in humans derives from the study of patients with synthesis or leptin reception deficit by the mutation of the ob gene or of the gene which codifies its receptor. All the three members (two female patients aged 6 and 34 and young man aged 22) of one family in Turkey in which the mutation of codon 105 from the ob gene was emphasized (mutation identical to the one responsible for the occurrence of the ob/ob phenotype in mice) presents hyperphagia, marked obesity and low levels of serum leptin. Among the three affected family members, the 34-year woman had primary amenorrhea and the 22-year man did not present any pubertal sexualization, displaying significant signs of hypogonadism. At this young man, the answer of gonadotropins to GnRH was normal, suggesting that the sexualization deficit was secondary to the incapacity of the hypothalamus to secrete proper GnRH levels [58]. Another report regarding three members of a family in which a mutation at the level of leptin receptor gene was described – three 19-year old girls – reveal the coexistence of marked and precocious obesity (settled from the first month of life) with lack of pubertal sexualization: at the age of 19 none of the women present pubarche, mammary development or menarche [59]. The delay in pubertal sexual development in patients with leptin synthesis or reception deficit suggests that this hormone plays an important part in the complex process of puberty initiation in humans. Moreover, it may be necessary that a certain threshold level of leptin concentration be reached for puberty to begin. Thus, recombined human leptin treatment for 12 months in a 9-year old girl who presented the mutation of

the ob gene was followed by the increase of the serum levels of gonadotropins (which at the beginning of the treatment were in the prepubertal field), but mostly by the occurrence of the pubertal pulsatile pattern of FSH and LH secretion [60].

In humans, the beginning of pubertal development is preceded at both sexes by an increase in nocturne leptin secretion, maintenance of a 24-hour pattern of secretion, unchanged as compared to the prepubertal stage [61, 62]. In girls, leptinemia levels grow progressively from the age of 5 [63], menarche settlement being preceded by one month approximately by an increase in leptinemia which reaches a serum concentration of 1ng/ml [64]. Montzoros and collab. longitudinally monitored eight boys during the pubertal development, finding that the beginning of puberty, defined as an increase in the nocturne secretion of testosterone, is preceded by an increase in leptin concentration at peak or close to peak levels in most of the cases [65], observation which has not been confirmed, however, by subsequent studies [66, 67]. During the process of sexual maturation, different patterns of leptin secretion were noticed in the two sexes [68- 70]. While in girls the levels of leptinemia grow as pubertal development advances, in boys the highest level of serum leptin is described precociously at the beginning of puberty, decreasing subsequently, at the same time with the increase in testosterone concentration. Thus, testosterone levels are inversely correlated with leptinemia in males, suggesting a negative feedback mechanism between leptin and the hypothalamo-hypophyso-gonadal axis. This different pattern of leptin secretion in the two sexes, which occurs during the final stages of puberty and which is also maintained subsequently, during adulthood, seems to be independent of the amount of adipose tissue, being also present after the correction according to BMI (with the reserve that BMI is at the growing child a less accurate index of the amount of adipose tissue as compared to the adult) [62]. Leptinemia levels are lower in healthy children (both in the prepubertal stage and during puberty) as compared to children with delayed puberty [61]. While in healthy children leptinemia is higher during puberty than during the prepubertal stage, this observation is not valid for boys with delayed puberty. These results suggest that an increase in leptinemia is not essential to puberty development and that puberty can occur at various levels of serum leptin [71].

Starting from the results of these clinical studies, one can infer that leptin has a permissive role in pubertal development, and it is likely that it acts as trigger per se, a certain level of leptinemia being required (however, not sufficient) for puberty progression [72, 73]. In fact, women with lipoatrophic diabetes do not present any disorders of the reproductive system even if the level of serum leptin is very low due to the absence of the adipose tissue [74].

Initial studies revealed in women during their reproductive period a similar secretion profile of leptin and estradiol during the menstrual cycle, indicating that estrogens could stimulate leptin synthesis [75].

