Possible Role of Neuromedin S in Male Reproduction

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POSSIBLE ROLE OF NEUROMEDIN S IN MALE REPRODUCTION

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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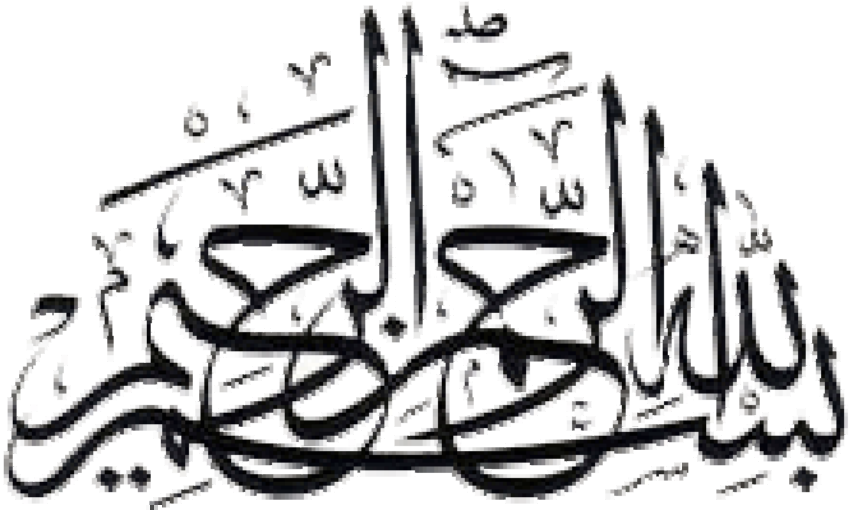

IN THE NAME OF ALLAH

THE MOST MERCIFUL

THE MOST BENEFICENT

AND

THE MOST COMPASSIONATE

CERTIFICATE

This dissertation **"Possible Role of Neuromedin S in Male reproduction"** submitted by **Mr. Shakeel Ahmed** is accepted in its present form by the Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirements for the degree of Doctor of Philosophy in Neuroendocrinology/ Reproductive Physiology.

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DECLARATION

I hereby declare that the material contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Shakeel Ahmed

Dedication

This thesis is dedicated

To

My Loving Parents

(My Father & My Mother)

Who always raised their hands to pray

for me

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Acknowledgement

From the depth of my heart I express my deep sincere gratitude to the Almighty omnipresent ***Allah Ta'ala*** *for the Blessings He had bestowed upon me, for answering my prayers, for giving me the strength to plod on despite my constitution wanting to give up. Countless salutation upon the* ***Holy Prophet Muhammad*** *(peace be upon him), who gave us path to recognize our self and the Creator.*

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List of Abbreviations

AC	Adenylate cyclase
ACTH	Adrenocorticotrophic hormone
AgRP	Agouty related peptide
ANOVA	Analysis of Variance
ARC	Arcuate nucleus
AVP	Arginine vasopressin
BW	Body weight
˚C	Celsius
CAMP	Cyclic adenosine-monophosphate
CLIP	Corticotrophin-like intermediate peptide
CNS	Central nervous system
Conc.	Concentration
CREB	Camp response element-binding
CRH	Corticotrophic releasing hormone
CRHR1	Corticotrophic releasing hormone receptor type 1
CRHR2	Corticotrophic releasing hormone receptor type 2
CSF	Cerebrospinal fluid
DA	Dopamine
DMH	Dorsomedial hypothalamus
DNA	Deoxyribo Nucleic Acid
EAA	Excitatory amino acid
EIA	Enzyme immuno-assay
ELISA	Enzyme-Linked Immuno-Sorbent Assay
EOP	Endogenous opioids peptides
FSH	Follicle stimulating hormone
GABA	Gamma aminobutyric acid
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GnIH	Gonadotropin inhibiting hormone
GnRH	Gonadotropic releasing hormone
hCG	Human chorionic gonadotropin
HCl	Hydrochloric acid
HPA	Hypothalamic pituitary adrenal
HPG	Hypothalamic pituitary gonadal
HPT	Hypothalamic pituitary testicular
Hr	Hour
icv	Intra cerebroventricular
IGF-1	Insulin-like Growth Factor 1
im	Intramuscular
IU	International unit
iv	Intravenous
kg	Kilogram
LH	Luteinizing hormone
ME	Median eminence
Min	Minutes

MPOA	Medial preoptic area
mpPVN	Medioparvocellular area of PVN
mRNA	Messenger Ribonucleic Acid
n	Number
ng	Nanogram (10^{-9} gram)
nmol	Nanomol (10^{-9} mol)
NMDA	N-Methyl-D-aspartic acid
NMS	Neuromedin S
NMU	Neuromedin U
NPY	Neuroprptide Y
OVX	Ovariectomized
Pe	Periventricular
PKA	Protein kinase A
POMC	Pro-opiomelanocortin
PRL	Prolactin
PVN	Paraventricular nucleus
rpm	Revolutions per minute
SCN	Suprachiasmatic nucleus
SEM	Standard Error Mean
SPOA	Septal preoptic area
subPVZ	Sub paraventricular zone
T	Testosterone
TIDA	Tuberoinfundibular dopaminergic
VIP	Vasoactive intestinal peptide
Yr	Year
α-MSH	α-Melanophore stimulating hormone
β-END	β-Endorphin

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Abstract

Background: Neuromedin S (NMS), a 36 amino acid anorexigenic neuropeptide was discovered in rat brain. It is a ligand for receptor FM4/TGR-1 which is also called as NMU receptor type II (NMU2R). Mainly it is expressed in suprachiasmatic nucleus (SCN) and involved in regulation of food intake and dark light circadian rhythms. In rodents its stimulatory role in HPG axis is reported but in higher primates its reproductive role is yet to be explored. In present study we examined involvement of NMS-NMU2R signaling pathway in the metabolic regulation of the HPG axis in adult male rhesus monkey. We used three different approaches in this regard. First, we observed the peripheral effect of NMS on HPG axis in two metabolic states (normal fed and 48-hrs fasting) as it was known that metabolic status is key factor in HPG axis regulation. Second, we studied the role of NMS in HPA regulation and its influence on reproductive hormone secretion in male rhesus monkeys and third, we investigated the role of exogenous NMS on some other metabolic hormones which are involved directly or indirectly in the regulation of HPG axis. For this purpose various hormones concentrations such as testosterone (T), cortisol, growth hormone (GH), Prolactin (PRL), adiponectin, resistin, leptin and insulin were determined after NMS administration in normal fed and 48-hrs fasting monkeys.

Materials and Methods: Four adult male rhesus monkeys (6-8 yr Age: 7-10 kg BW) were used in this study. Fifty nmol of NMS was injected through a cannula affixed in saphenous vein. Blood samples were collected individually at 15 min intervals, before and after NMS/saline/hCG administration. Plasma concentrations of T, cortisol, GH, PRL, adiponectin, leptin, resistin and insulin were estimated by using specific Enzyme Immunoassay (EIA) kits.

Results: Short term fasting significantly ($P<0.001$) decreased T while increased cortisol concentrations. NMS induced significant ($P<0.05$) increase in T and cortisol concentrations in both fed and 48-hrs fasting monkeys. No significant ($P>0.05$) change was observed in saline treated animals. In fasting conditions T response to NMS was delayed and suppressed. 48-hrs fasting significantly ($P<0.001$) decreased PRL but did not affect GH levels significantly. NMS also induced significant ($P<0.01$) rise in GH levels in both fed and fasting conditions while PRL

concentrations significantly ($P<0.05$) increased only in fed conditions. No significant change ($P>0.05$) in GH and PRL concentrations was noticed in saline treated animals. Short term fasting significantly increased ($P<0.001$) adiponectin concentrations while decreased leptin ($p<0.001$), resistin ($P<0.01$) and insulin ($P<0.001$) concentrations. NMS administration did not effect the adiponectin and insulin levels significantly ($P>0.05$) in both fed and fasting conditions. However NMS induced significant ($P<0.01$) increase in resistin levels, while suppressed leptin ($P<0.01$) secretion in both fed and 48-hrs fasting conditions. In saline treated animals no significant ($P>0.05$) changes in adiponectin, resistin, leptin and insulin levels were observed after saline administration.

