LEPTIN HORMONE PROFILES IN ADULT AND ITS PHYSIOLOGICAL FUNCTIONS IN JUVENILE SYRIAN HAMSTERS (*Mesocricetus auratus*)

FATMA PEHLİVAN

SEPTEMBER 2003

TO MY PARENTS

LEPTIN HORMONE PROFILES IN ADULTS AND ITS PHYSIOLOGICAL FUNCTION IN JUVENILE SYRIAN HAMSTERS

(*Mesocricetus auratus)*

by

FATMA PEHLİVAN

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Prof. Dr. Davut KÖŞKER
Director

I certify that this thesis satisfies all the requirements as a thesis for the Degree of Master Science.

Prof. Dr. Tekin BABAÇ
Head of Department

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Biology.

Assoc. Prof. Dr. Bülent GÜNDÜZ
(Supervisor)

Examining Committee Members

Assist. Prof. Dr. Erdinç SERİN .....

Assist. Prof. Dr. Tülay TURGUT .....

Assoc. Prof. Dr. Bülent GÜNDÜZ .....

ABSTRACT

LEPTIN HORMONE PROFILES IN ADULT AND ITS PHYSIOLOGICAL FUNCTIONS IN JUVENILE SYRIAN HAMSTERS (*Mesocricetus auratus*)

Pehlivan, Fatma

M.Sc., Department of Biology

Supervisor: Assoc. Prof. Dr. Bülent GÜNDÜZ

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Essentially adipose tissue derived hormone leptin plays a regulatory role in energy metabolism, body weight, food intake, infection, inflamation, skeletal system, reproduction and the time of puberty. Syrian hamster is a photoperiodic species which response strongly to daylength changes. It is suggested that, lipid metabolism in this species is also affected from daylength changes. In the present study, leptin hormone status in adults and the effects of leptin in juvenile Syrian hamsters were investigated.

In the first study, the melatonin dependent and independent plasma leptin profiles of adult female Syrian hamsters were invastegated in gestation and lactation. Short and long day exposed female hamsters were paired. From the fourth day of the gestation, blood samples were collected four times until the day of the birth. Bloods were taken in two different time zone; at 24:00 hr when the leptin levels are the lowest and at 12:00 hr when the leptin levels are the highest in the plasma in this species. Blood collection was done during lactaion

four times, totally 8 times with the same method. Blood was taken from the orbital sinus of each animal under light ether anesthesia. Samples during dark phase were taken under light ether anesthesia under dim red light. To prevent the loss of circulating plasma volume, 0,9 % NaCl was injected immediately after each blood collection in the same volume as that drawn. Blood samples were centrifuged at 4 °C for 30 minutes at 4000 rpm. Serum aliquots were aspirated and frozen at -20°C. Hormones were measured by commercial ELİSA kits according to the manufacturer's instructions (ICN, CA, USA). Optical dencities were determined at 450 nm in an automatic microplate reader. In gestation period, when the leptin levels were compared in short and long photoperiods, there was a drop in both control and pinealectomized animals. These values were the lowest at the end of the gestation (from 40 ng/ mg to 30 ng/ mg). In lactation period, although there was no difference in leptin levels between short and long photoperiods, significant differences were determined between control and pinealectomy groups ($p < 0.05$). These results have shown that the decrease in leptin levels during gestation is independent from photoperiodic effect and pineal gland. However, the presence of pineal gland exhibit some differences in the leptin profile during lactation.

In the second study, the influences of pineal gland and leptin on the onset of puberty of juvenile male Syrian hamster was examined. Adult Syrian hamsters (2-3 months of age) were mated at 14L:10D photoperiod. Male pups were weaned at the age of 21 days, three different groups were made; control, leptin injected and pinealectomized + leptin injected ($n = 10$). Daily food intake, weekly body weights and testes weights of three groups were taken throughout 8 weeks. Leptin injections (except the control group) were made intraperitonaly

at 1μl/kg/day (the dose was increased according to the increase in body weight) dose every morning during 8 weeks. Consequently, the developments of body weights and testes weights of leptin injected groups showed faster increase than the control group but no statistically differences of food intake values were found among three groups ($p>0.05$). Leptin hormone accelerates the onset of puberty in juvenile male Syrian hamsters in the presence and absence of melatonin hormone. However, the onset of puberty in pinealectomized + leptin injected group was earlier than the other groups ($p< 0.001$). These results show that, leptin hormone has an important effect on onset of puberty time of juvenile male Syrian hamsters and may play a critical role in the regulation of gestation and lactation in adults.

In conclusion, the precise role of leptin plays in the function of each of these reproductive organs may be species dependent. Employing a variety of paradigms have strongly suggested that leptin may serve a number of important regulatory roles throughout gestation and lactation.

Key words: Leptin, Pineal gland, Gestation, Lactation, Puberty, Syrian hamster

ÖZET

YETİŞKİN SURİYE HAMSTERLERİNDE (*Mesocricetus auratus*) LEPTİN HORMON PROFİLİ VE YAVRULARDA FİZYOLOJİK FONKSİYONLARI

Pehlivan, Fatma

Yüksek Lisans Tezi, Biyoloji Bölümü

Tez Danışmanı: Doç. Dr Bülent GÜNDÜZ

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Leptin hormonu başlıca yağ doku hücrelerinden salgılanan bir hormon olup, enerji metabolizması, vücut ağırlığı, besin alınımı, infeksiyon, inflamasyon, iskelet sistemi, üreme ve puberteye giriş zamanı gibi pekçok olayda düzenleyici roller oynamaktadır. Suriye hamsteri gün uzunluğundaki değişimlere kuvvetli cevaplar veren fotoperiyodik bir türdür. Bu türde yağ doku metabolizmasınında fotoperiyodik değişimlerden etkilendiği düşünülmektedir. Bu çalışma ile yetişkin ve yavru Suriye hamsterlerinde leptin hormonu ve etkileri araştırıldı. Birinci çalışmada, Suriye hamsterlerinin hamilelik ve laktasyon dönemlerinde melatonine bağımlı ve bağımsız meydana gelen leptin hormon profili incelenmiştir. Uzun (14L) ve kısa (10L)

fotoperiyodlarda tutulan intakt ve pinealektomili yetişkin dişi hamsterlar çiftleştirildi. Hamile kalan hayvanlardan 4. günden itibaren doğuma kadar 4 defa kan alındı. Kanlar bu türde leptin seviyesinin en düşük değere indiği gece 24: 00 ve en yüksek değere çıktığı öğlen 12:00 saatlaerinde olmak üzere iki farklı dönemde toplandı. Laktasyon döneminde aynı dişi hayvanlardan bu dönem boyunca dört defa gece ve gündüz olmak üzere kan toplandı. Örnekler (~1ml) hafif eter anestezisi altında gözün orbital sinüsünden, ince heparinize kapiller tüp yardımıyla toplandı. Gece kanları ise parlak olmayan kırmızı ışık altında temin edildi. Kan alımından sonra plazmada oluşabilecek hacim kaybını önlemek amacıyla alınan kan miktarı kadar % 0,9'luk NaCl solüsyonu her bir hayvana ayrıca enjekte edildi. Örnekler 4000 rpm'de, 4 °C de, 30 dakika santrifüj edildikten sonra serum kısmı toplandı ve analiz edilinceye kadar -20 °C de saklandı. Leptin hormonu üretici firmanın (Assay Designs, USA) önerdiği tekniğe göre ELİZA aletinde, 450 nm de ölçüldü. Hamilelik döneminde, uzun ve kısa fotoperiyodlarda leptin seviyeleri karşılaştırıldığında gerek kontrol ve gerekse pinealektomili hayvanların leptin seviyelerinde bir düşme gözlendi. Bu değerler hamileliğin son döneminde en düşük değere indi. Laktasyon döneminde, leptin seviyeleri uzun ve kısa fotoperiyotlarda fark göstermemekle birlikte, kontrol ve pinealektomili gruplar arasında belirgin farklar gözlendi ($p< 0,05$). Hamilelik boyunca leptin seviyesinde meydana gelen düşme hem fotoperiyodik etkiden hemde pineal bezinden bağımsız gerçekleşmektedir. Laktasyon döneminde ise pineal bezinin olup olmaması leptin hormon profilinde farklılıklar ortaya çıkarmıştır.

İkinci çalışmada, pineal bez ve leptin hormonunun yavru erkek Suriye hamsterlerinin puberteye giriş zamanı üzerine olan etkileri incelendi. 2-3 aylık

yetişkin Suriye hamsterleri 14L: 10D fotoperiyodunda çiftleştirildi. Erkek yavrular 21 günlük olduklarında annelerinden ayrılıp kontrol, leptin enjeksiyonu ve piealektomi+ leptin enjeksiyonu olmak üzere 3 grup oluşturuldu. Bu üç grubun günlük besin tüketimleri, haftalık vücut ve testis ağırlık gelişimleri 8 hafta boyunca ölçüldü. Leptin enjeksiyonları intraperitonal olmak üzere her sabah 1μg/kg/gün (Leptin dozu vücut ağırlığındaki artışa bağlı olarak artırıldı) dozunda 8 hafta boyunca yapıldı. Sonuç olarak üç grubun besin tüketimleri arasında istatistiksel olarak belirgin bir fark bulunamadı ($p > 0,05$). Ancak leptin enjeksiyonu yapılan grupların vücut ve testis ağırlık değişimleri kontrol grubundakilere göre daha hızlı bir artış göstermiştir. Leptin hormonu yavru erkek Suriye hamsterlerinin puberteye giriş zamanını pineal bezden bağımsız olarak hızlandırmaktadır. Ancak pinealektomi + leptin enjeksiyonu yapılan grupta puberteye giriş daha erken olmuştur ($p < 0,001$). Bu sonuçlar göstermektedir ki, bu türde saptanan ilk veri olarak, leptin hormonu yavru hamsterlerin puberteye giriş zamanını belirgin bir farkla erken başlatmaktadır ve yine yetişkin Suriye hamsterlerinde hamilelik ve laktasyonun düzenlenmesinde kritik bir role sahip olabilmektedir.

Sonuç olarak, leptinin üreme oraganları üzerinde oynadığı spesifik role türe bağlı olarak farlılık gösterebilmektedir. Değişik yöntemler uygulanarak elde edilen sonuçlar leptinin hamilelik ve laktasyon dönemlerinde düzenleyici bir role sahip olduğuna işaret etmektedir.

Anahtar kelimeler: Leptin, Pineal bez, Hamilelik, Laktasyon, Puberte, Suriye hamsteri

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1.INTRODUCTION

1.1 PHOTOPERIODISM

Several species regulate their body weight, reproduction and energy expenditure according to the external environmental factors such as daylength, temperature, rainfall, humidity and food availability (Chapmann, 1970; Reiter, 1974; Stetson et al., 1975; Turek and Campbell, 1979). Mammals which utilize information to appraise their internal organs of the season are described as being photoperiodic. Photoperiod (day length) has been shown to be the most critical environmental cue regulating seasonal reproductive rhythms in mammals of the temperate zone. Mammals living in these ever-changing environments have evolved means to increase the chances of their survival, and more importantly, the survival of their young. The latter is achieved by having the young of the species born during the time of the year that is maximally supportive of their survival. This critical time is the spring of the year.

In photoperiodic species, mating occurs according to the length of gestational period. Animals which have a short gestation period (~16-20 days) and reproduce in spring and summer are called long day breeders; for example hamster, ferret and vole. Animals which have a long gestation period and reproduce in autumn and winter are called short day breeders; for example sheep, goat and deer which have a gestation length up to 6 months.

Reproduction and gonadal maturation in hamsters are very sensitive to photoperiod. Exposure to short photoperiods results in a gonadal regression and loss of sexual behaviour (Pospichal et al., 1991; Schilatt et al., 1993). Lack of responsiveness of reproductive system to photoperiodic changes has been reported for male laboratory

rats (Wallen et al., 1987), prairie voles (Nelson, 1985) and Shaw's jirds (El-Bakry et al., 1999). Studies on hamster species (Hoffmann, 1981), deer mice (Nelson et al., 1992) and marsh rice rat (Edmonds and Stetson, 1994) revealed that short photoperiods reduced testicular mass. Gonadal regression in short photoperiods has been reported for the Indian palm squirrel (Haldar and Pandey, 1988). It was demonstrated that constant light resulted in reproductive inhibition whereas constant darkness was stimulatory in the Indian desert gerbil (Sinhasane and Joshi, 1997; Thomas et al., 2001).

Reproductive development in many photoperiodic species occurs only when the animal is exposed to photoperiods greater than threshold (critical photoperiod), while reproductive development is greatly prolonged by photoperiods less than critical. Critical photoperiods, often between 12 and 14 hr of light per day, have been characterized for several rodent species. In the Syrian hamster, this photoperiod is 12,5 hours of light per day, in the Siberian hamster it is about 13 hours. In Syrian hamsters daylength shorter than 12,5 hours causes gonadal atrophy whereas the daylength longer than this promote gonadal maintance (Elliot, 1976; Hoffmann, 1979; Hoffmann, 1981). So that, during the short days of the winter the testes are involuted and the animals are sexually incompetent (Reiter, 1974). Conversely, during the spring and summer the animals possess large functional gonads equivalent in size to those normally seen in hamsters maintained under long days in the laboratary setting. Similar relationships between daylength and the status of the reproductive organs exist in female hamsters (Reiter, 1974).