Ludwig M. and collab. find a higher concentration of the serum level at the middle of the luteal phase as compared to the follicular phase, suggesting that progesterone can be involved in the control of leptin secretion [76]. Subsequent studies did not reveal any changes in serum leptin concentration during the menstrual cycle in healthy women, a significant correlation between leptin and estrogens being emphasized only subsequent to ovulation stimulation in view of in vitro fertilization [77]. With regard to the impact of estroprogestatives administration on leptin secretion, the results of the studies are also contradictory. Rechberger and collab. do not identify any change in the level of basal leptinemia after three, respectively six months of oral contraceptive treatment [78], while Messinis describe an increase in leptinemia subsequent to estrogen and progesterone administration in normal-weight women [79]. Most of the studies indicate the decline of leptin levels in post-menopause, especially in

overweight women, probably in the context of change in the steroid hormones status [80]. However, hormonal substitution therapy did not determine any significant increases in leptin levels in most of the studies carried out , but there are however several exceptions [81,82].

Leptin acts directly at the level of gonads [83], leptin receptors being identified at the level of follicular ovarian granulosa, tecal and interstitial cells [84], as well as at the level of Leydig cells [85]. Leptin levels at the level of the follicular fluid are comparable to serum levels [86]. Leptin reduces ovulation directly, but also independent of the influence on steroid hormones secretion and without modifying the number of preovulatory follicles [87]. This indirect mechanism is based on the metabolic effect induced by leptin: the increase of glucose takeover, of hepatic gluconeogenesis and of fat acids and carbohydrates oxidation [88]. With regard to the correlation between leptin and fertility there are studies which reveal that hyperleptinemia suppresses steroidogenosis and has a potentially negative effect on gametes maturation . Brannian and collab. found that an increased leptin / BMI ratio indicates a decrease in the success rate of in vitro fertilization [89]. Similarly, it was found that a low follicular level of leptin is a success indicator of the GIFT in vitro fertilization procedure (gamete intra-fallopian transfers) [90].

During pregnancy the levels of serum leptin reach values much higher than in its absence [91]. The increase of leptinemia occurs precociously, before the occurrence of any weight changes [92] and reaches a peak during the second pregnancy trimester, the serum concentration remaining unchanged subsequently until the due date when it decreases briskly. Leptin can have an important role in regulating maternal nutrition and in the adaptation mechanisms during intense energy consuming processes, such as pregnancy and lactation [93,94]. Pregnancy with its associated hormonal modifications (especially regarding changes in the serum levels of insulin, glucocorticoids, estrogens and prolactin) seem to be a status of physiological *hyperprolactinemia* and leptin resistance with a decoupling of the alimentary behavior and of the metabolic activity. Through its lipolytic effect, leptin can favor an increase in the mobilization of fat acids from the deposits of adipose tissue, with the concomitant increase in their use at the periphery [95].

The leptin from the umbilical cord has double origin: placental secretion and fetal synthesis. Both the long and the short form of leptin receptors are present in the placenta and are in contact with leptin at the level of the syncytiotrophoblasts of the maternal interface, indicating an autocrine or paracrine potential effect of leptin on the placental function [96]. It was found that leptin stimulates the release of placental hCG, being responsible for the induction and increase in the amplitude of hCG peaks. Leptin also activates the release of pro-inflammatory cytokines (such as prostaglandins) at the level of the placental situs, also suggesting the existence of a modulating role of the endocrine-placental function [97]. The presence of leptin and of its receptors in the human placenta and in the endometrium, the observation that the endometrial secretion of leptin is high in the presence of the viable blastocyst ties this polypeptide to the precocious development of the product of conception and suggests its place in the group of active regulators from the implantation apposition and adhesion phases [98]. As previously mentioned, the identification of leptin receptors at the level of the syncytiotrophoblast, in its turn leptin producer, suggests the existence of autocrine and paracrine mechanisms, at the level of this tissue with important role in the synthesis of a large number of hormones involved in maintaining the viability of the product of conception. This can partially explain the observation that the level of leptinemia is 8% lower in patients with spontaneous abortions during the first trimester as compared to those who successfully bear the pregnancy [39].

Leptin concentration is correlated to the level of progesterone from the luteal phase of the menstrual cycle and with the hCG concentration in pregnancy, indicating the existence of a possible interaction between placental

steroids and polypeptidic hormones for pregnancy maintenance. Leptin secretion at the level of placental vilosities during the first trimester of pregnancy is 50 times higher than in tissue sampled at the due date. This period of the leptin peak corresponds to that of increase in the hCG concentration. Even though leptin levels at placental level decrease afterwards, the plasmatic concentration of leptin increases concomitantly to the gestational age. This can be consecutive to leptin synthesis at the level of other pregnancy-specific tissues (decidua, cori-amnios) which increases as gestation progresses or the action of leptin binding proteins or leptin soluble receptors, abundantly present during this period [10]. Interaction with such a receptor can be responsible, at least partly, for the hyperlectinemia specific to pregnancy and can contribute to inhibiting the optimal interaction with the hypothalamic receptors, determining a leptin resistant state similar in terms of effect to that conferred by the suppressors of the cytokine signal in the hypothalamus (SOCS-3) [12]. Also, a change in the placental contribution and / or of the maternal adipose reserves determined by high hormone concentrations (estrogens especially) typical to advanced pregnancy, may be responsible for the leptin concentration increase [11].