Discussion: Our results demonstrated that exogenous NMS administration rescues suppression of the HPG axis during conditions of metabolic fuels deficiency. In fasting conditions the NMS induced T response was both delayed and suppressed. Present results indicate that although NMS stimulated T secretion under fasting conditions, the response appears to be delayed and suppressed suggesting that fasting-induced suppression of the HPG axis in the adult male rhesus monkey may involve, at least in part, a reduction in the sensitivity of GnRH neuronal network to endogenous NMS stimulation. Cortisol is generally considered as negative regulator of HPG axis in males so it was assumed that in fasting conditions the delayed response of T secretion in present study is, due to the increased concentrations of cortisol. However in normal fed monkeys, elevated cortisol levels did not suppress T secretion. So it is not yet confirmed, whether increased cortisol levels caused suppression of T release or fasting itself has some deleterious effects on NMS expression in hypothalamus. In previous studies no association was found between cortisol and T secretion in monkeys. So it was concluded that in present study elevated cortisol levels are not responsible for delayed and suppressed T response to NMS. In our study increased GH and PRL secretions after NMS administration suggest that NMS exerts its regulatory actions on HPG axis and testicular steroidogenesis by affecting the secretions of these two pituitary hormones. Our results also demonstrated that exogenous NMS administration has no significant effect in the secretion of insulin and adiponectin but the same dose of NMS significantly inhibited leptin while

stimulated plasma resistin levels. These findings suggest a potential involvement of NMS in the physiology of adipose tissue.

Conclusion: NMS-NMU2R signaling appears to be critical in the regulation of reproductive axis in mammals including primates, during metabolically stressed conditions. Whether it is just another redundant pathway or the master conduit for relaying such information to GnRH neurons, the research will tell exactly very soon.

General Introduction

Hypothalamus, the neuroendocrine center of the brain, controls a diverse number of homeostatic processes, such as metabolic control, feeding, drinking, reproduction, lactation, sleep-wake cycle, cardiovascular function, thermoregulation, and hormone secretion (Everitt and Hokfelt, 1990; Bernardis and Bellinger, 1993, 1998). Factors are delivered through the hypophyseal portal system from the hypothalamus to the anterior pituitary gland. This gland receives stimulatory and inhibitory hormones secreted from important hypothalamic nuclei, such as the paraventricular nucleus (PVN), arcuate nucleus (ARC), as well as the septal preoptic area (SPOA) and the medial preoptic area (MPOA), which in turn regulate the secretions of other endocrine glands (Everitt and Hokfelt, 1990; Bernardis and Bellinger, 1993, 1998).

Regulation of normal reproductive development and physiology is a complex process involving the coordinated interaction of neurotransmitter systems, hypothalamic releasing factors, pituitary hormones, gonadal sex steroid hormones and various growth factors. The reproductive system is part of the endocrine system, which contains an elegant feedback system with control centers at the level of the hypothalamus and the pituitary gland, and with target organs such as the testes or ovaries. There are also smaller local feedback loops involving paracrine and autocrine signals at the levels of the pituitary, testes and ovaries, which maintain organ or cell homeostasis (Knobil, 1981).

Normal testicular function comprising steroidogenesis and spermatogenesis requires the establishment of a complex network of endocrine–paracrine–autocrine systems for optimum cell-cell communication and coordination. The testis is subject to a hierarchy of controls like other endocrine-regulated glands. The systemic hormones are the first step regulators while the local paracrine and autocrine factors produced by the cellular components of the testis work to transform the microenvironment essential for germ cell development. There is a complex interplay of endocrine, autocrine and paracrine signals which regulate the processes of spermatogenesis, steroidogenesis and testicular function. (Heindel and Treinen, 1989; Spiteri-Grech and Nieschlag, 1993; Gnessi *et al*., 1997; Abney, 1999; Hull and

Harvey, 2000; Roser, 2001; Welt *et al*., 2002; Huleihel and Lunenfeld, 2004; Holdcraft and Braun, 2004a; Petersen and Soder, 2006).

The hypothalamic pituitary gonadal (HPG) axis plays a vital role in controlling of reproductive functions. This axis regulates secretion of pituitary gonadotropins, Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) by pulsatile release of hypothalamic decapeptide Gonadotropin-releasing hormone (GnRH). All these hormones play a major role in gonadal maturation and its functions (Plant, 2008). Ernest Knobil was first to demonstrate the episodic release of GnRH into hypophyseal portal vein and then subsequent pulsatile release of pituitary gonadotropins (Knobil *et al*., 1980). Episodic hormone secretion is a characteristic feature of the HPG axis (Maeda *et al*., 2010). GnRH pulse generator, localized in the ARC region of the mediobasal hypothalamus, is suggested to be responsible for pulsatile release of GnRH (Maeda *et al*., 2010). It is an intrinsic property of GnRH neurons to secrete GnRH in rhythmic way (Krsmanović, 1992; Weiner and Martinez de la Escalera, 1993; Moenter *et al*., 2003).

Female rhesus monkeys, in which endogenous GnRH production had been abolished due to bilateral lesions in the mediobasal hypothalamus, the significance of pulsatile GnRH secretion was demonstrated effectively (Knobil *et al*., 1980). Continuous infusion of GnRH, failed to sustain gonadotropin secretion in these animals. While at physiological frequency, the pulsatile administration of GnRH, re-established the pre-lesion levels of gonadotropins. All these findings have been repeated in man and several other species and led to the development of various therapeutic remedies (Maeda *et al*., 2010). A large number of excitatory and inhibitory neurotransmitters and neuropeptides govern the activities of GnRH pulse generator either by mediating through internal or external factors (Terasawa and Fernandez, 2001; Plant and Shahab, 2002; Plant and Barker-Gibb, 2004; Ebling, 2005).

Many internal and external factors may affect the proper functioning of HPG axis. The most important factor is the nutritional status of an individual (Bronson, 1985; Cameron, 1996; Wade *et al*., 1996; Wade and Jones, 2004). The relationships between nutritional status and reproduction are not clearly understood. The

observations of Pirke and colleagues suggested the possible role of specific nutrients on reproductive functions in humans. The vegetarian diets with low protein content disrupted the reproductive cycle in human females more than a non-vegetarian diet, suggesting the important role of nutrients in reproduction (Pirke *et al*., 1986). Later on it was confirmed by certain evidences that suppression of reproductive activity is not due to deficiency of a particular nutrient but due to the deficiency of oxidizable metabolic fuels (Foster and Olster, 1985; Foster *et al*., 1989). The suppressed status of HPG axis in food restricted animals can be reversed in few hours after a single meal (Foster *et al*., 1989; Bronson and Manning, 1991; Cameron, 1996). In monkeys the normal FSH and LH levels were maintained even after several week utilization of isocaloric protein deficient diet (Cameron, 1996). This study further supported the idea that a deficiency of dietary protein does not provide the signal leading to reproductive dysfunction. Similarly neither lipid nor carbohydrate deficiencies resulted in suppression of gonadotropin levels (Cameron, 1996). All these evidences suggested that HPG axis functioning is negatively affected by any activity, situation, or condition which restrict the supply of metabolic fuels for oxidation (Wade and Jones, 2004). In some situations like diabetes mellitus and obesity, excess of metabolic fuel is present but as this fuel is not available for oxidation resulting in reproductive dysfunction (Wade and Jones, 2004).