In several species it has been shown that photoperiod may also regulate gonadal development in juveniles (Johnson and Zucker, 1980; Hoffmann, 1978; Hoffmann,

1981). Most of the rodents which is born in spring get mature and can mate in the same season, with their birth. In Montane vole, deer mice and Siberian hamster, short photoperiod exposure appears to be responsible for the prevention of pubertal development. (Horton, 1984; Stetson et al., 1986; Lee and Zucker, 1988). Siberian hamsters maintained from birth in long photoperiods have large and fully functional testes at the age of 40 days, while in short photoperiods, testicular development is delayed up to about 180 days of age (Gaston and Menaker, 1967). In Turkish hamster, the response of intact prepubertal hamsters to photoperiods depends on the lactational period (Gündüz and Stetson, 1998). On the other hand, in young lemmings, photoperiod did not affect testicular development but had an effect on pelage color in the same animal (Hassler et al., 1976). However, the prepubertal gonadal maturation and body weight gain in Syrian hamster are independent from environmental daylenght.

1.2 PINEAL GLAND

Pineal gland is a small pine-shaped structure located in the epithalamus of the brain. It originates as an envagination of the diencephalon and in most species retains a "stalk" connection to the habenular commissure. Frequently it is closely associated with the third ventricle of the brain and the area of the third ventricle receiving the pineal stalk is known as the pineal recess. The mammalian pineal is a secretory organ whereas in fish and amphibians it is directly photoreceptive and in reptiles and in birds it has a mixed photoreceptor and secretory function. Most important product of the pineal gland is the hormone melatonin. Melatonin is secreted and released from pineal gland in a rhytmic manner. Pineal gland functions as a chemical neurotransducer which converts

the neural stimuli to a hormonal product as melatonin. And also in some birds and lower vertebrates it serves as a rhythm generating system or a biological clock.

There are two dominant environmental oscillators shaping the living conditions of our world: The day-night cycle and the succession of the season. Organisms have adapted to these by evolving internal clocks slaved to the master pacemaker of the suprachiasmatic nuclei (SCN) synchronizes the body to the daily 24 hour cycle. However, this clock's work closely interacts with the seasonal time-teller. Recent experiments show that photoperiod, the dominant zeitgeber of the circannual clock might be decephered by the organism using the tools of the circadian clock itself. Destruction of the SCN in the Syrian hamster by lesions, abolishes three well documented circadian rhythms, locomotor activity, estrous cyclicity and photoperiodic photosensitivity. Entrainment of these rhythms by light cycles fails in lesioned hamsters; females become persistently estrous. Locomotor activity in both sexes is abolished and is totaly arrhythmic when lesioned animals are exposed to continuous darkness. The photoperiodic gonodal response (gonodal regression induced by short daylengths) is abolished, lesioned animals remain reproductively mature irrespective of photoperiodic treatment.

Light information is first perceived by the retina of the eye. Then this information is transferred to suprachiasmatic nuclei (SCN) by the retinohypothalamic tract. SCN is a very important brain component because it generates all the internal rhythms in the body and also it conducts the light information to the superior cervical ganglia (SCG). At the end, light information reaches to the pineal gland (Arent, 1995).

Pinealectomy (the remove of the pineal gland) is one of the methods to investigate the effect of melatonin in photoperiodic animals. Pinealectomy eliminates the melatonin hormone from blood circulation. It prevents the animal from responding against the changes in daylength (Hoffmann and Reiter, 1965; Hoffmann, 1974). The effects of pinealectomy in some hamster species have been well documented. Pinealectomy prevents the regressional effect of short photoperiods while gonadal maintenance on long photoperiods is not affected in Syrian hamster (Hoffmann and Reiter, 1965). The effect of pinealectomy on Siberian hamsters is more complex. Pinealectomy blocks short photoperiod induced gonadal regression of hamsters previously housed on long photoperiod (Hoffmann, 1974). The pineal gland is also involved in tranducing long photoperiod effects in these species: Pinealectomy prevents long photoperiod induced gonadal growth of hamsters previously housed on short photoperiod. Thus, the general effect of pinealectomy in adult Siberian hamster is either to prevent the perception of a change in daylength or to maintain the daylength measurement system in preoperative mode, the gonads are maintained in a state appropriate to the previous photoperiod (Gündüz and Stetson, 1994). The responses to pinealectomy in Turkish hamsters are much more different. In this species, pinealectomy causes gonadal atrophy in long photoperiods but in short photoperiods, pinealectomy does not prevent the gonadal regression (Carter et al., 1982). Also there is an effect of pinealectomy on juvenile sexual development in Siberian hamsters. Removal of the pineal gland deletes the effect of long and short photoperiod on gonadal development. The development of pinealectomized juveniles is more rapid than the intact juveniles (Hoffmann, 1973; Brackmann, 1977). Moreover, pinealectomy inhibits

the stimulatory effect of long photoperiods (Hoffmann and Kuderling, 1975). Besides the effects on reproductive system, pineal gland may affect the body weight and lipid mass changes. It was reported in Syrian hamsters, Siberian hamsters and praire voles that pinealectomy may block the photoperiod related changes in body weight.

The nature of the endocrine message communicated via the melatonin rhythm has been the subject of much investigation. Two different possibilities have been proposed to explain how melatonin conveys the photoperiod information to the reproductive axis (Reiter, 1991a,b; 1993). The duration hypothesis, implies that the photoperiod induced modification in the length of the nocturnal melatonin rise, is the message that synchronises biological and reproductive functions to the different seasons of the year (Reiter, 1987; Bartness et al., 1993). Evidence in support of this hypothesis has come from melatonin infusions in pinealectomized Siberian and Syrian hamsters. The internal coincidence hypothesis implies that melatonin exerts an effect only when its circadian secretion is coincident with target tissue sensitivity. This hypothesis supposes that the time of presence of melatonin is important. Support for this hypothesis has come from experiments in intact adult Syrian hamsters (Stetson and Tay, 1983), Turkish hamster (Hong and Stetson, 1987) and Siberian hamsters (Stetson et al., 1986), pinealectomized adult Syrian hamsters (Stetson and Watson-Whitmyre, 1986; Watson-Whitmyre, 1985) and intact and pinealectomized juvenile Siberian hamsters (Gündüz and Stetson; 2001a, b).

Body weight responses show a species-specific situation. Seasonal body mass changes are demonstrated by seasonal non-hibernating breeders, and, in general, body mass of individuals differs in breeding (favorable) and non-breeding (unfavorable)

seasons. Some species show a significant decrease in body mass in winter or dry season whereas the other species demonstrate the opposite trend (Hoffmann, 1981; Gower et al., 1994). There are also seasonal breeders that do not change body mass seasonally (Bartness and Wade, 1984). It is an expected seasonal adaptation for some animals living at higher latitudes to increase their energy stores before winter, like graung squirrel (*Spermophilus beldingi*), Syrian hamster (*Mesocricetus auratus*), and praire voles (*Microtus ochrogaster*). Conversely, some species like, meadow voles (*Microtus pennsylvanicus*), deer mice (*Peromycus maniculatus*) and Siberian hamster (*Phodopus sungorus)* decrease their body weight when they exposed to short winter like days. In three gerbil species, *M. crassus*, *G. dasyurus* and *G. henleyi*, there was a significant reduction in body weight in winter, ranging from %8,9-%20,2 (Vander Wall, 1990). The increase of body weight and lipid mass might seem intuitive before winter but decreases are explained as an adaptive strategy that reduces energy requirements during the harsh conditions (adaptation hypothesis) (Stebbins, 1978) and a response to changes in food-water availability (Abramsky, 1990). The adaptation hypothesis received support from studies on Meadow voles (*Microtus montanus*) and Siberian hamster in which body weight was found to be regulated by photoperiod (Heldmaier and Steinlechner, 1981). Siberian hamster reduces its body weight before winter some %45 for about 45 g to 25 g. Meadow voles also change their energy intake and body weight in response to photoperiod. Reduction in body weight was due to a decrease in total body water and in lean body mass, but brown adipose tissue remained unchanged. However, there was a reduced activity of white adipose tissue lipoprotein lipase. The loss in body weight during the short photophase is in agreement with other studies on

winter acclimated voles (Wunder et al., 1977). Also *Cricetus cricetus* born in autumn were unable to increase their body weight in winter even with excess food under stable conditions (Conguilhem and Marx, 1973). As it seen in different animal examples, photoperiod plays a major role on the regulation of body weight. And pineal gland is the most important component in this photoperiodic regulation.

1.3 MELATONIN

This indolamine is a highly conserved molecule that may exist in organisms ranging from algae to humans. Melatonin is produced and released from the pineal gland into the blood especially at night and is referred to as the chemical experience of darkness (Reiter,1981; Stetson and Watson-Whitmyre,1984). Other reported sites of melatonin synthesis are the retina, the harderian gland, the gut and blood platelets (Dubocovich, 1988). Melatonin readily gains access to every fluid and cell in the organism (Reiter, 1993). It also functions in the control of endocrine and reproductive physiology, and has been shown to be a potent free radical scavenger (Reiter, 1980).

The melatonin activity of the pineal gland is determined primarily by the release of norepinephrine (NE) (Axelrod, 1974) from postganglionic sympathetic nerve endings that terminate in the gland. The release of NE is associated with darkness, when the SCN are relieved of the inhibitory signal from the eyes due to the interaction of photons with the retinas. Inasmuch as NE release onto the pinealocytes occurs at night, melatonin synthesis likewise occurs primarily during darkness. Therefore, the concentration of melatonin in the blood is greater at night than during the day.

Three different patterns of nocturnal melatonin production have been describes (Reiter, 1991a,b). Type A pattern of melatonin production is typified by a delay of

several hours in the onset of melatonin synthesis after darkness onset; thereafter, the melatonin level rises to its peak quickly and soon thereafter returns to daytime values, at about the time of lights on. An example of a species with this type of melatonin rhythm is the Syrian hamster. Animals with a type B melatonin pattern experience a gradual rise in pineal production of the indole beginning at about the time of lights off; peak melatonin levels are reached during the middle of the dark phase followed by a gradual reduction in indole production during the second half of the night. The two best known species that exhibit this common pattern of nocturnal melatonin synthesis are the domestic rat and the human. Mammals with type C pattern of nocturnal melatonin synthesis are also common and they experience high melatonin levels for virtually the entire night. Thus, within 30 min after darkness onset peak melatonin production is reached; these high levels are maintained for the entire period of darkness. This pattern of melatonin production is present in the sheep and the cotton rat among other species.

Melatonin has a half life of nearly 20-40 minutes. It does not remain in the blood very long. Unless the pineal gland continues to produce and secrete melatonin, blood levels of the hormone drop quickly. Melatonin is removed from the blood in at least four ways. It is enzymatically degraded primarly to 6-hydroxy melatonin by the liver. Melatonin is taken up by other cells is non enzymatical degraded when it scavenges hydroxyl radicals. Also, melatonin in the blood rapidly escapes into other body fluids. Finally, melatonin attaches to specific receptors or binding sites located at various locations in the organism.

Melatonin receptors (or binding sites) have been most frequently reported (in mammals) in the central nervous system. The areas in which radioactive melatonin

binding has often been described include the SCN, pars tuberalis of the adenohypophysis, preoptic area, area postrema and various cerebrocortical structures Stankov et al., 1991).

The melatonin receptors involved in mediating the effects of melatonin on the reproductive and endocrine systems are presumed to be those located in the pars tuberalis of the anterior pituitary gland (Stankov et al., 1991). These cells are in close proximity to the primary portal plexus and the terminals of the hypothalamic releasing hormone neurosecretory cells in the median eminence. Melatonin theoretically controls the release of substances, e.g., gonadotropins or other factors, that act in a paracrine manner in the nearby median eminence thereby regulating the release of the hypothalamic releasing hormones, e.g., gonadotropin releasing hormone (GnRH). In this manner melatonin could obviously regulate the functional status of the gonads and control the reproductive capability of an animal on a seasonal basis.

1.4 LEPTIN and ADIPOSE TISSUE

Leptin (167-amino acid polypeptide hormone), the product of the ob gene (Zhang et al., 1994) is an adipocyte-secreted protein (Frederich et al., 1995) whose circulating levels in the fed state reflect body fat content in mice (Frederich et al., 1995; Caro et al., 1996) and humans (Considine et al., 1997) and signal the status of energy stores to the brain. Moreover, leptin levels in the blood change in response to fasting and overfeeding independently of changes in adipose tissue stores (Boden et al., 1996). In addition to its role in regulating energy balance, leptin mediates the neuroendocrine response to fasting-starvation (Ahima et al., 1996), may play a role in haematopoiesis (Bennett et al., 1996), functions as the metabolic signal to the reproductive system

(Chehab et al., 1996) and appears to signal the onset of puberty in rodents (Ahima et al., 1997; Chehab et al., 1997) and humans (Mantzoros et al., 1998).