Leptin receptors have been identified at the level of the anterior fetal hypophysis, leptin stimulating GH secretion, thus modulating the increase of the product of conception [3]. There have been confirmed the initial observations which correlated leptin levels in the umbilical cord with the cranial length and circumference of the newborn [1]. Leptin is also involved in angiogenesis and hematopoiesis, as revealed by studies carried out by Cioffi and Hirose [22,23]. Preeclampsia, which affects approximately 5-10% of the pregnancies represents one of its most common complications. Hypertension with arteriolar vasoconstriction is the major clinical manifestation which determines the reduction of the utero-placental flow, producing placental hypoxia, as well as fetal hypotrophy. Leptin secretion is high at the level of the trophoblastic cell line in conditions of hypoxia [7]. Placental ischemia can explain the rapid increase in leptin concentration during the third trimester of pregnancy, in preeclampsia, leptin increase representing an adaptive answer of the fetoplacental unit to flawed placental perfusion [18]. Moreover, it was found that leptinemia increases even more precociously before the twentieth week of gestation in patients which develop preeclampsia, suggesting that leptin can be considered a precocious marker of this affection [9]. It was found in preeclampsia that the leptin mRNA / actine $^{\beta}$ mRNA ratio is significantly higher in the tissue of placental vilosities in preeclamptic patients than in the healthy ones which have the same gestational age [48]. In pregnancies complicated with preeclampsia, several angiogenetic factors (the vascular endothelial factor and the growth placental factor) are in low concentration in maternal serum, probably explaining characteristic placentation, leptin participating in the capacity of angiogenetic regulator in this process. Also, high leptin secretion can contribute to the intensification of the sympathetic activity in preeclampsia.

In female patients with insulin-treated diabetes, leptin synthesis at placental level is high as is the concentration of serum leptin and a mechanism for regulating fetal growth has been advanced, exerted by insulin by up-regulating leptin secretion. On the other hand, in gestational diabetes, leptinemia levels are significantly lower as compared to the reference group of healthy pregnant women, with a similar amount of adipose tissue and similar levels of basal insulinemia [62].

Besides its role in regulating fetal and placental development, leptin can also be associated to certain mechanisms which mediate lactation and the growth of the newborn. Leptin, whose serum level decreases fast postpartum, is inversely correlated with the levels of serum prolactin. Mukherjea and collab. find that leptinemia is significantly higher in women who breastfeed as compared to those who do not, the authors

suggesting the role of leptin in mobilizing the energy reserves necessary to lactation [40]. Moreover, receptors for this hormone have been identified at the level of the mammary epithelial cells in sheep, indicating leptin involvement in the development of the mammary gland [11]. Leptin has also been identified in colostrum and in milk, result of the synthesis at the level of the epithelial mammary cells, as well as the diffusion in maternal circulation. At the newborn, the level of leptinemia decreases sharply after the birth, this decline being probably involved in breastfeed stimulation and in maintaining energetic homeostase [16].

Amenorrhea represents one of the symptoms characteristic to nervous anorexia (NA), hormone changes (which appear when more then 30% of the initial weight is lost) being complex and involving the entire endocrine system, the hypothalamo-hypophyso-gonadal axis included [7]. The amenorrhea from nervous anorexia is almost exclusively of central, hypothalamic origin . It was found that leptinemia levels in NA patients are significantly lower as compared to normal weight women and unaltered alimentary behavior [93]. The increase in serum leptin concentration, as a response to dietetic treatment measures is significantly correlated to LH and FSH serum levels, suggesting that the hypothalamo-hypophyso-gonadal axis can be activated by the increase in leptin levels consecutive to weight gain [10]. In spite of these, leptin represents a necessary signal, however not enough for the reoccurrence of menses in NA patients, weight gain not being always followed by menses resettlement; moreover, often, significant differences are not found between leptin levels in amenorrheic patients and those who do not have menstruation disorders, indicating that it is also necessary to normalize other endocrine axes such as the GH/IGF1 [21]. In nervous bulimia, serum leptin concentration is also low and seems to be in this case also correlated to menstrual cycle disorders, described in these patients [24]. Women who perform intense physical activity (ballet, performance athletics) are often amenorrheic, as a result of the suppression of GnRH pulsations. In these cases, significantly lowers levels of leptinemia are described, as well as an altered pattern of leptin secretion as compared to eumenorrheic patients, suggesting that low levels of leptinemia, next to a negative energy balance, can lead to the appearance of amenorrhea [23].