Energy imbalance can impair reproduction as it is an energy demanding process (Schneider, 2004). Energy imbalance and metabolically stressed conditions delay the onset of puberty in pre-pubertal animals (Kennedy and Mitra, 1963; Foster and Olster, 1985), while it hobbles the pulsatile release of GnRH with concomitant hypogonadotropic hypogonadism in post-pubertal animals (Cameron, 1996; Wade *et al*., 1996; Wade and Jones, 2004), and strangle the sexual behavior (Gill and Rissmann, 1997). In large number of mammalian species, the effect of metabolic imbalance have also been studied in relation to seasonal breeding, cyclicity, gestation, lactation, and major reproductive hormones secretion (Delgado *et al*., 1978; Merry and Holehan, 1979; Chakravarty *et al*., 1982; Knuth and Friesen, 1983; Cameron, 1996; Wade *et al*., 1996; Wade and Jones, 2004). During pubertal period, the metabolic reserves of body influence the pubertal awakening of the HPG axis (Kennedy and Mitra 1963; Foster and Olster, 1985). Pubertal onset is interrupted by

energy imbalance and severe metabolic stress conditions (Roa *et al*., 2009). Many studies in rats revealed that during the critical reproductive period, the metabolic stress prevents or delays the awakening of the HPG axis (Kennedy and Mitra, 1963; Schenck *et al*., 1980). Similar results were found in mice (Marstellar and Lynch, 1983; Perrigo and Bronson, 1983; Hamilton and Bronson, 1985; 1986), lamb (Foster and Olster, 1985; Foster *et al*., 1989), syrian hamster, (Morin, 1975) and humans (Kulin *et al*., 1984; Biederman *et al*., 1986).

Chronic metabolic stresses (such as dieting, extensive exercise in the athletes, prolonged undernutrition or fasting) during adulthood, cause irregularity or acyclicity in menstrual cycle in the human female (Chakravarty *et al*., 1982; Knuth and Friesen, 1983; Manning and Bronson, 1989), while these are associated with decrease in plasma concentrations of sex hormones and gonadal atrophy (Crewal *et al*., 1971; Smith *et al*., 1975). Disruption of the HPG axis function produced delayed menarche, uterine bleeding, and secondary amenorrhea among anorexic women (Eisenberg, 1981). The consistent metabolic deficiency induced effects, on reproductive system, are decreased concentrations of plasma FSH, LH and T. These effects are studied in rodents (Howland, 1972; 1975; 1980), non-human primates (Dubey *et al*., 1986), and humans (Hoffer *et al*., 1986). Metabolic stresses also affect embryonic survival in rats (Pond *et al*., 1989) and primiparous swine (Pond *et al*., 1988), due to decrease in the supply of carbohydrates and lipids. Metabolic deficiencies also prolonged the breeding intervals in mammals (Merry and Holechan, 1979). Such metabolic deficiencies caused lactational anovulation in cattle (Echternkamp *et al*., 1982; Easdon *et al*., 1985), rats (Woodside, 1991), and women (Delgado *et al*., 1978). In some cases, such conditions prolonged lifespan and duration of reproductive period in addition to delayed puberty (Merry and Holechan, 1979). Many studies on rats, cattle and hamsters, have also shown that the metabolic deficiencies affect the testicular size and sperm production, as caused by other environmental factors such as photoperiod and social cues (Lincoln and Short, 1980; Walkden-Brown *et al*., 1994).

The exact mechanisms of such responses to metabolic deficit are not clearly understood. However, this is evident that hypothalamus perceives such energy deficiencies and controls the gonadotropins and gonadal hormone secretion by regulating GnRH release (Ebling *et al*., 1990; Foster *et al*., 1998; Ichimaru *et al*.,

2001). It is confirmed through many studies in different animals that energy availability puts great impact on gonadotropin secretions, by regulating the release of GnRH. It was suggested through many studies that this reproductive suppression, is due to a decrease in release of GnRH and not by decrease in pituitary-gonadal axis sensitivity to GnRH (Bergendahl *et al*., 1991; Cameron and Nosbisch, 1991; Aloi *et al*., 1997). So any neurotransmitters or neuromodulators, which are known to regulate GnRH neurons, may mediate the effects of energetic imbalance on GnRH secretion.

To understand the mechanism of the effects of metabolic status on reproductive functions, various animal models are used in different studies (Cameron 1996; Blache *et al*., 2000; Barb *et al*., 2002). Rhesus monkeys have been widely used as an animal model to deduce the mechanisms that suppress the HPG axis in situation of metabolic stresses (Cameron, 1996). Indeed being a primate, it is the best and the most-relevant experimental animal model to decipher the metabolic regulation of reproduction in humans. Various studies showed that metabolic deficiencies negatively affect the secretion of LH and T (Cameron and Nosbisch, 1991; Helmreich and Cameron, 1992). This decrease in LH and T secretion in response to metabolic stress is due to suppression of GnRH release (Cameron and Nosbisch, 1991; Helmreich and Cameron, 1992). This concept is further supported by the evidence that suppression in the frequency of pulsatile LH secretion, during brief periods of metabolic fuels deficiency, is not accompanied by a decrease in the LH pulse amplitude (Cameron and Nosbisch, 1991). All these studies suggest that the hypothalamic GnRH pulse generator may be the main site of action for metabolic signals to trespass on the reproductive axis in primates (Cameron, 1996). So on the basis of these evidences, it may be suggested that the suppression of pituitary gonadotropin and testicular T due to metabolic deficit, involves a gradual decrease of the GnRH release to the reproductive axis.

A direct effect of metabolic signals at the pituitary level in suppressing LH secretion is less likely, as the pituitary maintains its normal response to GnRH stimulation, during metabolic fuels deficient conditions. It was observed that under these stress situations, very low doses of exogenous GnRH were enough to enhance LH levels (Cameron and Nosbisch, 1991). This study further supported the idea that under metabolic fuels insufficiency, decrease in hypothalamic stimulation of the

pituitary is the main reason in the decrease of gonadotropin secretion. Of note, such suppression of LH and T secretion can be reversed by refeeding (Parfitt *et al*., 1991; Schreihofer *et al*., 1993a; 1993b). This restoration depends directly on the amount of the refed meal (Parfitt *et al*., 1991). All these data show that the primate HPG axis is very sensitive to energy conditions and changes its activity accordingly.

The exact mechanism of how metabolic fuel deficiencies arrest the neural networks, which regulate the intermittent GnRH discharge, is not completely understood. The master position of the hypothalamic GnRH in the hierarchy of signals controlling the reproductive axis, makes it a target of multiple regulators of both central and peripheral origins. Neurotransmitters, glial factors and neuropeptides are included in central modulators, while leptin and sex steroids are included in peripheral modulators. A wide variety of excitatory and inhibitory neurotransmitters/neuropeptides have been identified and it is suggested that these may play a vital role in HPG axis arrest under metabolic fuels deficiency (Terasawa and Fernandez, 2001; Plant and Shahab, 2002; Plant and Barker-Gibb, 2004; Ebling, 2005). This idea is further supported by the observation of reduced sensitivity of the HPG axis to NMDA administration in fasting (Shahab *et al*., 1997) condition. In the mammalian hypothalamus, Glutamate and GABA are the most abundant excitatory and inhibitory neurotransmitters, respectively (Cotman and Iversen, 1987; Thind and Goldsmith, 1997; Terasawa and Fernandez, 2001), and in several species the neurons containing both these neurotransmitters, synapse with GnRH neurons in the hypothalamus (Jennes *et al*., 1983; Leranth *et al*., 1992). Metabolic deficiency suppressed GnRH secretion, is associated with increased levels of GABA due to over expression of GABA synthesizing enzymes (Leonhardt *et al*., 1999). The study of Mahesh and Brann showed that EAA stimulates LH secretion (Mahesh and Brann, 2005). Similarly the energy deficiency (short term fasting) induced HPG axis inactivity, is terminated by EAA (Shahab *et al*., 1997). Glutamate, which is known to stimulate LH secretion, decreased during restricted supply of food intake (Tal *et al*., 1983), and aspartate derivatives administration to hypogonadotrophic lambs on an insufficient diet, results in increased plasma LH levels (Ebling *et al*., 1990). Similar stimulatory effect of aspartate on LH concentrations was noticed in lambs, with lowered LH secretion due to food insufficiency (Bucholtz *et al*., 1996).