Serum leptin concentrations are positively correlated with the white adipose tissue (Wade et al., 1996). The leptin amount synthesized by different adipose tissue localizations offer regional differences, being the subcutaneous fat tissue more visceral tissue in secreting leptin. Leptin expression in adipose tissue and circulating leptin depends upon fat/ energy stores, which are affected by sex and dietary patterns. Females have higher plasma leptin concentration than males, even after being adjusted the values by body weight, fat content or age. This could be attributed to: 1) a higher efficiency of the female adipocyte in producing leptin, 2) the leptin secretion is induced by estrogen, and 3) a higher subcutaneous fat content in women than men. A possible explanation of this phenomenon has been attributed to the need to cover the high requirements of lactation and pregnancy in women (Licinio, et al., 1998).

The activity of reproductive axis is sensitive to the sufficient quantity of food and the store of the metabolic sources. Leptin hormone that is secreted from adipose tissue is correlated with the regulation of the food intake and body mass. It functiones as a metabolic gate to reproductive system (Wade et. al., 1996).

Genetically obese ob/ob mice (lacking endogenous leptin) are infertile due to the absence of the leptin hormone. Leptin treatment to these mice restores the fertility (Chehab et. al., 1996). Food restricted animals have reduced circulating levels of leptin, which are associated with markedly reduced secretion of gonadotropins (Matthew et. al., 1999). Refeeding restores the normal leptin levels. Moreover, fasting or food restriction

decrease the level of circulating leptin being the reduction accompanied by an increase in food intake and a fall in energy expenditure (Li et al., 1997).

Overmuch food restricted animals have decreased circulating levels of leptin, this situation is associated with markedly decreased secretion of the gonadotropins (Matthew et al., 1999). Treatment of food-restricted mice, rats, sheep and monkeys with exogenous leptin, reverses the diet-induced inhibition gonadotropin secretion. In Siberian hamsters, exposure to short photoperiod decrease leptin gene expression and hormone release related to decrease in adipose tissue (Klingenspor et.al., 1996; Klingenspor et.al., 2000). Leptin might be affected independently upon to food deprivation in seasonal behaviors of animals mediated with photoperiod.

Diurnal pattern of plasma leptin concentrations were defined in human (Sinha et al., 1996). Nevertheless, it has been understood that this pattern entrains to time of eating rather than a circadian circulation. Leptin levels increase after eating and decrease to the lowest level in the morning (Schoeller et al., 1997). This diurnal pattern is also determined in rats and mice (Ahren, 2000; Saladin et al., 1995).

There is considerable debate concerning the potential role of leptin as a physiological trigger for initiating the onset of puberty. Because leptin is a metabolic signal that indicates the level of somatic development, it may signal the brain that the body is capable of supporting pubertal development and subsequent reproductive function. As such, leptin could potentially serve as a trigger for the initiation of sexual maturation (Vogel, 1996).

Leptin-treated prepubertal female mice reproduced at an earlier age than did nontreated controls, indicating that leptin may help to trigger puberty (Chehab et al.,

1997). Administration is associated with elevated LH concentrations and dramatic changes in ovarian and uterine weights and histology. Leptin-associated mechanisms appear to be conserved across species. Leptin stimulates gonadotropin release in rhesus macaques, identifies the polypeptide as a mediator of reproduction in multiple species, from rodents to primates. Leptin may also serve as a permissive regulator of human reproductive maturity (Foster and Nagatani, 1999; Kiess et al., 1999), as increases in peripheral levels are associated with the onset of menarche (Matkovic et al., 1997). Women of reproductive age typically exhibit higher levels than men of comparable age or adiposity (Castracane et al., 1998).

During pregnancy, maternal serum leptin concentrations are greater than those in nonpregnant women (Butte et al., 1997). Substantial increases in early pregnancy, before the occurrence of any notable increase in body weight due to progressive gestation, imply that factors other than increased adiposity mediate maternal leptin levels (Highman et al., 1998). Numerous studies have demonstrated that maternal peripheral leptin levels are enhanced during pregnancy (Sivan et al., 1998) and collectively suggest that leptin concentrations peak in the second trimester and remain elevated until parturition. In the pregnant mouse, serum leptin levels peak on Day 17 and are many fold higher than nonpregnant levels. Leptin is indeed produced during pregnancy, expressed by the placenta, the importance of leptin is not clear. In the pregnant rat, serum leptin increases during pregnancy (Garcia et al., 2000), perhaps declining before parturition (Chien et al., 1997).

During pregnancy and lactation, major adaptations in maternal energy metabolism must occur to meet the requirements of fetal growth and milk production.

Although these energetic adaptations have been studied extensively during pregnancy, the extracellular factors responsible for their onset have not been well-defined. Leptin, because of its role in the regulation of energy metabolism, has been implicated in the coordination of maternal adaptations during pregnancy and lactation. Maternal plasma leptin concentration is elevated during pregnancy in rats, mice and humans and falls during the transition to lactation (Masuzaki et al., 1997; Butte et al., 1997). Leptin is regulated by adiposity and energy balance and it is unclear whether factors specifically associated with pregnancy or lactation can alter plasma leptin concentration independently of changes in energy balance or adiposity.

1.5 THE RELATIONSHIP BETWEEN LEPTIN AND MELATONIN

The recent studies have shown that there is a direct or indirect relationship between leptin and melatonin hormones. Melatonin application in rats causes a significant reduction in serum leptin concentrations (Mastronardi et al., 2000). In contrast, leptin levels increase after pinealectomy (Rasmussen et al., 1999; Wolden-Hanson et al., 2000). Acccording to this data melatonin declines the secretion of leptin hormone. On the other hand, leptin and melatonin hormones exhibits an antogonistic influence in some physiological processes such as the onset of puberty, pain transmission, food intake and reproduction (Chehab et al., 1997).

Puberty onset is accelerated by leptin whereas it is delayed by melatonin. One possible mechanism for this; melatonin suppresses the leptin secretion from pituitary. Serum melatonin levels decrease before puberty and this led the increase in serum leptin concentrations. Another antogonistic influence is seen in the regulation of pain transmission. When melatonin was applied, the pain treshold is increased and by this

way decreases the sensitivity to pain. Although the studies about anelgezic effects of leptin are unclear, it has been shown in mice that leptin application decreases the treshold of pain and increases the sensitivity to pain. Appetive suppressive effects of leptin is well known. Apart from that, leptin also increases the energy consumption. On the contrary, melatonin increases the apetite. The decrease in food intake in pinealectomized mice is returned to normal amounts with melatonin application. Another antogonistic interaction between leptin and melatonin hormones is seen in oxidative stress. It has been shown that, melatonin decreases the oxidative stress by rendering free radicals and increasing the activity of antioxidative enzymes (Reiter, 1997). Conversaly, leptin increases the oxidative stress in endotelial cells. Reproductive physiology is also affected from leptin and melatonin hormones in an antogonistic character. Melatonin inhibits the reproduction of photoperiodic animals. Whereas leptin stimulates (Matthew et al., 1999).

1.6 SYRIAN HAMSTERS

Syrian hamsters have been prefered as a laboratory model species because of their strong photoperiodic characteristics that illuminate effect various mechanims of the day length. However, the body weights and gonadal development of prepubertal individuals of this species are independent from ambient photoperiod (Gaston and Menaker, 1967; Stetson, 1978).

Syrian hamsters display a 4-day estrous cycles that are exceptionally predictable and easy to monitor compared to the estrous cycles of other laboratory animals. On day 4 of the estrous cycle, adult females show streotypical estrous behaviour (lordosis) proptly upon being introduced to a sexually experienced male. Ovulation occurs during the

night of day four. The next day, is characterized by the appereance of a consipicious vaginal discharge (Orsini, 1961).

The hamster has the shortest gestation period in eutharian mammals. Gestation length depends on the age of the mated hamsters. It is about 373 hours for one month-old mated hamster while 402 hour for 14 months of age (Soderwall et.al., 1960). There is a sharp drop in mating and deliveries after 14 months of age.

Sexual maturation and the onset of puberty are aspects of mammalia reproduction which have received considerable attention. Rather than being under strict genetic control, the timing of the prepubertal events can be modulated for environmental conditions such as nutrition, temperature and noise. Photoperiod which is an important regulator of annual breeding cycles in many adult mammals, also has been thought to play a role in the sexual maturation process. Although the reproductive system of adult syrian hamster is exquisitely sensitive to photoperiod being inactivated when daylength is less than 12,5 h. It has not been clear from early work whether the prepubertal sexual maturation process is under photoperiod control. Syrian hamsters born in the spring and summer have been reported to reach puberty more rapidly than those born in the fall under conditions of decreased day length (Czyba, 1968). Yet male hamsters blinded at birth demonstrated gonadal weights at 6 weeks of age similar to those of sighted controls (Reiter et al., 1975) suggesting a lack of photoperiodic involvement in early sexual development.

Body weights and fat content of this species is preciaely controlled by photoperiodic changes. They reduce body weight in long photoperiods but increase in short photoperiods; opposite to other counterparts (e.g. Siberian hamsters). This

mechanism in altering body fat and weight is not well known and is suggested that leptin may adjust this changes (Karakaş and Gündüz, 2002).

1.7 OBJECTIVES

It has been demonstrated in many physiological process that there is an antogonistic relationship between adipose derived leptin and pineal gland derived melatonin hormones. However, maternal serum leptin levels in gestation and lactation periods, the influence of leptin application on puberty and the effect of pinealectomy on these processes were not defined very well.

The objectives of the studies reported here was to determine the serum leptin profiles of adult female Syrian hamsters during gestation and lactation periods and to determine the effects of leptin injections on the onset of puberty in juvenile male Syrian hamsters with or without pineal gland.

2. MATERIALS AND METHODS

2.1 Animal care

Syrian hamsters (*Mesocricetus auratus*) were obtained from our laboratory colony maintained at the Abant Izzet Baysal University. They were exposed from birth to 14L (14 hour of light, 10 hour of darkness, lights off at 20:00 hr). Animals were maintained in plastic cages (16x31x42 cm) with pine shavings used as bedding. Food pellets (Purina rodent chow) and top water were accessible ad libitum. Principles of laboratory animal care and specific national laws were followed. All lightning was provided by cool-white fluorescent tubes controlled by automatic programmable timers. Ambient temperatures in the animal facilities were held constant at 22 ± 2 °C in air-ventilated rooms.

2.2 Anesthesia

Before surgery, hamsters were anesthetized subcutaneously with Ketamine (20 mg/kg BW, Sigma Chemical Company, MO, USA) and intraperiotoneally with pentobarbitol (32.5 mg/kg BW). Depth of anesthesia was monitored by frequent testing for the presence of leg flexion reflexes and active muscle tonus.

2.3 Surgical Procedures

Pinealectomy Pinealectomy of hamsters was performed according to the method of Hoffmann and Reiter (1965); aspiration was used to control the hemorraging. Anesthetized hamsters were placed in a stereotaxic apparatus to stabilize the head during surgery. After the head was shaved the surgical area was sterilized with 70% ethanol, an incision was made in the scalp. Muscle attachments were removed from the dorsal skull. After drying the skull, an incomplete circular cut was made with a dental drill

burr at the λ (lambda) suture and a piece of cranium covering the pineal gland was folded forward anteriorly. Fine-tipped forceps was used to extend into the confluence of the sinuses to grasp and remove the pineal gland. After removal of the pineal gland, the bone flap was replaced and a small square of absorbable gelatin sponge (Gelfoam, Up John, Kalamazoo, MI) was applied to the skull surface to help promote clotting. The scalp was closed with a stainless steel surgical clips. After surgery, the incision was treated with Newskin adhesive to prevent any contamination.

Testes Measurements The length and the width were measured with calipers to the nearest 0.1 mm by external palpation of the left testis in the scrotum. In most cases, only one of the testes was measured, alternating sites on successive animals. However, if the testes are suspected of being disparate in size (as assesed by gentle palpation) both were measured. Paired testicular volume was calculated from this measurement using the formula for a prolate spheroid (Watson-Whitmyre and Stetson, 1985);

$$\text{Volume} = 0.5236\ (\text{length})\ \text{width})$$

This single testis volume (STV) was then converted to paired testes weight (PTW) using a pre-determined linear regression formula;

$$\text{PTW} = 1.846\ (\text{STV}) - 0.015$$

All data from in situ testicular measurements are reported in the form of paired testes weights derived by this method.

2.4 Hormone Measurement

Blood samples from each group were obtained for leptin measurements. Blood was taken from the orbital sinus of each animal under light ether anesthesia. Samples during dark phase were taken under light ether anesthesia using dim red light. To

prevent the loss of circulating plasma volume, 0.9 % NaCl was injected immediately after each blood collection in the same volume as that drawn. Blood samples were centrifuged at 4 °C for 30 minutes at 4000 rpm. Serum aliquots were aspirated and frozen at -20°C. Hormones were measured by commercial ELİSA kits according to the manufacturer's instructions (ICN, CA, USA). Optical densities were determined at 450 nm in an automatic microplate reader. Serum concentrations of hamster leptin were measured in duplicate, with a lower detection limit of 0.5 ng/ml. The intra and inter-assay coefficient of variation (CV) were less than 10%.