Obesity in humans, in most of the cases is due to a leptin resistance syndrome secondary to a down-regulation of receptors or to a post-receptor defect rather than to a leptin synthesis deficit [56]. Obesity is frequently associated to disorders of the reproductive function. It was found that in obese girls, menarche settles earlier as compared to normal weight girls, argument which supports the hypothesis that high levels of serum leptin contribute to the process of puberty initiation [25]. On the other hand, obesity is correlated to the increase in frequency of the anovulatory cycles, overweight women having a high number of atretic follicles. This is due to the direct effect of ovarian steroidogenesis inhibition by high leptin levels with the alteration of the follicular maturation process [6]. Thus, it can be assumed that increased levels of leptinemia, found in overweight patients can contribute to the dysfunction of the hypothalamo-hypophyso-gonadal axis at various levels: the central effect of leptin increase leads to the precocious settlement of menarche, which is later followed by a resistance of the gonadotropins answer to GnRH which, combined with a peripheral effect of ovulation inhibition, predispose to anovulation [83].

The virilizing polycystic ovary syndrome (polycystic ovary syndrome – PCOS) is a heterogeneous condition characterized by irregular menses and hyperandrogenism, frequently associated to metabolic disorders, such as obesity and insulin resistance, as well as to an abnormal pattern of the GnRH secretion, leading to the increase of the LH/FSH ratio. Although it is known that leptin can modulate gonadotropin secretion, its precise role in PCOS physiopathology is not known, leptinemia levels being unchanged in most of the cases [27, 28]. Moreover, treatment with antiandrogens,

estrogens, or medicines which increase sensitivity to insulin, do not influence leptin levels in humans [27], a single study existing in which short-term treatment with metformin in overweight women with PCOS was followed by the decrease in leptin concentration [79]. The high levels of leptinemia described in certain cases can be correlated to infertility. Mantzoros and collab. found that in women who successfully underwent in vitro fertilization (irrespectively of whether they had PCOS or not), follicular leptin level was much lower as compared to those in which in vitro fertilization was not followed by pregnancy [33].

Leptin plays an important role in the physiology of the reproductive system, having complex interactions at all the levels of the hypothalamo-hypophyso-gonadal axis. Observational studies have proved that both excess and secretion deficiency or leptin resistance can be associated to anomalies of the reproductive function. Interventional studies, involving leptin administration, will elucidate these complex correlations and will provide potential therapeutic options in these cases.

References

1.Fanutzzi G, Faggioni R. Leptin in the regulation of immunity, inflammation and hematopoiesis. J Leukoc Biol 2000; 68:437-46.

2. Matthew J. Cunningham, Donald K. Clifton, 3 and Robert A. Steiner. Leptin's Actions on the Reproductive Axis: Perspectives and Mechanisms. Biology of reproduction 1999; 60:216-222.

3. Moschos S, Chan JL, Mantzoros S. Leptin and reproduction: a review. Fertil Steril 2002; 77;433-44.

4. David R Mann, Tony M Plant.Leptin and pubertal development. Semin Reprod Med 2002; 20(2): 93-102.

5. Duggal PS, Van Der Hoek KH, Milner CR,et al. The in vivo and in vitro effects of exogenous leptin on ovulation in the rat. Endocrinology 2000; 141: 1971-6.

6. Cervero A, Dominguez F, Horcajadas JA, Quinonero A, Pellicer A, Simion C. The role of leptin in reproduction. Curr Opin Obstet Gynecol 2006; 18(3): 297-303.

7.Vera Popovic, Felipe F Casanueva. Nutrition and reproduction: new insights. Hormones 2000; 1(4): 204-217.

8. Susann Bluher Christos S Mantzoros The Role of Leptin in Regulating Neuroendocrine Function in Humans. J. Nutr. 2004; 134: 2469S-2474S.