The idea that metabolic fuel deficiency induced LH suppression, is mediated by Endogenous opioids peptides (EOP) looks promising as EOP have been known to be inhibitory to LH secretion. However, this idea was ruled out as naloxone, an opioid antagonist, failed to relieve suppression of LH pulse frequency in fasting conditions (Helmreich and Cameron, 1992). It has been suggested that one day fasting in adult male rhesus monkey, has only mildly activated the hypothalamic-pituitary-adrenal axis and there is no evidence that LH suppression is caused due to the increased activity of the hypothalamic-pituitary-adrenal axis (Helmreich *et al*., 1993). Gonadotropin inhibiting hormone (GnIH), characterized in several mammalian species including primates, is known to be as putative suppressor of gonadotropin secretion (Tsutsui, 2010). Theoretically it can be suggested that such a peptide is involved in suppression of GnRH neurons, however, such a possibility has not been examined to date. In recent years, a role of glial cells produced molecules, in regulation of GnRH secretion has been indicated (Ojeda *et al*., 2008). However, its involvement in metabolic regulation of GnRH secretion has not been investigated until now. Certain metabolic hormones, like insulin, adiponectin, leptin and cortisol may play a contributory role in metabolic deficiency induced LH suppression indirectly by determining the concentrations of certain neurotransmitters in the central nervous system (CNS) (Qi *et al*., 2004; Smith *et al*., 2006; Kos *et al*., 2007; Ahima and Lazar, 2008; Xu *et al*., 2009).

Adipokines (Adiponectin, leptin, and resistin) play important role in the regulation of energy homeostasis and metabolism (Fischer-Posovszky *et al*., 2007). Leptin acts as a surfeit factor and its systemic concentration, is in proportion to body fat mass. Adiponectin is found most abundantly in the circulation. Adiponectin, unlike leptin, is negatively correlated with body fat mass. Adiponectin boosts insulin sensitivity while leptin and resistin decrease it (Ahima and Lazar, 2008). These adipokines have also some impacts on the reproductive axis. Leptin and adiponectin act antagonistically on the output of key reproductive hormones (Lado-Abeal *et al*., 2000; Fischer-Posovszky *et al*., 2007; Rodriguez-Pacheco *et al*., 2007; Caminos *et al*., 2008). Resistin role in reproduction is less understood. Changes in plasma concentrations of adipokines play some role in energy imbalance (Arita *et al*., 1999; Hotta *et al*., 2000; Kadowaki and Yamauchi, 2005; Fischer-Posovszky *et al*., 2007;

Ahima and Lazar, 2008). Short-term fasting decreases the plasma leptin and resistin secretion, while increases adiponectin secretion (Rajala *et al*., 2004; Kadowaki and Yamauchi, 2005; Guevara *et al*., 2008). Cortisol and insuline levels are also affected by energy imbalance (Lado-Abeal *et al*., 2002; Guevara *et al*., 2008). So the metabolic hormones may serve as a possible link between metabolism and neuroendocrine regulation of reproductive functions.

The neuromedins belong to one of the largest family of neuropeptides i.e. tachykinin family (Helke *et al*., 1990). Neuromedins were first studied in the porcine CNS (Minamino *et al*., 1983) and were given the name on the basis of their receptor preference (B: bombesin-like, K: kassinine-like, N: neurotensin- like, etc.). Those CNS sites which are involved in the autonomic, behavioural and endocrine processes have abundant expression of these peptides (Minamino *et al*., 1985). Neuromedin B and C are involved in above mentioned processes (Ohki-Hamazaki, 2000) while Neuromedin N has a critical role in HPA axis (Malendowicz *et al*., 1993), and plays an important role in thermoregulation (Dubuc *et al*., 1988).

Neuromedin U is a brain-gut peptide, discovered in porcine spinal cord and was considered to be involved in contraction of uterine smooth muscle (Minamino *et al*., 1985). NMU plays very important role in both peripheral as well as central regulation. Its peripheral activities are blood pressure elevation, smooth muscle contraction and modification of ion transport in intestine while its central regulation include food suppression, stimulation of ACTH and corticosterone secretion (Minamino *et al*., 1985; Honzawa *et al*., 1987; Malendowicz *et al*., 1994; Nakazato *et al*., 2000; Chu *et al*., 2002; Wren *et al*., 2002; Hanada *et al*., 2003). Further studies confirmed that CRH neurons are involved in NMU regulated central processes. All the processes like inhibition of gastric acid release, behavioural and HPA activation, are looked to be CRH-dependent (Wren *et al*., 2002; Hanada *et al*., 2003; Mondal *et al*., 2003).

Neuromedin S (NMS), highly expressed in SCN of the hypothalamus has close resemblance with NMU (Mori *et al*., 2005). NMS and NMU have similarity in their C-terminals but they are very different to each other in N-terminal sequence. Moreover these two neuromedins are coded by two different genes (Mori *et al*.,

2005). Furthermore, NMS and NMU act trough the same receptors i.e. FM-3/GPR66 (NMU1R) and FM-4/TGR-1(NMU2R). Both these neuropeptides have similar affinity to NMU1R, indicating similarities in their effects but as NMS has more affinity to NMU2R (Mori *et al*., 2005) so it was suggested that NMS mediates it physiological activities through NMU2R. The expression of NMS receptor is restricted almost only to the central nervous system having abundant expressions in SCN and PVN (Guan *et al*., 2001; Nakahara *et al*., 2004). The presence of receptor within SCN, suggests its ligand role in regulation of circadian rhythm of temperature, sleep/wake cycle and hypothalamic hormones like CRH and GnRH secretion (Mori *et al*., 2005) while its PVN presence implies its role in feeding and the regulation of HPA axis. NMS receptors are also abundantly present outside the hypothalamus such as thalamus, amygdala, hippocampus and cerebellum (Raddatz *et al*., 2000), which indicates its presumptive role in motor phenomena, emotions and regulation of behaviour. It has been demonstrated that NMS has higher expression than that of NMU in the hypothalamus (Rucinski *et al*., 2007), which suggests the predominance of NMS in central regulatory processes.

NMS mRNA has higher expressions in the hypothalamus, testes and spleen (Mori *et al*., 2005). In the brain, NMS mRNA has predominant expressions in SCN with slight expressions in other hypothalamic regions (Fujii *et al*., 2000; Mori *et al*., 2005). NMS has been demonstrated to influence the feeding (Ida *et al*., 2005; Shousha *et al*., 2006), circadian rhythm (Mori *et al*., 2005), and pituitary LH secretion (Vigo *et al*., 2007). It was proposed that activation of CRH release from PVN and pro-opiomelanocortin (POMC) from the ARC nucleus are crucially involved in NMS mediated processes (Ida *et al*., 2005). The CRH and dopamine play very important role in regulation of certain behaviours e.g. stress related motor phenomena, anxiety and NMS evoked hypophagia (Majovski *et al*., 1981; Vale *et al*., 1981; Monnikes *et al*., 1992; Menzaghi *et al*., 1994; Skutella *et al*., 1994; Ida *et al*., 2005).

In rats NMS administration activates SCN neurons. It acts through its receptor in a paracrine or autocrine manner within the SCN and acts as a nonphotic motor factor of circadian rhythm (Mori *et al*., 2005). SCN is the major region of circadian pacemaker in mammals and regulates circadian rhythms of physiological and behavioural processes (Lowrey and Takahashi, 2000; Reppert and Weaver, 2001,

2002). SCN is divided into two regions i.e. core and shell, corresponding to the ventrolateral and dorsomedial portions of the SCN, respectively (Moore *et al*., 2002). These SCN regions are very important in regulation of circadian entrainment and generation of strong rhythms (Hanada *et al*., 2001). Several neuropeptides in SCN are involved in circadian pacemaker regulation (Reppert and Weaver, 2001). For example, in the core region of SCN, Vasoactive intestinal peptide (VIP) plays very important role in both photic entrainment and maintenance of the circadian rhythms (Piggins and Cutler, 2003).