2.5 Statistics

Data were analyzed using SPSS (SPSS Statistical Software, SPSS Inc., Los Angeles, CA, USA, Ver. 10.0). Testes and body weights were analyzed using a repeated measures two-way analysis of variance (ANOVA). Hormone levels were analyzed by one way analysis of variance followed by Duncan's multiple range test. Differences between means within or between groups were determined by t-tests. Values were considered statistically significant at $p<0.05$. Data are presented as mean ± SEM.

2.6 Studies

2.6.1. *Leptin profiles during the period of gestation and lactation*

Melatonin dependent and independent plasma leptin concentrations of adult female Syrian hamsters were investigated in gestation and lactation periods.

Short (10L) and long (14L) day exposed female hamsters were paired. Pregnancy was confirmed by checking the estrus cyclicity. Blood samples from each group were obtained for leptin measurement every fourth day of gestation until the birth of babies. Samples were taken at 12:00 hr and 24:00 hr; these times reflects the highest

and lowest leptin levels in the blood of this species (Gündüz, 2002). Blood collection on females continued thorugh lactation period until the pups were weaned.

2.6.2. *Leptin's effect on puberty*

The aim of this study was to examine the influences of pineal gland and leptin on the onset of puberty in juvenile male Syrian hamster.

*A*dult Syrian hamsters (2-3 months of age) were paired at 14L: 10D photoperiod. Male pups were weaned at the age of 21 days. Three different groups were made; control, leptin injected and pinealectomized + leptin injected (n = 10). Daily food intakes, weekly body weights and testes weights of three groups were taken throughout 8 weeks. Leptin injections (except the control group) were made intraperitonally at 1 μg/ kg / day dose at 12:00 hr from day 21 throughout 8 weeks.

3. RESULTS

3.1 In gestation period when the leptin levels were compared in short and long photoperiods, there was a drop in both control and pinealectomized animals. These values were the lowest at the end of the gestation. In control group at long photoperiod it droped from 42 to 30 ng/ml in pinealectomized group it droped from 40 to 27 ng/ml (fig 3.1-3.2). In control group at short photoperiods it droped from 41 to 28 ng/ml, in pinealectomized group it droped from 38 to 30 ng/ml (fig 3.3-3.4). In lactation period, leptin levels were different between control and pinealectomized groups in day and night. Leptin levels were different during the first three measurements between control and pinx animals in 14L-day (fig 3.5). These values were changed in 14L-night; the difference were observed only in the second measurement (fig 3.6). On the other hand, leptin levels were significantly different during the first two measurements between control and pinx groups in 10L-day (fig 3.7). By contrary, the difference between groups was observed only in the first measurement in 10L-night (fig 3.8). These results have shown that the decrease in leptin levels during gestation is independent from photoperiodic effect and pineal gland.

Control Mean ± SE	Pinx Mean ± SE
42.4 ± 2.1	46.1 ± 2.2
37.8 ± 1.9	34.1 ± 1.8
31.4 ± 1.5	32.4 ± 1.5
35.2 ± 1.5	25 ± 1.3

Table 3.1: Serum leptin values (ng/ml) of 14L-day exposed adult female Syrian hamsters in gestation. SE: Standart Error, Pinx: Pinealectomy

Control Mean ± SE	Pinx Mean ± SE
39.7 ±1.8	33.1 ± 1.4
37.5 ± 1.6	34.2 ± 1.5
24.9 ± 1.1	27.6 ± 1.3
27 ± 1.3	26.4 ± 1.25

Table 3.2: Serum leptin values (ng/ml) of 14L-night exposed adult female Syrian hamsters in gestation. SE: Standart Error, Pinx: Pinealectomy

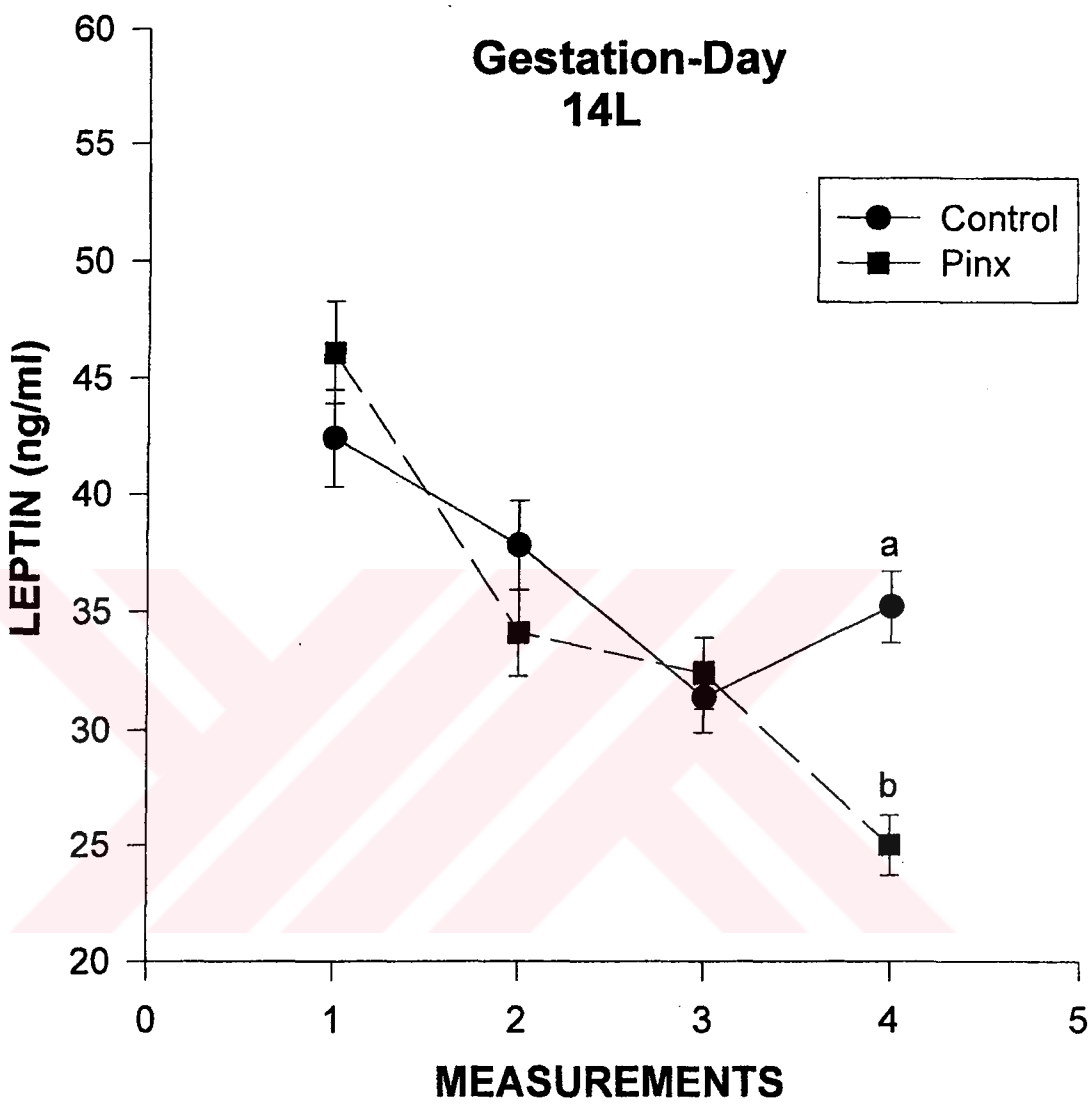

Figure 3.1: Serum leptin values (ng/ml) of 14L-day exposed adult female Syrian hamsters in gestation. Different letters represent significant differences among groups. ($p<0.05$) Mean ± SE, n=10.

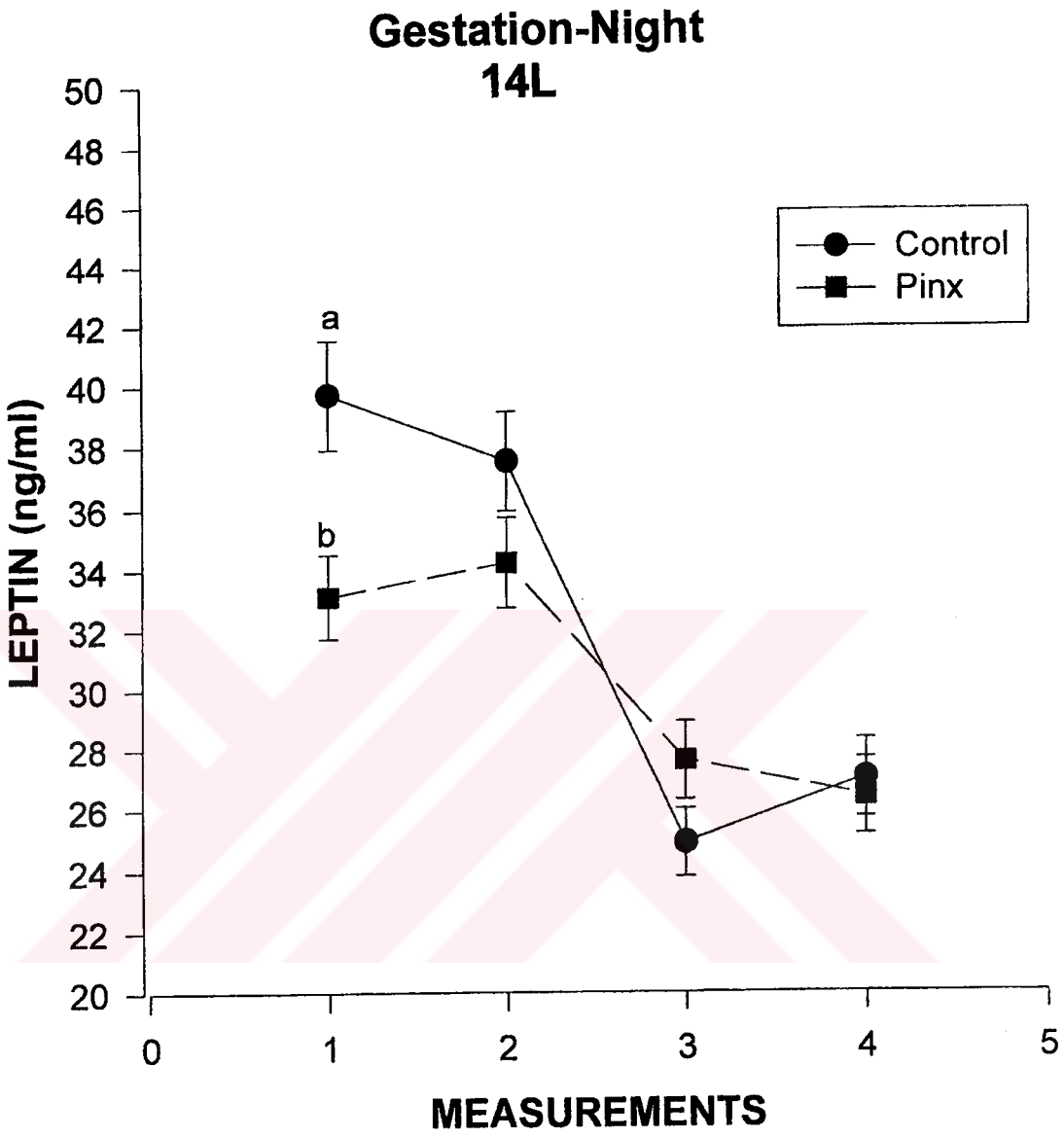

Figure 3.2: Serum leptin values (ng/ml) of 14L-night exposed adult female Syrian hamsters in gestation. Different letters represent significant differences among groups. ($p<0.05$). Mean ± SE, n=10.