9. Mantzoros CS, Dunaif A, Flier JS. Leptin concentrations in the polycystic ovary syndrome. J Clin Endocrinol Metab 1997; 82: 1687-91.

10. Michael C, Henson V. Daniel Castracane Leptin in Pregnancy. Biology of reproduction 2000; 63: 1219-1228.

11. Friedman JM. Leptin, leptin receptors, and the control of body weight. Nutr Rev, 1998;56:S38-S46.

12. Considine RV, Caro JF. Leptin and the regulation of body weight. Int J Biochem Cell Biol, 1997;29:1255-1272.

13. Coleman DL. Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 1973;9:294-298.

14. Pelleymounter MA, Cullen MJ, Baker MB, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995;269:540-543.

15. Chehab FF, Lim ME, Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with human recombinant leptin. Nat Genet 1996;12:318-320.

16. Barash IA, Cheung DK, Weigle DS, et al. Leptin is a metabolic signal to the reproductive system. Endocrinology 1996; 137:3144-3147.

17.Badger TM, Lynch EA, Fox PH. Effects of fasting on luteinizing hormone dynamics in the male rat. J Nutr 1985; 115:788-797.

18. Foster DL, Olster DH. Effect of restricted nutrition on puberty in the lamb:patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge system. Endocrinology 1985; 116:375-381.

19. Cameron JL, Nosbisch C. Suppression of pulsatile luteinizing hormone and testosterone secretion during short term food restriction in the adult male rhesus monkey (Macaca mulatta). Endocrinology 1991; 128;1532-1540.

20. Cameron JL, Weltzin TE, McConaha C, Helmereich DL, Kaye WH. Slowing of pulsatile luteinizing hormone secretion in men after fortyeight hours of fasting. J Clin Endocrinol Metab 1991; 73:35-41.

21. Bronson FH, Heideman PD. Short-term hormonal responses to food intake in peripubertal female rats. Am J Phisiol 1990; 259:R25-R31.

22. Bergendhal M, Perheentupa A, Huhtaniemi I. Starvation-induced suppression of pituitary-testicular function in rats is reversed by pulsatile gonadotropin-releasing hormone substitution. Biol Reprod 1991; 44:413-419.

23. **Aloi JA, Bergendahl M, Iranmanesh A, Veldhuis JD**. Pulsatile intravenous gonadotropin-releasing hormone administration averts fasting-induced hypogonadotrpism and hypoandrogenemia in healty, normal weight men. J Clin Endocrinol Metab 1997; 82:1543-1548.

24. Magni P, Vettor R, Pagano C, Calcano C, Beretta A, Motta M. Expression of a leptin receptor in immortalized gonadotropin-releasing hormone secreting neurons. Endocrinology 1999; 140:1581-1585.

25. Nagatani S, Guthikonda P, Thompson RC, Tsukamura H, Maeda K-I, Foster DL. Evidence for GnRH regulation by leptin: leptin administration prevents reduced pulsatile LH secretion during fasting. Neuroendocrinology 1998; 67:370-376.

26. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS. Role of leptin in the neuroendocrine response to fasting. Nature 1996; 382:250-252.

27. Finn PD, Cunningham MJ, Pau K-YF, Spies HG, Clifton DK, Steiner RA. The stimulatory effect of leptin on the neuroendocrine reproductive axis of the monkey. Endocrinology 1998; 139:4652-4662.

28. Tartaglia LA. The leptin receptor. J Biol Chem, 1997; 272:6093-6096.

29. Barb R, Hausman G, Lenz C. Feed Intache Regulation: Interface with the reproductive axis. Research 2008; 43 Suppl. 2:324-30.

30. McDonald JK. Role of neuropeptide Y in reproductive function. Ann NY Acad Sci 1990; 611:258-272.

31.Wilding JPH, Gilbey SG, Bailey CJ, Batt RAL, Williams G, Ghatei MA, Bloom SR. Increased neuropeptide-Y messenger ribonucleic acid (mRNA) and decreased neurotensin mRNA in the hypothalamus of the obese (ob/ob) mouse. Endocrinology1993; 132:1939-1944.

32. Licinio J, Negrado A, Mantzoros C et al. Synchronicity of frequently sampled 24h concentrations of circulating leptin, luteinizing hormone and estradiol in healthy women. Proc Natl Acad Sci 1998; 95:2541-2546.