In medioparvocellular area of the PVN (mpPVN), where CRH and arginine vasopressin (AVP) expressing neurons are present, the SCN shows some direct projections (Antoni *et al*., 1983; Swanson *et al*., 1983, Sawchenko *et al*., 1984a,b; Vrang *et al*., 1995; Engeland and Arnhold, 2005). SCN also innervates different adjacent regions in the PVN which may contact the pituitary neurons (Berk and Finkelstein, 1981; Stephan *et al*., 1981;Watts *et al*., 1987; Buijs *et al*., 1993; Vrang *et al*., 1995), the dorsomedial nucleus of the hypothalamus (DMH) and the subparaventricular zone (subPVZ), which in turn send projections to the mpPVN (Ter Horst and Luiten, 1986; Buijs *et al*., 2003; Engeland and Arnhold, 2005). All these SCN projections enable simultaneous regulation of circadian changes in various brain outputs (Watts *et al*., 1987; Buijs *et al*., 1993) and integration with other environmental factors (Saeb-Parsy *et al*., 2000; Buijs *et al*., 2003; Saper *et al*., 2005). The abundant expression of NMS in SCN, PVN and ARC suggest that NMS like other neuromedins, plays important role in the regulation of hypothalamic functions (Raddatz *et al*., 2000; Mori *et al*., 2005).

Intracerebroventricular (*icv*) injection of NMS in rats reduced 12- hr food intake during the dark period in a dose-dependent manner. Similarly *icv* injection of 3 nmol NMS and NMU into rats caused a significant decrease in 12-h food intake. On the other hand, at low doses (0.5 nmol and 1 nmol), only NMS administration suppressed food intake (Miyazato, 2008). NMS administration in rats augmented POMC mRNA in the ARC and CRH mRNA in the PVN, inducing c-Fos expression in POMC neurons in the ARC. These results suggest that POMC in the ARC and CRH in the PVN are involved in NMS action on feeding (Miyazato *et al*., 2008).

The presences of NMS mRNA in testes (Fujii *et al*., 2000) suggest its possible role in reproduction. Only few studies are available on the role of NMS on reproduction. Central administration of NMS in female rats has a stimulatory role in LH secretion (Vigo *et al*., 2007) and peripheral administration of NMS induced T secretion in rhesus monkeys in a dose dependant manner (Jahan *et al*., 2011).

Objectives of the study

This project was designed to investigate the possible role of NMS in metabolic regulation of reproductive axis in intact adult male rhesus monkeys by:

- Investigating the involvement of NMS in the regulation of HPG axis and HPA axis under normal fed and metabolically stressed conditions.
- Determining the role of NMS on the secretion of two pituitary hormones i.e. GH and PRL under normal fed and fasting conditions.
- Analyzing the effects of NMS on the secretion of adipokines (adiponectin, leptin and resistin), important for the functioning of the HPG axis, under fed and fasting conditions.
- Studying the effects of NMS on insulin release under fed and metabolically stressed conditions.

Materials and Methods

Animals

The animals used in the concerned study were, four adult normal male monkeys (*Macaca mulatta*) of age and weight ranging from 6-8 years and 7-10 kg respectively. All the animals were kept in specific colony environment of primate facility at Department of Animal Sciences, Quaid-i-Azam University Islamabad, Pakistan. The animals were daily provided with feed comprising of fresh fruits, boiled potatoes, eggs and bread at specific times according to their body weights, and water was available *ad libitum*. Prior to the start of experiment, appetite monitoring was carried out for a month. It was observed that all animals used to finish their food within 10-15 min. All experiments were approved by the Departmental Committee for Care and Use of Animals.

Chair restraining

Prior to initiation of experiment, the animals were chair restrained for one month in order to minimize stress factor on blood sampling and time period of this restraint was progressively increased up to 4-5 hrs a day. The chemical, Ketamine HCl, was used to sedate the animals during the procedure.

The experiment was launched after the approval of Departmental committee for care and use of animals.

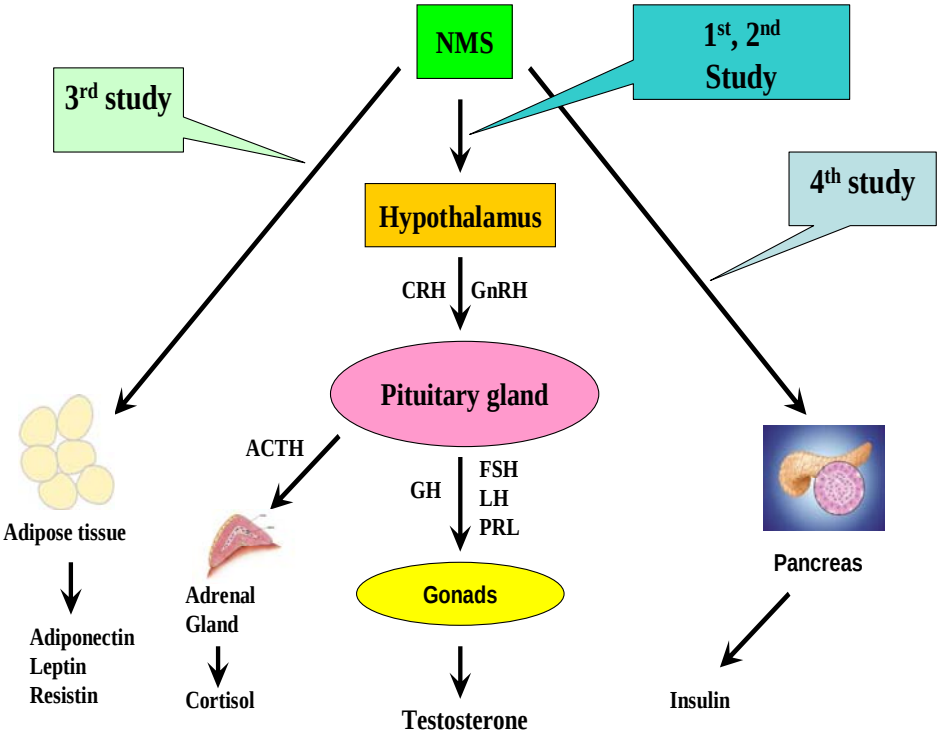
Venous Catheterization

A cathy cannula (Silver surgical complex, Karachi, Pakistan; 0.8 mm O.D/22 G×25mm) was affixed in the sephnous vein after anesthesizing the animals with Ketamine HCl (10 mg/kg BW, *im*), to bring about all the chemical administration and sequential blood sampling. A butterfly tubing (24 G×3/4″ diameter and 300 mm length; JMS Singapore) was attached with free end of the cannula. All the sampling was performed after full recovery of animals from sedation.

Experimental Design

This project was designed to evaluate the effect of an anorexigenic peptide NMS, on the regulation of HPG-axis in both physiological and metabolically stressed

conditions. The whole project comprised of four sub-studies. First study was designed to investigate the possible involvement of NMS on T and cortisol secretion. In the second study the effect of NMS was evaluated on the regulation of GH and PRL secretion. In the third study the effect of NMS was investigated on three vital adipokines i.e. leptin, adiponectin and resistin in both fed and fasting animals. In the fourth study the plasma insulin concentrations were determined before and after NMS administration. All the experiments were performed on normally fed and 48-hrs fasting male rhesus monkeys.



Pharmacological Reagents

Pharmacological reagents used in the study are listed below:

Heparin (Sinochem Ningbo, China)

Ketamine HCl (Rotexmedica, Trittau, Germany)

Human Neuromedin S (Anaspec, USA)

hCG (Gonacor®, Instituto Massone S.A Argentina)

All the working solutions were prepared in saline solution (0.9% NaCl).