Control Mean ± SE	Pinx Mean ± SE
40.3 ±1.8	38.5 ± 1.3
37.5 ± 1.6	37.3 ± 1.55
33.6 ± 1.4	32.1 ± 1.35
30.1 ± 1.2	30.3 ± 1.2

Table 3.3: Serum leptin values (ng/ml) of 10L-day exposed adult female Syrian hamsters in gestation. SE: Standart Error, Pinx: Pinealectomy

Control Mean ± SE	Pinx Mean ± SE
41.1 ± 1.8	35.6 ± 1.5
38.3 ± 1.6	31.1 ± 1.4
29.1 ± 1.2	29.4 ± 1.3
27.7 ± 1.3	28.3 ± 1.23

Table 3.4: Serum leptin values (ng/ml) of 10L-night exposed adult female Syrian hamsters in gestation. SE: Standart Error, Pinx: Pinealectomy

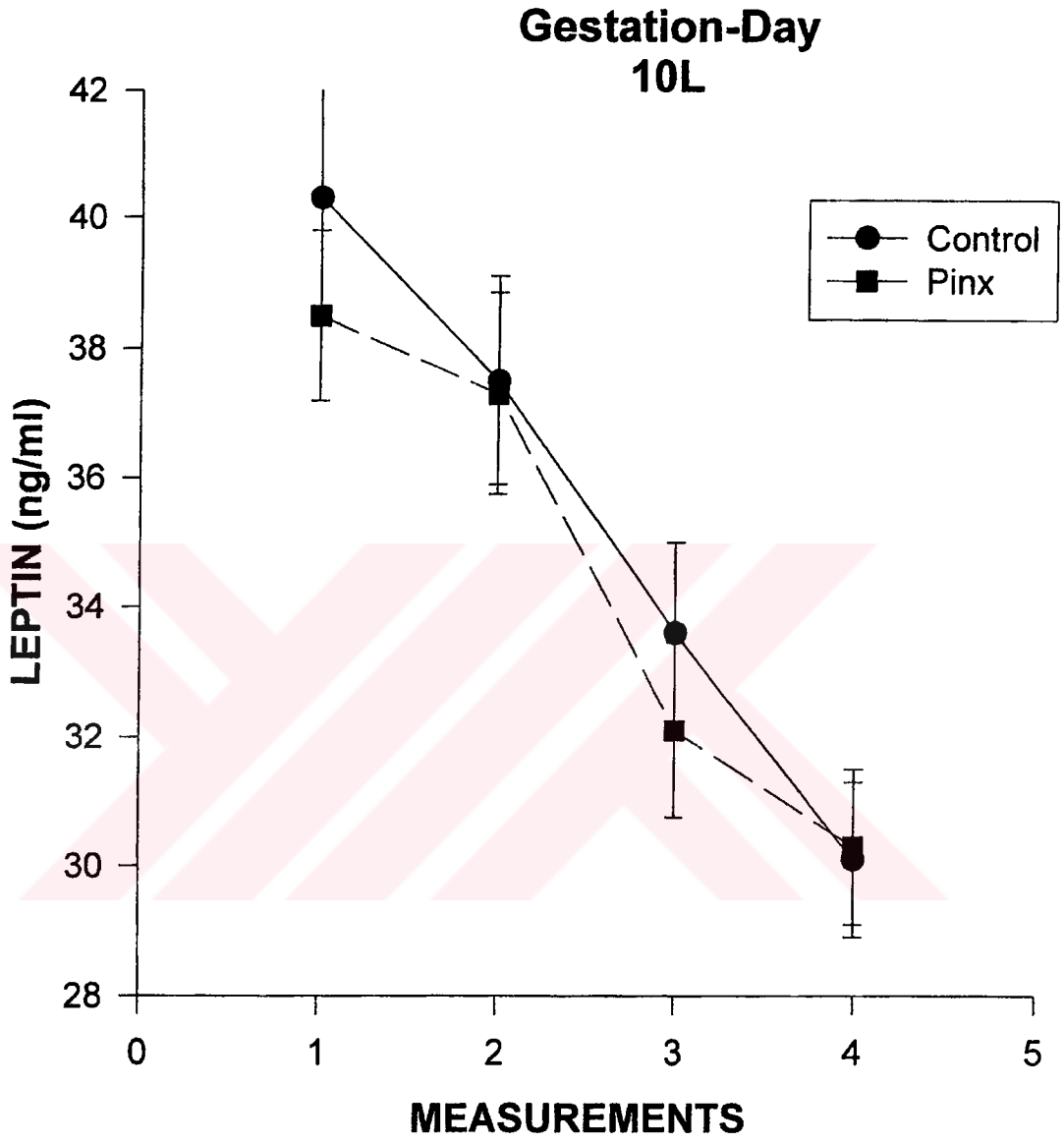

Figure 3.3: Serum leptin values (ng/ml) of 10L-day exposed adult female Syrian hamsters in gestation. Mean ± SE, n=10.

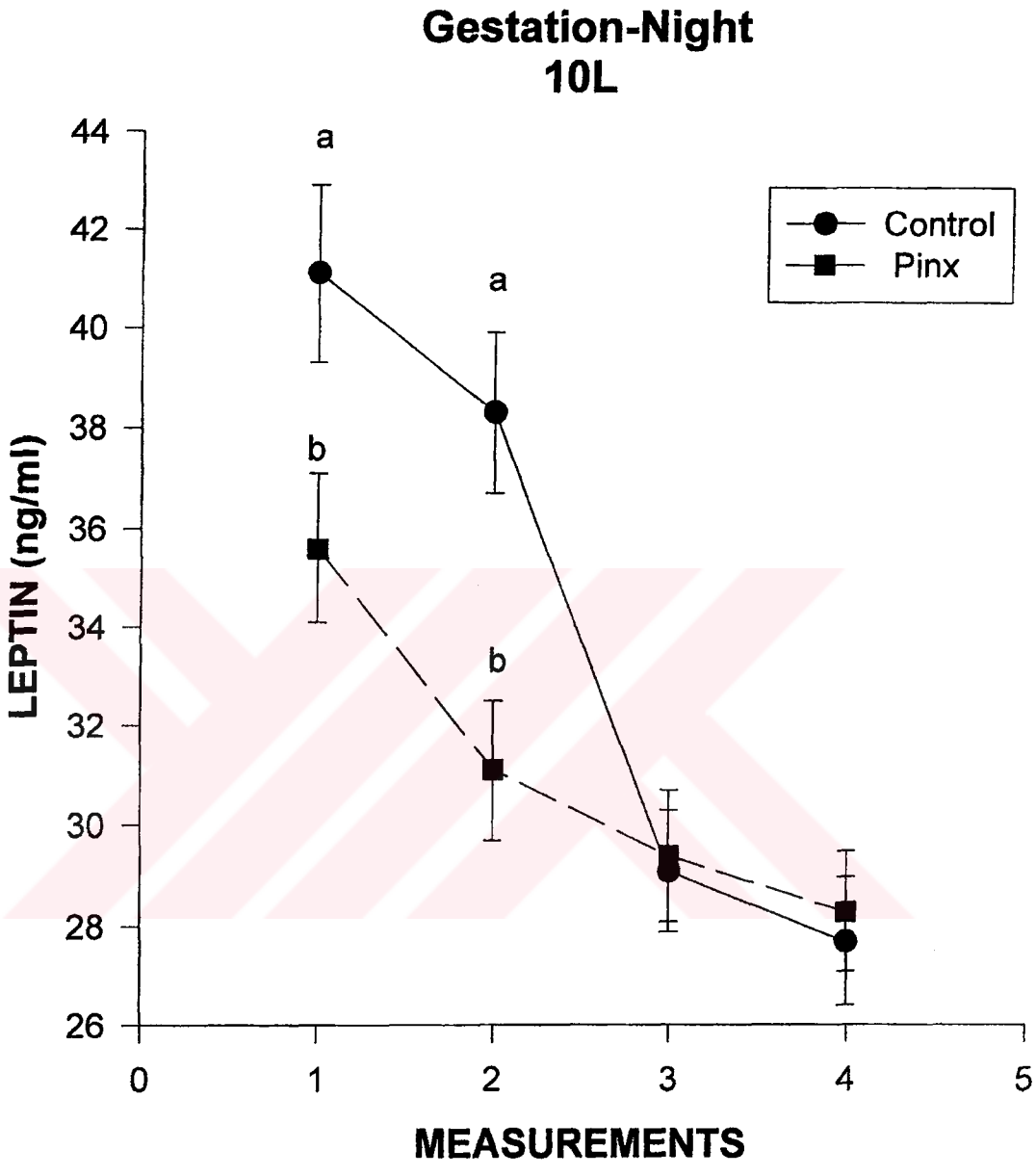

Figure 3.4: Serum leptin values (ng/ml) of 10L-night exposed adult female Syrian hamsters in gestation. Different letters represent significant differences among groups ($p<0.05$). Mean ± SE, n=10.

Control Mean ± SE	Pinx Mean ± SE
30.1 ± 2	26.6 ± 1.8
29.5 ± 2.4	27.3 ± 2
27.2 ± 2.5	29.6 ± 2.1
28.9 ± 2	28 ± 2.3

Table 3.5: Serum leptin values (ng/ml) of 14L-day exposed adult female Syrian hamsters in lactation. SE: Standart Error, Pinx: Pinealectomy

Control Mean ± SE	Pinx Mean ± SE
29.3 ± 2.5	28.6 ± 2.3
30.3 ± 2.5	27.5 ± 2.3
27.8 ± 2.6	27 ± 2.5
28.3 ± 2.4	27.2 ± 2.4

Table 3.6: Serum leptin values (ng/ml) of 14L-night exposed adult female Syrian hamsters in lactation. SE: Standart Error, Pinx: Pinealectomy

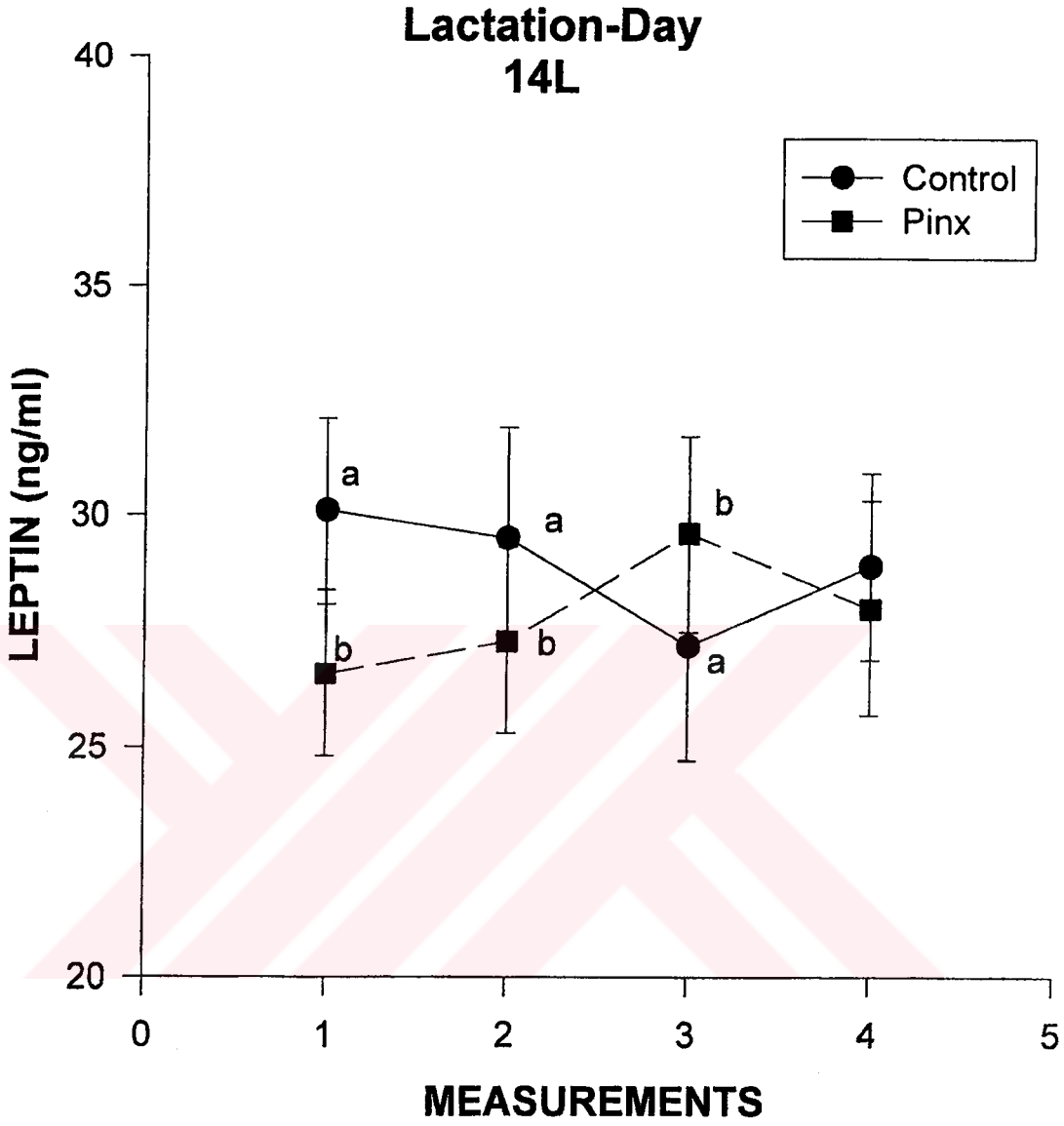

Figure 3.5: Serum leptin values (ng/ml) of 14L-day exposed adult female Syrian hamsters in lactation. Different letters represent significant differences among groups ($p<0.05$). Mean ± SE, n=10.