33. Natagani S, Zeng Y, Keisler D, Foster D, Jaffe C. Leptin regulates pulsatile LH and GH secretion in scheep. Endocrinology 2000; 141:3965-3975.

34. Considine RV, Cooksey RC, Williams LB, Fawcett RL, Zhang P, Ambrosius WT, Whitfield RM, Jones RM, Inman M, Huse J, McClain DA. Hexosamines regulate leptin production in human subcutaneous adipocytes. J Clin Endo Metab 2000; 85:3551-3556.

35. Cavagnini F, Croci M, Putigano P, Petroni ML, Invitti C. Glucocorticoids and neuroendocrine function. Int J Obesity, 2000; 24 Suppl 2:S77-S79.

36.Shimizu H, Shimomura Y, Nakanishi Y, et al. Estrogen increases in vivo leptin production in rats and human subjects. J Endocrinol 1997; 154;285-92.

37. Luukkaa V, Pesonen U,. Huhtaniemi I, et al. Inverse correlation between serum testosterone and leptin in men. J Clin Endocrinol Metab 1998; 83: 3243-6.
38. Saad MF, Damani S, Gingerich RL, et al. Sexual dimorphism in plasma leptin concentration. J Clin Endocrinol Metab 1997; 82:579-84.
39. Licinio J,Negrao AB, Mantzoros C, et al. Sex differences in circulating human leptin pulse amplitude:clinical implications. J Clin Endocrinol Metab 1998; 83: 4140-7.
40. McConway MG, Johnson D, Kelly A, et al. Differences in circulating concentrations of total, free and bound leptin relate to gender and body composition in adult humans. Ann Clin Biochem 2000; 37: 717-23.
41. Montague CT, Prins CB, Sanders L, et al. Depot-and sex-specific differences in human leptin mRNA axpression: implications for the control of regional fat distribution. Diabetes 1997; 46:342-7.
42. Tritos & Mantzoros. Leptin: its role in obesity and beyond. Diabetologia 1997; 40:1371-1379.
43. Rosenbaum M, Leibel RL. Role of gonadal steroids in the sexual dimorphisms in body composition and circulating concentrations of leptin. J Clin Endocrinal Metab 1999; 84:1784-1789.
44. Bronson FH. Mammalian reproductive biology 1989. University of Chicago Press; 88.
45. Chehab FF, Mounzih K, Lu R, Lim NE. Early onset of reproductive function in normal female mice treated with leptin. Science 1996; 275:88-90.
46. Ahima RS, Dushay J, Flier SN, Prabakaran D, Flier JS. Leptin accelerates the onset of puberty in normal female mice. J Clin Invest 1997; 99;391-395.
47. Aubert ML, Gruaz-Gumowski NM, Heritier A, Pierroz DD, Lalaoui M, Vuagnat B, Aebi A, Pralong FP, Englaro P, Blum WF & Sizonenko PC. Leptin could represent a metabolic signal for sexual function and GH secretion: studies during sexual maturation and in adverse metabolic conditions. In Leptin-the Voice of the Adipose Tissue. Eds WF Blum, W Kiess & W Rascher.Heidelberg: J&J Edition, JA Barth Verlag, 1997.
48.Cheung CC, Thornton JE, Kuijper JL, Weigle DS, Clifton DK, Steiner RA. Leptin is a metabolic gate for the onset of puberty I the female rat. Endocrinology 1997; 138: 855-858.
49. Gruaz NM, Lalaou M, Pierroz DD, et al. Chronic administration of leptin into the lateral ventricle induces sexual maturation in severely food-restricted female rats. J Endocrinol 1998; 10 :627-633.
50. Cheung CC, Thornton JE, Nurani SD, Clifton DK, Steiner RA. A reassessment of leptin's role in triggering the onset of puberty in the rat and mouse.Endocrinology 2001; 74:12-21.
51. Lado –Abeal J, Hickox JR, Cheung TL, Veldhius JD, Hardy DM, Norman RL.Neuroendocrine consequences of fasting in adult male macaques: effects ofrecombinant rhesus macaque leptin infusion. Neuroendocrinology 2000; 71: 196-208.
52. Barker-Gibb ML, Sahu A, Ramaswamy S, Plant TM. Peripheral and centralinfusion of leptin in juvenile male monkeys does not elicit precocious GnRH release. Annual Meeting of the Society for Neuroscience, San Diego, 2001. Abstract 466.9.
53. Plant TM, Durant 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: 4505-4508.
54. Urbanski HF, Pau K-YF. A biphasic developmental pattern of circulating leptin in the male rhesus macaque (Macaca mulatta). Endocrinology 1998; 139:2284-2286.
55. Mann DR, Akinbami MA, Gould KG, Castracane VD. A longitudinal study of leptin during development in the male rhesus monkey: the effect of body composition and season on circulating leptin levels. Biol Reprod 2000; 62: 258-291.