Blood sampling

Blood sampling (2-3 ml) was conducted, at regular intervals of 15 min, using heparinized syringes. An equivalent quantity of heparinized (5 IU/ml) saline was injected after each sample withdrawal. Samples were collected 60 min before and 120 min after NMS administration. The time of NMS (50 nmol) administration was considered as 0 min. In case of hCG treatment, samples were collected 60 min before and 180 min after hCG administration (30 IU/kg BW). In this case NMS (50 nmol) was injected after 60 min of hCG infusion. All blood samples were obtained between 1100-1500 hrs. All experiments were performed in a couple of weeks in order to reduce the alterations in hormonal levels associated with seasonal changes. Samples were centrifuged for 10 min at 3000 rpm, and then plasma was pipetted out and stored at -20˚C until analyzed.

Analysis of hormones

Plasma hormonal concentrations were assessed using specific Enzyme linked immunosorbent assay (ELISA) kits according to the assay protocol provided with the kits. Specific enzyme immunoassays kits (Amgenix inc. USA) were used to determine plasma T, cortisol, GH and PRL concentrations. The minimum limits of detectable T and cortisol levels were upto 0.05 ng/ml and 1.5 ng/ml respectively; intra-assay and inter-assay coefficients of variation for both T and cortisol were <12%. For both GH and PRL the minimum detectable levels were 0.05 ng/ml; intra-assay and inter-assay coefficients of variation were <8%. Specific EIA kits (AssayMax Human ELISA; Assaypro 41 Triad south drive St. Charles, USA) were used for determining the changes in plasma leptin, adiponectin and resistin levels. The minimum limit of detectable level of leptin was upto 0.12 ng/ml; intra-assay and

inter-assay coefficients of variation were 4.5% and 7.2% respectively. The minimum limit of detectable adiponectin levels was upto 0.5 ng/ml; intra-assay and inter-assay coefficients of variation were 4.2% and 7.3% respectively. In case of resistin the minimum detectable level was upto 0.2 ng/ml; intra-assay and inter-assay coefficients of variation were 4.2% and 7.3% respectively. Human insulin EIA kit (Calbiotech Inc. CA) was used to determine plasma insulin levels. The minimum limit of detectable insulin levels was upto 1.47 μIU/ml; Intra-assay and inter-assay coefficients of variation were 8.1% and 8.5% respectively.

All EIA procedures were used according to the manufacturer instructions.

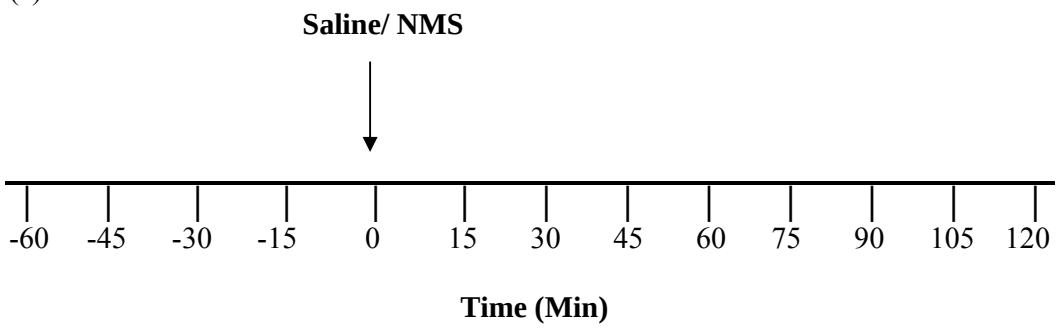
Statistical analysis

All the data were presented as mean±SEM. Hormonal concentrations after NMS and saline administration were compared by one-way ANOVA followed by post hoc Dunnett's multiple comparisons test. Student's t test was employed to compare mean pre- and post-treatment hormonal levels, in 48-hrs fasting and normal fed conditions.

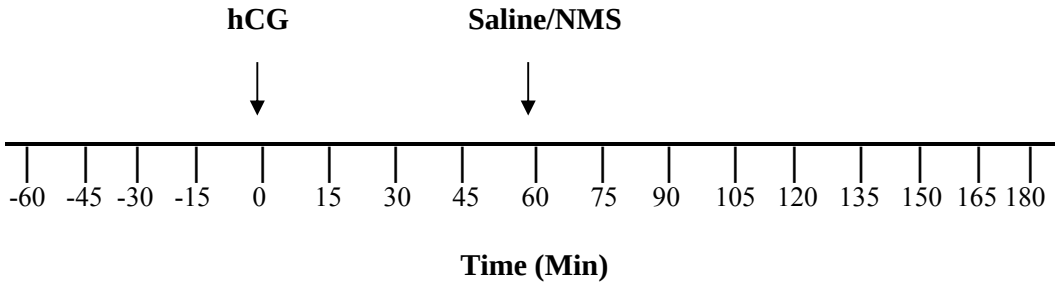
Statistical significance was set at $P \leq 0.05$. All the data were analyzed by using statistical software GraphPad Prism version 5.

Sampling protocol

(a)



(b)



(a) Saline/NMS, fed and fasting conditions (b) hCG, Saline/NMS fed condition

Chapter # 1

Possible involvement of Neuromedin S signaling in the regulation of HPG axis and HPA axis under normal fed and 48-hrs fasting adult male rhesus monkeys (*Macaca mulatta*)

Abstract

Background: Neuromedin S (NMS), an anorexigenic neuropeptide was discovered in rat brain. It is a ligand for receptor FM4/TGR-1 which is also called as NMU receptor type II (NMU2R). Mainly it is expressed in SCN and involved in regulation of food intake and dark light circadian rhythms. In rodents its stimulatory role in HPG axis is reported but in higher primates its reproductive role is yet to be explored. In the present study the stimulatory role of NMS was investigated in the regulation of HPG axis and HPA axis. For this purpose after NMS administration plasma T and cortisol levels were determined in normal fed and 48-hrs fasting monkeys.

Materials and methods: Four adult male rhesus monkeys were used in this study. Single dose of 50 nmol NMS was injected through a cannula affixed in saphenous vein. Blood samples were collected individually 60 min before and 120 min with 15 min intervals, after NMS/saline administration. In case of hCG administration, samples were collected 60 min before and 180 min at 15 min intervals, after hCG administration. Plasma T and cortisol concentrations were determined by using specific Enzyme Immunoassay (EIA) kits.

Results: 48 hrs fasting significantly ($P<0.001$) decreased plasma T and increased cortisol ($P<0.001$) levels compared to normal fed monkeys. In both fed and fasting conditions, NMS injection induced a significant increase ($P<0.05$) in T and cortisol concentrations compared to saline treated animals.

Conclusion: In summary our results suggested that NMS is a positive modulator of both HPG and HPA axis. In fasting conditions its effect on T secretion was delayed but in fed conditions no such response was observed suggesting that fasting has inhibitory role on HPG axis and NMS has ability to temporary terminate this inhibition. The exact pathway of its signaling is not clearly understood, so in future further studies are required to confirm the NMS involvement and its pathway in the regulation of reproductive axis.

Introduction

Reproductive functions are vitally controlled by HPG axis. This axis regulates secretion of pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) by pulsatile release of hypothalamic decapeptide gonadotropin-releasing hormone (GnRH). All these hormones play a major role in gonadal maturation and functions (Plant, 2008). Many internal and external factors may affect the proper functioning of HPG axis. The most important factor is the nutritional status of an individual (Bronson, 1985; Cameron, 1996; Wade *et al.*, 1996; Wade and Jones, 2004). The relationships between nutritional status and reproduction are not clearly understood. The observations of Pirke and colleagues suggested the possible role of specific nutrients on reproductive function in the human (Pirke *et al.*, 1986). Many studies on rats, cattle and hamsters, have also shown that the metabolic deficiencies affect the testicular size and sperm production, as caused by other environmental factors such as photoperiod and social cues (Lincoln and Short, 1980; Walkden-Brown *et al.*, 1994).