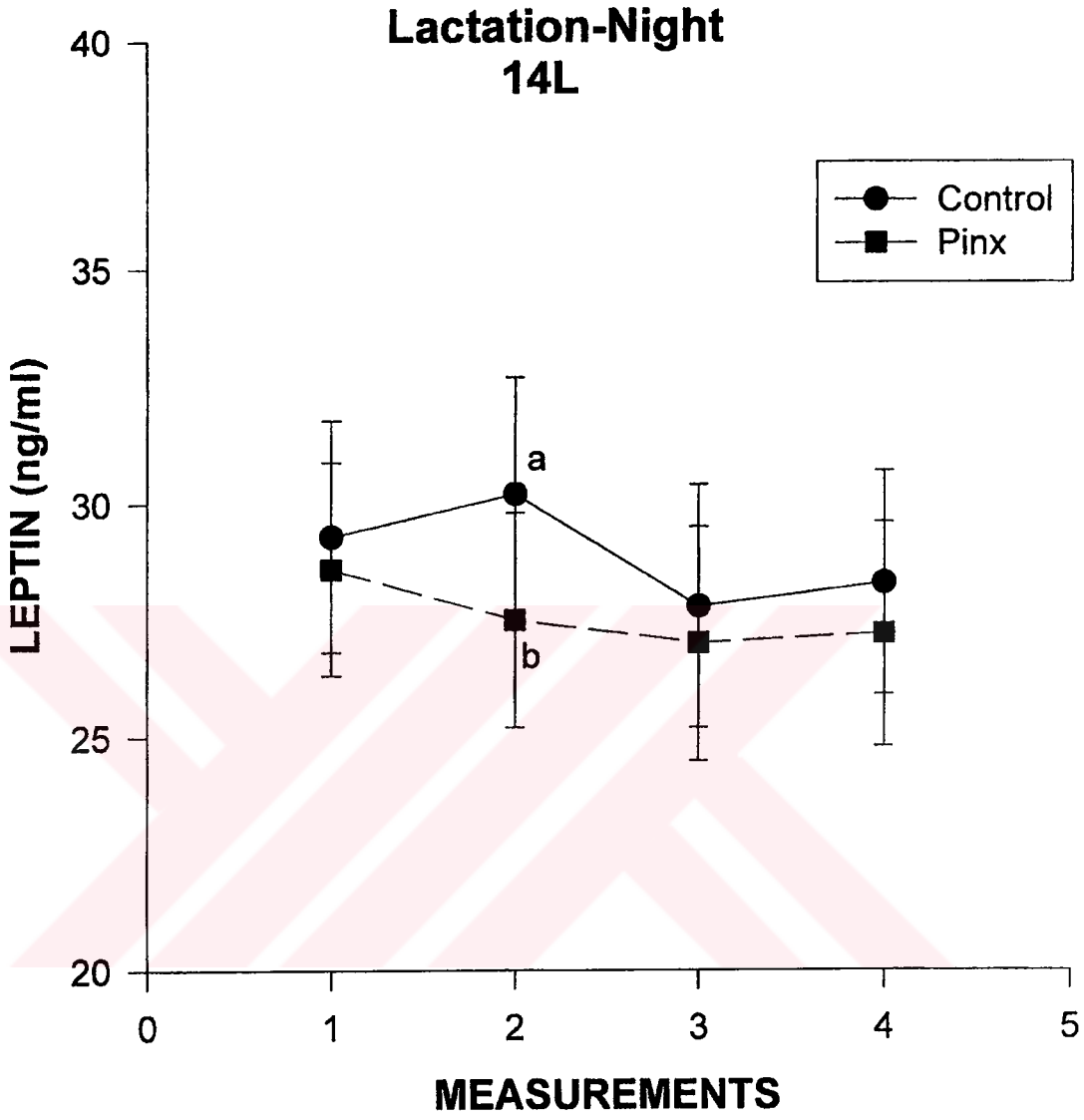

Figure 3.6: Serum leptin values (ng/ml) of 14L-night exposed adult female Syrian hamsters in lactation. Different letters represent significant differences among groups ($p<0.05$). Mean ± SE, n=10.

Control Mean ± SE	Pinx Mean ± SE
28.7 ± 2.1	32.8 ± 2.3
28.2 ± 2.3	32.2 ±2.1
28.9 ± 2.2	30.1 ± 2
29.6 ± 2.2	31.5 ± 2.2

Table 3.7: Serum leptin values (ng/ml) of 10L-day exposed adult female Syrian hamsters in lactation. SE: Standart Error, Pinx: Pinealectomy

Control Mean ± SE	Pinx Mean ± SE
30.1 ± 2.1	34 ± 2.3
31.2 ± 2,3	32.8 ± 2.4
33.3 ± 2.6	33.3 ± 2.5
34.4 ± 2.7	32.5 ± 2.5

Table 3.8: Serum leptin values (ng/ml) of 10L-night exposed adult female Syrian hamsters in lactation. SE: Standart Error, Pinx: Pinealectomy

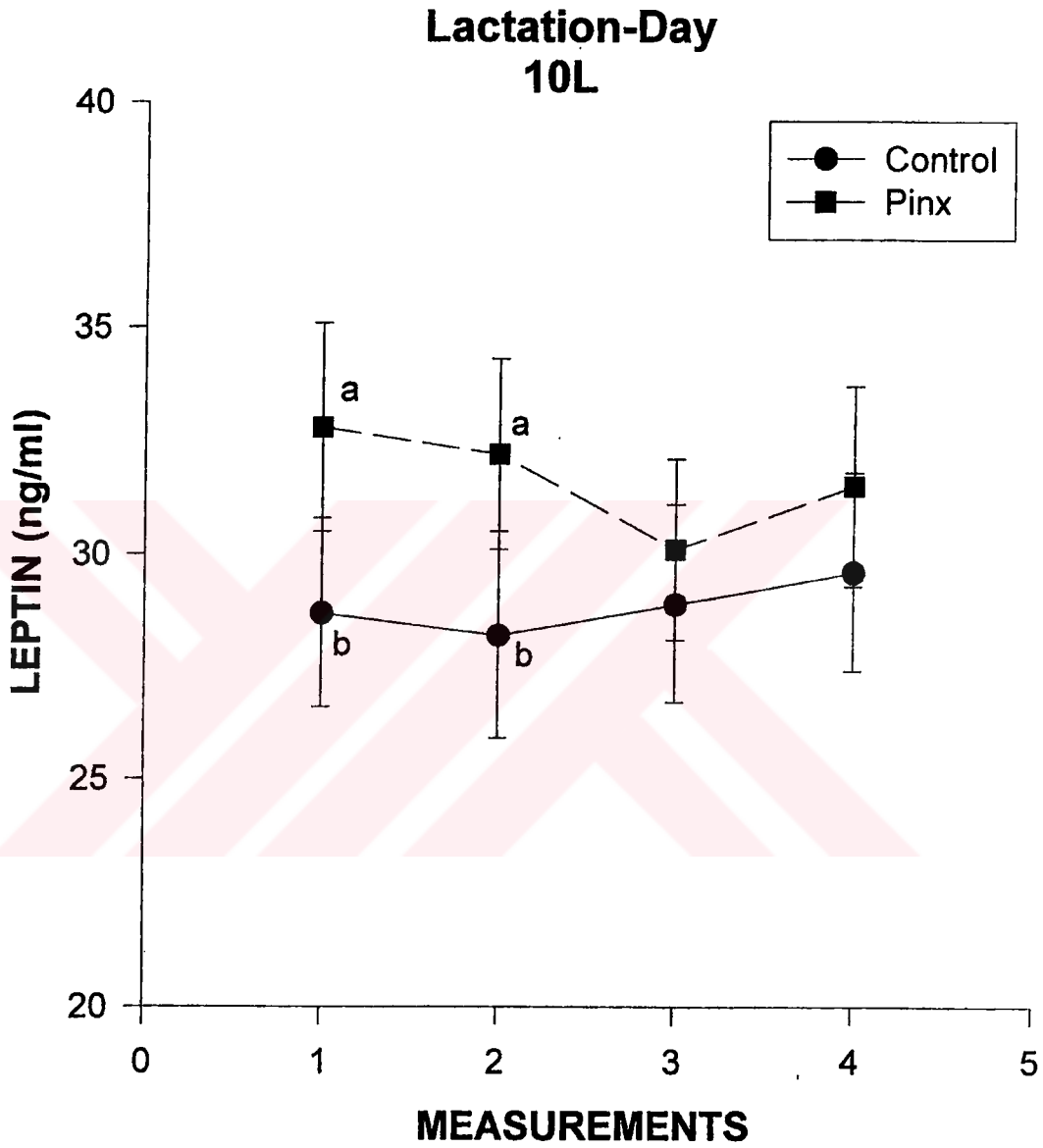

Figure 3.7: Serum leptin values (ng/ml) of 10L-day exposed adult female Syrian hamsters in lactation. Different letters represent significant differences among groups ($p<0.05$). Mean ± SE, n=10.

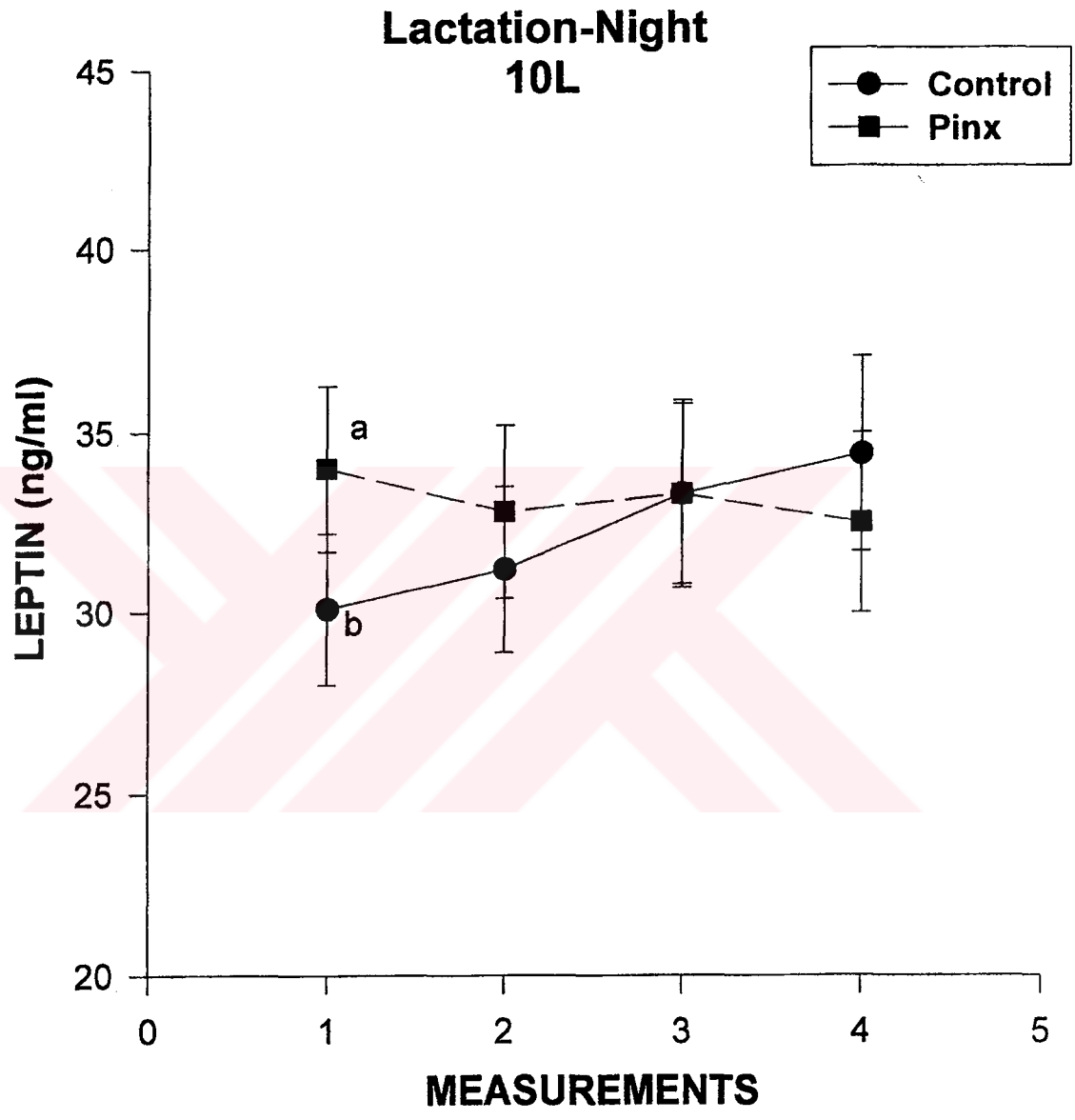

Figure 3.8: Serum leptin values (ng/ml) of 10L-night exposed adult female Syrian hamsters in lactation. Different letters represent significant differences among groups ($p<0.05$). Mean ± SE, n=10

3.2 Testes weights of juvenile male Syrian hamsters showed a continuous increase during the 8 weeks (fig 3.9). No differences were observed among the groups in the first and second weeks. Testes weight gain of pinealectomized + leptin injected group was statistically greater than the other two groups at the third week of the experiment ($p<0.001$). At the fourth week, testes weight gain of leptin injected group was bigger than control group, but smaller than the pinealectomized + leptin injected group ($p<0.05$). From the fifth week to the last week of the experiment, testes weights of control and leptin injected groups did not change but the testes weight gain went on in pinealectomized + leptin injected group ($p<0.001$) (fig 3.9). Body weight gain of the hamsters were nearly the same at the first two weeks of the experiment ($p>0.05$) (fig 3.10). The increase of body weight in pinealectomized + leptin injected group was bigger than the other two groups at the third week ($p<0.05$). At the fourth week, increase in body weight was similar in leptin injected and pinealectomized + leptin injected groups ($p>0.05$), but it was different in control group ($p<0.001$). From the fifth week of the experiment, body weights of control group did not change ($p>0.05$), but body weight increase continued in leptin injected and pinealectomized + leptin injected groups in a similar way and statistically different from the control group ($p<0.001$) (fig 3.10). When the food intake values were compared, no statistically differences was determined among the three groups. Food consumptions were similar in all groups during the experiment ($p>0.05$) (fig 3.11).