56. Frisch RE. Pubertal adipose tissue: is it necessary for normal sexual maturation? Evidence from the rat and human female. Fed Proc 1980; 39: 2395-2400.
57. Cameron JL. 1997 Search for the signal that conveys metabolic status to the reproductive axis.Curr Opin Endocrinol Diabetes. 4: 158-163.
58. Strobel A,Issad I, Camion L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. Nature Genrtics 1998; 18: 213-215.
59. Clement K, Vaisse C, Lahlou N,et al. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 1998; 392: 398-401.
60. Farooqi IS, Jebb SA, Langmack G, et al. Brief report: effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med 1999 ;341: 879-884.
61. Pombo M,Herrera-Justiniano E, Considine RV, et al. Nocturnal rise of leptin in normal prepubertal and pubertal children and in patients with perinatal stalk-transection syndrome. J Clin Endocrinol Metab 1997; 82: 2751-2754.
62 Horlick MB, Rosenbaum M , Nicolson M, et al. Effect of puberty on the relationship between circulating leptin and body composition. J Clin Endocrinol Metab 2000; 85: 2509-18.
63. Garcia-Mayor RV, Andrade A,Rios M , Lage M, Dieguez C, Casanueva FF.Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. J Clin Endocrinol Metab 1997; 82; 2849-2855.
64. Matkovic V, Ilich JZ, Skugor M,et al. Leptin is inversely related to age at menarche in human females. J Clin Endocrinol Metab 1997; 82: 3239-45.
65. 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. J Clin Endocrinol Metab 1997; 82: 1066-1070.
66. Mann DR, Plant TM. Leptin and pubertal development . Sem Reprod Med 2002;20: 93-102.
67. Farooqi IS. Leptin and the onset of puberty: insigts from rodent and human genetics. Sem Reprod Med 2002, 20: 139-144.
68. Clayton PE , Gill MS, Hall CM, Tillmann V, Whatmore AJ, Price DA. Serum leptin through childhood and adolescence. Clin Endocrinol (Oxf) 1997; 46: 727-733.
69. Ahmed ML, Ong KKL, Morrell DJ, et al. Longitudinal study of leptin concentrations during puberty: sex differences and relationship to changes in body composition. J Clin Endocrinol Metab 1999; 84: 899-905.
70. Blum WF, Englaro P, Hanitsch S, et al. Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. J Clin Endocrinol Metab 1997; 82: 2904-10.
71. Gill MS, Hall CM, Tillman V, Clayton PE. Constitutional delay in growth and puberty (CDGP) is associated with hypoleptinemia. Clinical Endocrinology 1999; 50: 721-726.
72. Foster DL, Nagatani S Physiological perspectives on leptin as a regulator of reproduction: role in timing puberty. Biol Reprod 1999; 60: 205-215.
73. Cunningham MJ, Clifton DK, Steiner RA. Leptin's actions on the reproductive axis: perspectives and mechanisms. Biol Reprod 1999;60: 216-222.
74. Andreelli F, Hanaire-Broutin H, Laville M, Tauber JP, Riou JP, Thivolet C. Normal reproductive function in leptin-deficient patients with lipoatropic diabetes. J Clin Endocrinol Metab 2000; 85: 715-719.
75. Licinio J,Negrado A, Mantzoros C, et al. Synchronicity of frequently sampled 24h concentrations of circulating leptin, luteinizing hormone and estradiol in healthy women. Proc Natl Acad Sci 1998; 95: 2541-2546.
76. Ludwig M, Klein HH, Diedrich K et al. Serum leptin concentrations throughout the menstrual cycle. Arch Gynecol Obstet 2000; 263: 99-101.
77. Butzow TL,Moilanen JM, Lehtovirta M, et al. Serum and follicular fluid leptin during in vitro fertilization: relationship among leptin increase, body fat mass, and reduced ovarian response. J Clin Endocrinol Metab 1999;84: 3135-3139.
78. Rechberger T,Baranowski W,Postawski K, et al. Serum leptinconcentrations in women taking oral contraceptives. Eur J Obstet Reprod Biol 1999;83: 105-8.
79. Messinis IE, Papageorgiu I, Milingos s,et al. Oestradiol plus progesterone treatment increases serum leptin concentrations in normal women. Hum Reprod 2001;16: 1827-32.
80. Haffner SM, Mykkanen L, Stern MP. Leptin concentrations in women in the San Antonio Heart Study: effect of menopausal status and postmenopausal hormone replacement therapy. Am J Epidemiol 1997; 146: 581-5.
81. Gower BA, Nagy TR, Goran mI, et al. Leptin in postmenopausal women: influence of hormone therapy, insulin ,and fat distribution. J Clin Endocrinal Metab 2000; 85: 1770-5.