The exact mechanism that how metabolic fuel deficiencies arrest the neural networks which regulate the intermittent GnRH discharge is not completely understood. In the hierarchy of signals controlling the reproductive axis, the position of the hypothalamic GnRH makes it a target for both peripheral and central regulators. Neurotransmitter, glial factors and neuropeptides are included in central modulators while leptin and sex steroids are peripheral modulators. A wide variety of inhibitory and excitatory neurotransmitters/neuropeptides may play a vital role in HPG axis arrest under metabolic fuels deficiency (Terasawa and Fernandez, 2001; Plant and Shahab, 2002; Plant and Barker-Gibb, 2004; Ebling, 2005). This idea is further supported by the observation of reduced sensitivity of the HPG axis to NMDA administration in fasting (Shahab *et al.*, 1997). In the mammalian hypothalamus, Glutamate and GABA are the most abundant excitatory and inhibitory neurotransmitters respectively (Cotman and Iversen, 1987; Thind and Goldsmith, 1997; Terasawa and Fernandez, 2001), and in several species the neurons containing both these neurotransmitters synapse with GnRH neurons in the hypothalamus (Jennes *et al*., 1983; Leranth *et al*., 1992).

Metabolic deficiency suppressed GnRH secretion, is associated with increased levels of GABA due to over expression of GABA synthesizing enzymes (Leonhardt *et al.*, 1999). The study of Mahesh and Brann showed that EAA stimulates LH secretion (Mahesh and Brann, 2005). Similarly the energy deficiency (short term fasting) induced HPG axis inactivity, is terminated by EAA (Shahab *et al.*, 1997). Glutamate, which is known to stimulate LH secretion, decreased during restricted supply of food intake (Tal *et al.*, 1983), and aspartate derivatives administration to hypogonadotrophic lambs, on an insufficient diet, results in increased plasma LH levels (Ebling *et al.*, 1990). Similar stimulatory effect of aspartate on LH concentrations was noticed in lambs with lowered LH secretion due to food insufficiency (Bucholtz *et al*., 1996).

Neuromedin S is a novel 36-amino acid peptide which binds with the G protein-coupled receptor FM4/TGR-1 also called neuromedin U receptor type-2 (NMU2R), and is highly expressed in the suprachiasmatic nucleus (SCN) of the hypothalamus (Mori *et al*., 2005). Structurally, NMS shows complete sequence homology with the C- terminal seven amino acid portion of a brain-gut peptide, NMU, originally isolated from porcine spinal cord (Minamino *et al*., 1985). The expression of NMS receptor is restricted almost only to the central nervous system having abundant expressionin SCN and PVN (Guan *et al*., 2001; Nakahara *et al*., 2004). The presence of receptor within SCN, suggests its ligand role in regulation of circadian rhythm of temperature, sleep/wake cycle and hypothalamic hormones like CRH and GnRH secretion (Mori *et al*., 2005) while its PVN presence implies its role in feeding and the regulation of HPA axis. NMS receptors are also abundantly present outside the hypothalamus such as thalamus, amygdale, hippocampus and cerebellum (Raddatz *et al*., 2000), which indicates its presumptive role in motor phenomena, emotions and regulation of behavior. It has been demonstrated that NMS has higher expression than that of NMU in the hypothalamus (Rucinski *et al*., 2007), which suggests the predominance of NMS in central regulatory processes. NMS mRNA has higher expression in the hypothalamus, testes and spleen (Mori *et al*., 2005).

In rats, SCN plays a stimulatory role in cortisol secretion (Cascio *et al*., 1987). AVP is one of the main neurotransmitters in the SCN having projections towards PVN/DMH (Buijs *et al*., 1993, 1999). AVP administration into these brain regions,

inhibited cortisol secretion in SCN removed rats (Kalsbeek *et al*., 1992), suggesting that PVN/DMH mediates this effect of SCN, and AVP alone can imitate the inhibition of this effect. In support of this hypothesis, when AVP antagonist was administered into these brain areas, at the peak time of AVP release, the cortisol secretion was stimulated (Kalsbeek *et al*., 1992, 1996a, b). It was also suggested that during second half of the light period, increase in cortisol and ACTH release after AVP antagonist administration, are most prominent and consistent activities (Kalsbeek *et al*., 1996b).

By evoking marked ACTH and cortisol release, NMS and SCN association is an effective activator of the HPA axis (Miklos *et al*., 2007). CRH release and activation of its receptor CRHR1 might be responsible for these effects, since the corticosterone secretion was inhibited after treatment of CRHR1 antagonist i.e. antalarmin. Moreover the administration of CRHR2 antagonist i.e. astressin 2B, did not inhibited the NMS stimulated HPA activation, supporting the hypothesis, that CRHR1 pathway is important in central regulation of the stress response (Miklos *et al*., 2007). The presence of NMS mRNA in testes (Fujii *et al*., 2000), suggest its possible role in reproduction. Central administration of NMS in female rats, stimulated LH secretion (Vigo *et al*., 2007) and peripheral administration of NMS, induced T secretion in rhesus monkeys in a dose dependant manner (Jahan *et al*., 2011). The present study was designed to investigate the possible involvement of NMS in T and cortisol secretion in normal and metabolically stressed conditions in adult male rhesus monkeys.

Materials and Methods

Animals

The animals used in the concerned study were, four adult normal male monkeys (*Macaca mulatta*) of age and weight ranging from 6-8 years and 7-10 kg respectively. All the animals were kept in specific colony environment of primate facility at Department of Animal Sciences, Quaid-i-Azam University Islamabad, Pakistan. The animals were daily provided with feed comprising of fresh fruits, boiled potatoes, eggs and bread at specific times according to their body weights, and water was available *ad libitum*. Prior to the start of experiment, appetite monitoring was carried out for a month. It was observed that all animals used to finish their food within 10-15 min.

Venous Catheterization

A cathy cannula (Silver surgical complex, Karachi, Pakistan; 0.8 mm O.D/22 G×25mm) was affixed in the sephnous vein after anesthesizing the animals with Ketamine HCl (10 mg/kg BW, *im*), to bring about all the chemical administration and sequential blood sampling. A butterfly tubing (24 G×3/4″ diameter and 300 mm length; JMS Singapore) was attached with free end of the cannula. All the sampling was performed after full recovery of animals from sedation.

Pharmacological Reagents

Pharmacological reagents used in the study are listed below:
Heparin (Sinochem Ningbo, China)
Ketamine HCl (Rotexmedica, Trittau, Germany)
Human Neuromedin S (Anaspec, USA)
hCG (Gonacor®, Instituto Massone S.A Argentina)
All the working solutions were prepared in saline solution (0.9% NaCl).

Blood sampling

Blood sampling (2-3 ml) was conducted, at regular intervals of 15 min, using heparinized syringes. An equivalent quantity of heparinized (5 IU/ml) saline was injected after each sample withdrawal. Samples were collected 60 min before and

120 min after NMS/saline administration. The time of NMS (50 nmol) administration was considered as 0 min. In case of hCG treatment, samples were collected 60 min before and 180 min after hCG administration (30 IU/kg BW). In this case NMS was injected after 60 min of hCG infusion. All blood samples were obtained between 1100-1500 hrs. All experiments were performed in a couple of weeks in order to reduce the alterations in hormonal levels associated with seasonal changes. Samples were centrifuged for 10 min at 3000 rpm, and then plasma was pipetted out and stored at -20˚C until analyzed.

Analysis of hormones

T and cortisol concentrations were quantitatively determined by using EIA kits (Amgenix Inc. USA). The minimum limit of detectable T levels was upto 0.05 ng/ml; intra-assay and inter-assay coefficients of variation were 6.4% and 4.4% respectively and the minimum limit of detectable cortisol levels was upto 1.5 ng/ml; intra-assay and inter-assay coefficients of variation were 9.4% and 10.2% respectively. All the procedures of EIA were followed as provided with the kits.