Control Mean ± SE	Leptin-Injected Mean ± SE	Pinx-Leptin Injected Mean ± SE
0.513 ± 0.024	0.491 ± 0.031	0.497 ± 0.020
1.005 ± 0.034	0.863 ± 0.010	0.823 ± 0.040
1.495 ± 0.051	1.068 ± 0.021	0.985 ± 0.050
1.853 ± 0.062	1.279 ± 0.023	1.649 ± 0.080
2.085 ± 0.070	1.770 ± 0.026	1.882 ± 0.090
2.169 ± 0.074	1.876 ± 0.039	1.960 ± 0.100
2.245 ± 0.077	2.018 ± 0.041	2.076 ± 0.100
2.435 ± 0.080	2.123 ± 0.045	2.254 ± 0.120
2.550 ± 0.080	2.270 ± 0.040	2.360 ± 0.090
2.720 ± 0.080	2.350 ± 0,040	2.520 ± 0,080

Table 3.9: Testes weight development of juvenile male Syrian hamsters. SE: Standart Error, Pinx: Pinealectomy

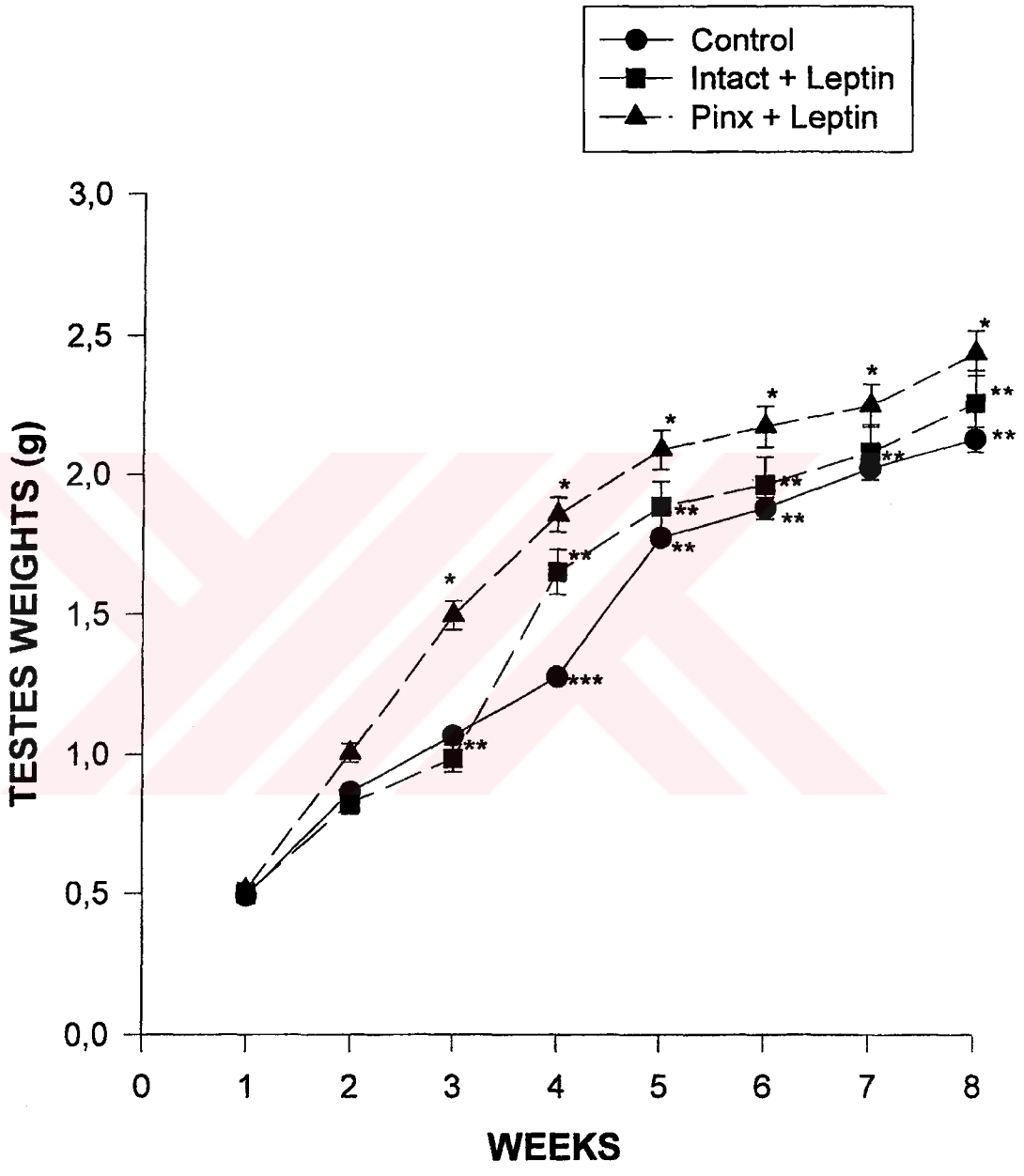

Figure 3.9: Testes weight development of juvenile male Syrian hamsters. * indicated statistical differences among groups ($p<0.001$). Mean ± SE, n=10.

Control Mean ± SE	Leptin Injected Mean ± SE	Pinx-Leptin Injected Mean ± SE
38.765 ± 0689	37.120 ± 2,433	38.500 ± 1.632
46.887 ± 1.055	46.200 ± 3.201	47.500 ± 2.082
60.662 ± 1.362	60.733 ± 3.311	60.640 ± 2.405
63.425 ± 1.420	62.933 ± 4.061	69.971 ± 2.765
67.937 ± 1.524	77.066 ± 4.711	80.357 ± 2.943
76.700 ± 1.718	84.533 ± 5.210	85.428 ± 3.071
80.437 ± 1.800	94.266 ± 5.518	89.128 ± 3.273
88.562 ± 1.890	100.000 ± 5.673	95.000 ± 3.326
88.100 ± 1.745	101.000 ± 3.201	96.500 ± 3.420
93.543 ± 1.545	105.130 ± 4.650	98.765 ± 3.120

Table 3.10: Body weight development of juvenile male Syrian hamsters. SE: Standart Error,

Pinx: Pinealectomy

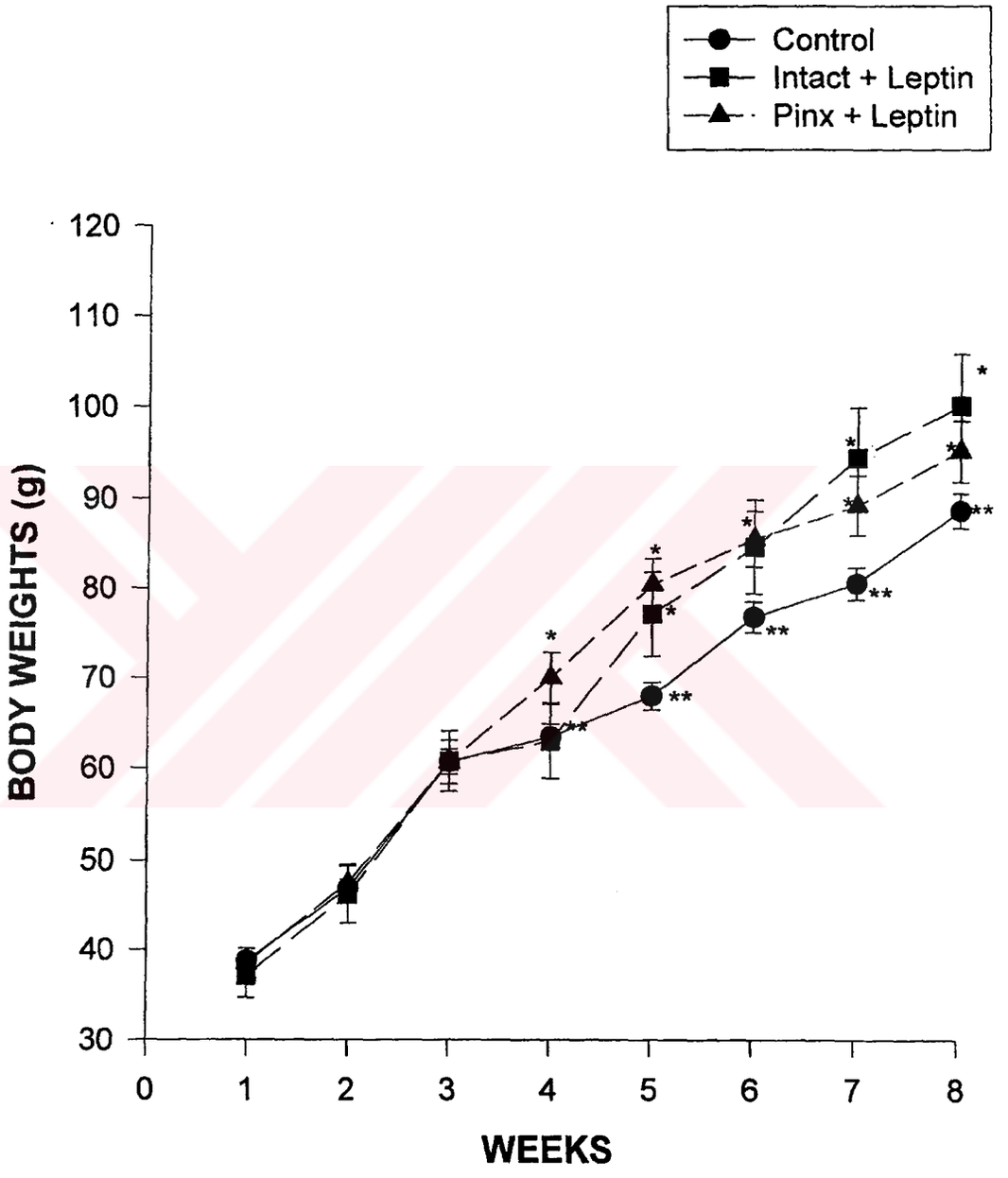

Figure 3.10: Body weight development of juvenile male Syrian hamsters. * indicated statistical differences among groups ($p<0.001$). Mean ± SE, n=10.

Control Mean ± SE	Leptin Injected Mean ± SE	Pinx-Leptin Injected Mean ± SE
3.500 ± 0.146	3.200 ± 0.450	3.500 ± 0.234
4.300 ± 0.172	4.100 ± 0.379	4.600 ± 0.266
5.500 ± 0.171	5.000 ± 0.459	5.800 ± 0.266
6.200 ± 0.153	5.700 ± 0.436	6.600 ± 0.272
6.800 ± 0.162	6.300 ± 0.435	7.300 ± 0.259
7.345 ± 0.154	7.200 ± 0.416	7.800 ± 0.234
7.898 ± 0.159	7.600 ± 0.453	8.300 ± 00.264
8.010 ± 0.177	8.410 ± 0.431	8.500 ± 0.255
8.050 ±0.175	8.300 ± 0.450	8.700 ± 0.245
8.121 ± 0.175	8.200 ± 0.320	8.500 ± 0.250

Table 3.11: Food intake of juvenile male Syrian hamsters. SE: Standart Error, Pinx: Pinealectomy

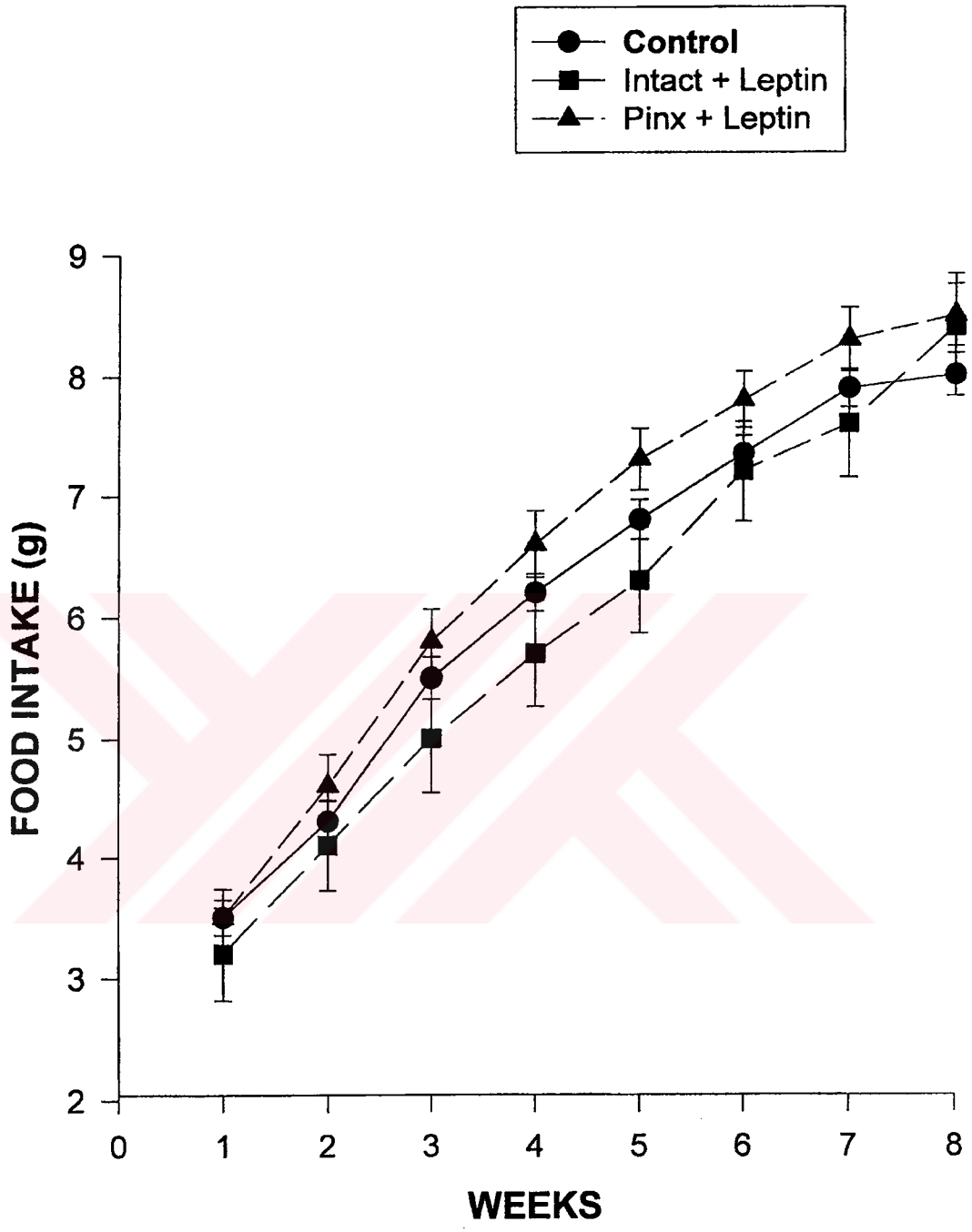

Figure 3.11: Food intake of juvenile male Syrian hamsters. Mean ± SE, n=10.