82. Konukoglu D, Serin O, Ercan M. Plasma leptin levels in obese and nonobese postmenopausal women before and after hormone replacement therapy. Maturitas 2000; 35: 203-7.
83. Fulvia Mancini, Domenico de Aloysio. Leptin and reproduction. Riv lt Ost Gin 2001; 4: 8-12.
84. Kitawaki J, Koshiba H, et al. Expression of leptin receptor in human endometrium and fluctuation during the menstrual cycle. J Clin Endocrinol Metab 2000; 85: 1946-50.
85. Caprio M, Isidori AM, Carta AR, et al. Expression of functional leptin receptors in rodent Leydig cells. Endocrinology 1999; 140: 4939-47.
86. Mantzoros CS, Cramer DW, Liberman RF; Barbieri RL. Predictive value of serum and follicular fluid leptin concentrations during assisted reproductive cycles in normal women and in women with the polycystic ovarian syndrome. Hum Reprod 2000; 15: 539-544.
87. Duggal PS, Van Der Hoek KH, Milner CR, et al. The in vivo and in vitro effects of exogenous leptin on ovulation in the rat. Endocrinology 2000; 141: 1971-6.
88. Kamohara S, Burcelin R, Halaas JL, et al. Acute stimulation of glucose metabolism in mice by leptin treatment. Nature 1997; 389: 374-7.
89. Brannian JD, Hansen KA. Leptin and ovarian folliculogenesis: implications for ovulation induction and ART outcomes. Sem Reprod Med 2002; 20: 103-112.
90. Unkila-Kallio L, Andersson S, Koistinen H, Karonen S, Yikorkala O, Titinen A, Leptin during assisted reproductive cycles: the effects of ovarian stimulation and of very early pregnancy. Human Reprod 2001 ,16: 657- 662.
91. Hardie L, Trayhurn P, Abramovich D,Fowler P. Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy Clin Endocrinol 1997; 47: 101-106.
92. Highman TJ,Friedman JE, Huston LP, Wong WW, Catalano PM. Longitudinal changes in maternal serum leptin concentrations, body composition, and resting metabolic rate in pregnancy. Am J Obstet Gynecol 1998; 178: 1010-1015.
93. Sivan E, Whittaker PG, Sinha D, Homko CJ, Lin M, Reece EA, Boden G. Leptin in human pregnancy: the relationship with gestational hormones. Am J Obstet Gynecol 1998; 179: 1128-1132.
94. Mukherjea R, Castonguay T, Douglass T, Moser Veillon P, Elevated leptin concentrations in pregnancy and lactation: possible role as a modulator of substrate utilization. Life Sci 1999,65: 1183-1193.
95. Mounzih K, Qiu J,Ewart-Toland A, Chehab F, Leptin is not necessary for gestation and parturition but regulates maternal nutrition via a leptin resistant state. Endocrinology 1998, 139: 5259-5262.
96. Sagawa N,Yura S, Itoh H, Mise H,Kakui K, Korita D,Takemura M, Nuamah MA, Ogawa Y, Masuzaki H, Nakao K, Fujii S, Role of leptin in pregnancy-a review. Placenta 2002; 23: (suppl A, Trophoblast Res 16) S80-S86.
97. Ashworth CJ, Hoggard N, Thomas L, mercer jG, Wallace JM, Lea RG. Placental leptin. Rev Reprod 2000; 5: 18-24.
98. Gonzalez rr, Caballero-Campo p, jasper M, Mercader A, Devoto L, Pellicer A, Simon C, Leptin and leptin receptor are expressed in the human endometrium and endometrial leptin secretion is regulated by the human blastocyst. J Clin Endocrinol Metab 2000; 85: 4883-4888.