These results show that, leptin has an important effect on the onset of puberty in juvenile male Syrian hamsters and may play a critical role in the regulation of gestation in adults.

4. DISCUSSION

The leptin receptor is expressed in various tissues, such as hypothalamus, muscle, liver and adipose tissue (Tartaglia et al., 1995; Haggard et al., 1997; Karlsson et al., 1997). In addition to adipose derived leptin, placenta derived leptin may act on the hypothalamus and regulate maternal energy expenditure and neuroendocrine functions (Cheab et al., 1996; Yura et al., 2000). On the other hand, placental leptin may also act on maternal peripheral tissues, such as muscle, liver or pancreas and regulate glucose metabolism and insulin sensitivity (Masuzaki et al., 1999; Ogawa et al., 1999; Ebihera et al., 2001). In addition, placental leptin is transferred to the fetus (Linnemann et al., 2000; Hoggard et al., 2001; Lepercq et al., 2001) and may regulate fetal development and growth (Hoggard et al., 1997).

In the present study, day and night serum leptin levels were determined in adult female Syrian hamsters during gestation and lactation periods in long and short photoperiods. It was also investigated that if pineal gland has an effect on leptin levels. According to the data, day and night leptin levels in long photoperiod exhibit a decline. At the last phase of the gestation, day and night leptin levels of control and pinealectomized animals are similar to each other. When we look at the short photoperiod, serum leptin levels are high at the first phase of the gestation while they are low at the last phase of the gestation. However, no significant difference was determined between day and night leptin levels in control and pinealectomized animals without the first and second phase of the gestation in short photoperiod.

It has been shown that leptin concentration is changed with photoperiod in adult Syrian hamsters and leptin level exhibited circadian rhythm (Gündüz, 2002). There is a

reverse relationship between plasma melatonin and leptin levels connected with photoperiod and/or pineal gland; leptin levels of hamsters in light phase high but melatonin levels are low (Gündüz, 2002). This data shows that melatonin has inhibitory effect on the production/release of leptin hormone. Daily rhythm of leptin changes in rodents (Ahima et. al., 1996) and humans (Sinha et. al., 1996). It was demonstrated in mice and rats that serum leptin concentrations and mRNA content of leptin in adipose tissue decrease in light phase and increase in dark phase (Ahima et. al., 1998; Pickavance et. al., 1998; Shimokawa and Higami, 1999). Studies on humans especially in children showed serum leptin levels increased in dark phase and decreased in the morning (Wolthers et. al., 1999; Schoeller et. al.,1997). It was demonstrated that plasma leptin level reaches its highest value at 24:00 and remains low between 09:00 and 12:00 in young humans. In an other study, it was demonstrated that leptin shows circadian rhythm and remains high levels in the morning and low level in the midday (Flier, 1998). It was exhibited that, leptin secretion profile in humans has a similar relationship with prolactin, triotropin and melatonin, on the contrary of that secretion profile of leptin has a reverse relationship with cortisol and adrenocorticotropic hormones (Mantzoros and Moschos, 1998). Although, Mantzoros and Moschos (1998) found similar rhythmic relationship between melatonin and leptin in human examples, this rhythmic relationship is reverse in adult Syrian hamsters (Gündüz, 2002).

Several adaptations must occur in maternal energy metabolism for the fetal growth and milk production in gestation and lactation periods. Leptin may have a role in the coordination of maternal adaptation due to its regulatory role in energy metabolism. In some species like mouse, bat, rat and human, both serum leptin levels

and food intake increase in gestation period. This situation exhibits that the leptin levels are regulated without the effect of energy balance and body lipid content but this is not consistent with the effect of leptin in food intake. However in Syrian hamster, decreasing leptin levels rise the apetite and the food intake during the gestation. According to this data, opposite to other species, regulation of leptin concentrations in gestation period is achieved by energy balance and body lipid content.

One might have expected that a low maternal leptin level would be desirable during gestation, since the metabolic effects of high leptin levels (metabolic inefficiency and decreased food intake) are undesirable, and indeed are not observed, when the mother is putting great nutritional effort into the fetus and preparation for lactation. If a low leptin could act as a safety valve, preventing leptin levels from reaching too low level, placental leptin might have a paracrine role in maintaining pregnancy or in preparation for parturation.

Since the endocrinology of pregnancy in general, and leptin in particular, are not well concerved between species, the functions of leptin during pregnancy may differ between species. In the mouse, the elevated total leptin levels may be misleading. If bound leptin is inactive and free leptin is actually reduced in amount, then there is infact less leptin available for signalling. This scenerio would be consistent with the expectation that the effects of leptin would not be desirable during pregnancy.

The lack of consistent correlation between circulating leptin and body adiposity in pregnant animals, however, suggest that leptin is secreted during pregnancy from a non adipose site. Consistent with this hypothesis, human placenta and cell lines derived from human choriocarcinomas express leptin mRNA at comparable or greater levels

than adipose tissue and they also produce leptin protein. In rodents, however, conflitting results have been reported. Kawai et al. (1997) reported that leptin mRNA was expressed in adipose but not in placental tissue. Other investigators have observed low levels of leptin mRNA in rat placenta, which in one case, increased as pregnancy progressed. In mice, leptin protein content in the placenta is high, but whether leptin mRNA is expressed in mouse placenta is equivocal. Thus, whether the placenta contributes to hyperleptinimia of pregnancy in rodents remains unresolved. The factors responsible for up-regulation adipose leptin expression and secretion during pregnancy are unclear, but two possible candidates are estradiol and glycocorticoids. As with leptin, the circulating concentrations of these hormones increase during pregnancy. Both hormones also increase leptin gene expression in rodent and women adipose tissue. For example, adrenalectomy and overiectomy decreases leptin mRNA levels in adipose tissue among rodents, and these effects are reserved by glycocoticoids or estradiol replacement, respectively.

In most mammals, the relatively high ratio of milk energy production to maintenance demands a cycle of energy storage during pregnancy, lipid mobilization and increased food intake during lactation, and restoration of body fat in the late lactation or after weaning.

When lactating leptin levels were examined, it has been shown that in long photoperiod, there is not a significant difference among the phases of the lactation period without the decrease at the second phase. No difference was determined between the leptin levels of control and pinealectomized groups at the end of the lactation. In short photoperiod, there was not any significant difference in serum leptin levels in

pinealectomized group while low levels of leptin concentrations at the first and second phases of the lactation significantly increases at the third and fourth phases of the lactation.

Two examples of metabolic control critical to support of lactation by adipose tissue are the regulation of lypogenesis by somatotropin and the regulation of hormone-sensitive lipase by the sympathetic nervous system. Somatotropin and its actions have been studied for decades, but the onset of potential commercial use of somatotropin in lactation has spurred intense analysis into the mode of the somatotropin action. In the late pregnancy and early lactation, adipose tissue comes resistant to the normal actions of insuline. The reduction in lypogenesis is associated with a reduction in insulin receptors on adipocytes.

In several species, elevated leptin levels in gestation decrease with the start of the lactation. However, in Syrian hamster, leptin levels decrease in gestation period and any significant reduction occurs in lactation period. The possible reason for this is the short gestational period of the Syrian hamster. In other species, lactation induced down-regulation of leptin was associated with autocrine-paracrine action of leptin in mammary and adipose tissues, and that the milk leptin, especially at the latter stages of lactation was not only ascribed to diffuse transport from maternal blood stream but also regional production and secretion by mammary epithelial cells.

Onset of puberty in animals is directly proportional with leptin hormone which is secreted from adipose tissue. One of the most important parameter which shows the onset of puberty increases mass of reproductive organs (Chehab et. al., 1997). For onset of puberty, individual must be reached obvious critique body and adipose tissue mass

(Kennedy and Mitra, 1963; Frisch, 1972, 1980; Frisch and McArthur, 1974). When it is looked at the comparisons in group as from third week most effective in leptin injections especially pinealectomized group, namely accelerate onset of puberty, is supported probability of relationships consequence between leptin and pineal gland. The removal of antogonistic effect of melatonin hormone by the reverse relationship can cause the occurence the stimulatory effect of leptin on body weight and testes dimentions.

The early onset of puberty which is happening on testes is also seen in the body weight, namely from third week weight gain happened more into pinealectomized + leptin injected group. It is known that onset of puberty comes true as from 45 days in Syrian hamsters (Darrow et. al., 1980), on account of our findings clearly bring up leptin's effect on puberty.

Leptin is the first peripheral hormone which is showed has an accelerated effect in normal rodents. It has been accepted that besides leptin has a function on transmitting food data, it has a trigger role of onset of puberty in rodents and it stimulates their reproductive functions (Yu et. al., 1997; Ahima et. al., 1997; Chehab et. al., 1997). While food restriction delays onset of puberty in rats and sheep, with refeeding the situation was converted into its exsituation (Kennedy and Mitra, 1963; Foster and Olster, 1985). In another study, it has been showed that only leptin could not initiate the variation of GnRH secretion which is necessary for onset of puberty (Cheung et. al., 1997; Yu et. al., 1997a,b). It was exhibited that leptin stimulates the gonadotropin secretion in vitro rats hypophysis. This data has supported the hypothesis that leptin may play a central role about the regulation of reproductive system. Magni et. al., (1999) claimed that leptin hormone could be a modulator as only GnRH level in

reproductive functions. There are leptin receptors not only in hypothalamus and thalamus but also in the other sides of the brain as cortex, cerebellum, hypocampus and croid plexus (Tritos and Mantzoros, 1997; Corp et. al., 1998). This situation gives an idea that besides the known physiological functions, leptin also can take on some other unknown important functions.

In photoperiodic animals the onset of puberty earlier or later is important for growing of that population and continuation of the species. Especially in animals whose reproduction activity and body weight are controlled by the light, it is very important for the continuity of the species to have normal time for the signal (GnRH) that starts the hormonal relationships. Syrian hamsters can reach puberty at every environmental condition regardless of pineal gland. On the other hand, in Siberian hamsters onset of puberty has been delayed by short photoperiods (<16L). The control of puberty was happened early by leptin injections which was showen in our study, but this did not affect food intake and that showed the dose of leptin has a critical role not only in food metabolism but also on onset of puberty.

We observed that there is a reverse relationship between leptin and melatonin and leptin has a characteristics of early onset of puberty in that experiment, all these are sign that leptin achieves its affect on the level of brain. It is known that melatonin hormone provides its effect on reproductive system thorugh the inhibitory effect on GnRH neurons. In this scenario, in the absence of pineal gland and melatonin hormone, leptin can be effective on GnRH neurons and can initiate the secretion of GnRH to stimulate the onset of puberty earlier than the expected time. Leptin injections in the morning are more effective due to the high leptin concentration, but low melatonin

concentration (Gündüz, 2002) at that time of the day. It has been shown that the effects of leptin injections are seen later in leptin injected group than pinealectomized + leptin injected group. This situation can show the preventive effect of melatonin on leptin activity. These results show that the physiological effects of leptin hormone should be investigated before puberty.

In conclusion, studies conducted over the past 6 years clearly indicate that leptin can influence reproductive functions in mammals. The reproductive organs that have leptin receptors and are influenced by leptin include the gonads, anterior pituitary, hypothalamus, uterus and placenta. The precise role of leptin plays in the function of each of these reproductive organs may be species dependent. Employing a variety of paradigms have strongly suggested that leptin may serve a number of important regulatory roles throughout gestation.

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