Statistical analysis

All the data were presented as mean±SEM. T and cortisol concentrations after NMS and saline administration were compared by one-way ANOVA followed by post hoc Dunnett's multiple comparisons test. Student's t test was employed to compare mean pre- and post-treatment T and cortisol concentrations, under 48-hrs fasting and normal fed conditions.

Statistical significance was set at $P \leq 0.05$. All the data were analyzed by using statistical software GraphPad Prism version 5.

Results

Basal plasma T concentrations in fed and fasting conditions

Basal plasma concentrations of T (ng/ml) during 1-hr before saline administration in fed and 48-hrs fasting monkeys are given in Fig. 1.1A-1.1B. Plasma T concentrations significantly ($P< 0.001$) decreased in 48-hrs fasting monkeys compared to normal fed monkeys.

Effect of NMS on plasma T secretion in normal fed adult male monkeys

The individual and mean plasma T concentrations (ng/ml) before and after saline/NMS administration in normal fed monkeys are given in table 1.1-1.2 and Fig. 1.2A. At 30 min after NMS administration significant ($P<0.05$) increase in T secretion was observed. Maximum levels of T concentrations were observed at 60 min of NMS treatment compared to 0 min sample (Fig. 1.2B). Comparison between pre- and post-treatment also showed a significant ($P<0.05$) increase in T secretion after NMS treatment (Fig. 1.3).

Effect of NMS on plasma T secretion in 48-hrs fasting adult male monkeys

The individual and mean plasma T concentrations (ng/ml) before and after saline/NMS administration in 48-hrs fasting monkeys are given in table 1.3-1.4 and Fig. 1.4A. At 60 min after NMS administration significant ($P<0.05$) increase in T secretion was observed. Maximum levels of T concentrations were observed at 75 min of NMS treatment compared to 0 min sample (Fig. 1.4B). Comparison between pre- and post-treatment also showed a significant ($P<0.05$) increase in T secretion after NMS treatment (Fig. 1.5).

Effect of NMS on hCG induced plasma T secretion in adult male monkeys

The individual and mean hCG induced plasma T levels (ng/ml) before and after saline/NMS administration are given in table 1.5-1.6 and Fig 1.6A. NMS administration did not cause any significant ($P>0.05$) change on hCG induced T levels compared to saline treatment (Fig. 1.6B).

Basal plasma cortisol concentrations in fed and fasting conditions

The pattern of basal plasma concentrations of cortisol (ng/ml) during 1-hr before saline administration in fed and 48-hrs fasting adult male monkeys are given in Fig. 1.7A-1.7B. Basal plasma cortisol concentrations significantly ($P< 0.001$) increased in 48-hrs fasting compared to normal fed monkeys.

Effect of NMS on plasma cortisol secretion in normal fed adult male monkeys

The individual and mean plasma cortisol concentrations (ng/ml) before and after saline/NMS administration in normal fed monkeys are given in table 1.7-1.8 and Fig. 1.8A. NMS induced significant ($P<0.01$) increase in cortisol secretions at 45 min compared to 0 min sample. Maximum cortisol concentrations ($P<0.001$) were observed at 105 min of NMS administration compared to 0 min sample (Fig. 1.8B). Comparison between pre- and post-treatment also showed a significant ($P<0.05$) increase in cortisol levels after NMS treatment (Fig. 1.9).

Effect of NMS on plasma cortisol secretion in 48-hrs fasting adult male monkeys

The individual and mean plasma cortisol concentrations (ng/ml) before and after saline/NMS administration in 48-hrs fasting monkeys are given in table 1.9-1.10 and Fig. 1.10A. NMS induced significant ($P<0.05$) increase in cortisol secretion at 60 min compared to 0 min sample. Maximum increase in cortisol concentrations ($P<0.001$) was observed after 90 min of NMS administration compared to 0 min sample (Fig. 1.10B). Comparison between pre- and post-treatment showed a significant ($P<0.05$) increase in cortisol concentrations in post-NMS treated animals (Fig. 1.11).

Table 1.1. Individual and mean (±SEM) plasma T concentrations (ng/ml) before and after saline administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	1.17	1.53	1.48	0.97	1.29±0.13
-45	1.06	1.67	1.41	1.48	1.40±0.13
-30	0.96	1.02	1.46	1.35	1.20±0.12
-15	0.94	0.87	1.03	1.39	1.06±0.11
0	1.10	1.18	1.30	1.40	1.24±0.07
15	1.30	1.22	1.23	1.44	1.30±0.05
30	1.39	1.26	1.31	1.58	1.39±0.07
45	1.25	1.54	1.20	1.73	1.43±0.13
60	1.50	1.66	1.36	1.85	1.59±0.10
75	1.42	1.47	1.39	1.91	1.55±0.12
90	0.96	1.28	1.42	1.64	1.32±0.14
105	0.98	1.23	1.58	1.81	1.40±0.18
120	0.79	1.11	1.38	1.21	1.12±0.12

Table 1.2. Individual and mean (±SEM) plasma T concentrations (ng/ml) before and after NMS administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	1.29	1.62	1.16	1.48	1.39±0.10
-45	1.75	1.68	1.07	1.46	1.49±0.15
-30	1.94	1.70	0.97	1.27	1.47±0.22
-15	1.00	1.97	1.49	0.79	1.31±0.26
0	1.66	1.82	1.11	1.04	1.41±0.20
15	1.89	1.10	1.69	1.38	1.51±0.17
30	1.81	2.39	1.86	1.97	2.01±0.13*
45	2.19	2.05	2.51	2.21	2.24±0.10**
60	2.28	2.01	2.17	2.77	2.31±0.16***
75	1.82	2.00	1.80	1.75	1.84±0.05
90	1.63	1.96	1.21	1.47	1.56±0.16
105	1.79	2.01	1.69	1.49	1.75±0.11
120	1.47	1.78	1.31	1.61	1.54±0.10

***P<0.05, **P<0.01, ***P<0.001 vs 0 min (ANOVA followed by post hoc Dunnett's test).**

Table 1.3. Individual and mean (±SEM) plasma T concentrations (ng/ml) before and after saline administration (at 0 min) in 48-hrs fasting adult male monkeys (n=3).

Time (Min)	Animal Numbers			Mean±SEM
	201	202	203	
-60	0.77	0.76	1.01	0.85±0.08
-45	0.64	0.78	0.85	0.76±0.06
-30	0.61	0.60	0.88	0.70±0.09
-15	0.63	0.54	0.98	0.72±0.13
0	0.71	0.70	1.06	0.82±0.12
15	0.62	0.67	0.82	0.70±0.06
30	0.53	0.70	0.70	0.64±0.06
45	0.66	0.68	0.64	0.66±0.01
60	0.74	0.82	0.59	0.72±0.07
75	0.78	0.85	0.72	0.78±0.04
90	0.99	0.81	0.75	0.85±0.07
105	0.94	0.78	0.86	0.86±0.04
120	0.97	0.65	0.62	0.74±0.11

Table 1.4. Individual and mean (±SEM) plasma T concentrations (ng/ml) before and after NMS administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	0.63	0.86	0.99	0.79	0.82±0.07
-45	0.77	0.91	0.69	0.77	0.79±0.05
-30	0.64	0.82	0.68	0.49	0.66±0.07
-15	0.60	0.84	0.59	0.69	0.68±0.06
0	0.69	0.95	0.58	0.67	0.72±0.08
15	0.61	0.81	0.68	0.48	0.65±0.07
30	0.72	0.98	0.89	0.83	0.85±0.05
45	0.78	0.96	0.92	1.01	0.92±0.05
60	0.83	1.16	0.98	1.12	1.02±0.07*
75	0.93	1.18	1.23	1.31	1.16±0.08***
90	1.04	1.03	1.11	1.22	1.10±0.04**
105	0.96	0.84	0.91	1.14	0.96±0.06
120	0.87	0.85	1.10	1.02	0.96±0.06

***P<0.05, **P<0.01, ***P<0.001 vs 0 min (ANOVA followed by post hoc Dunnett's test).**

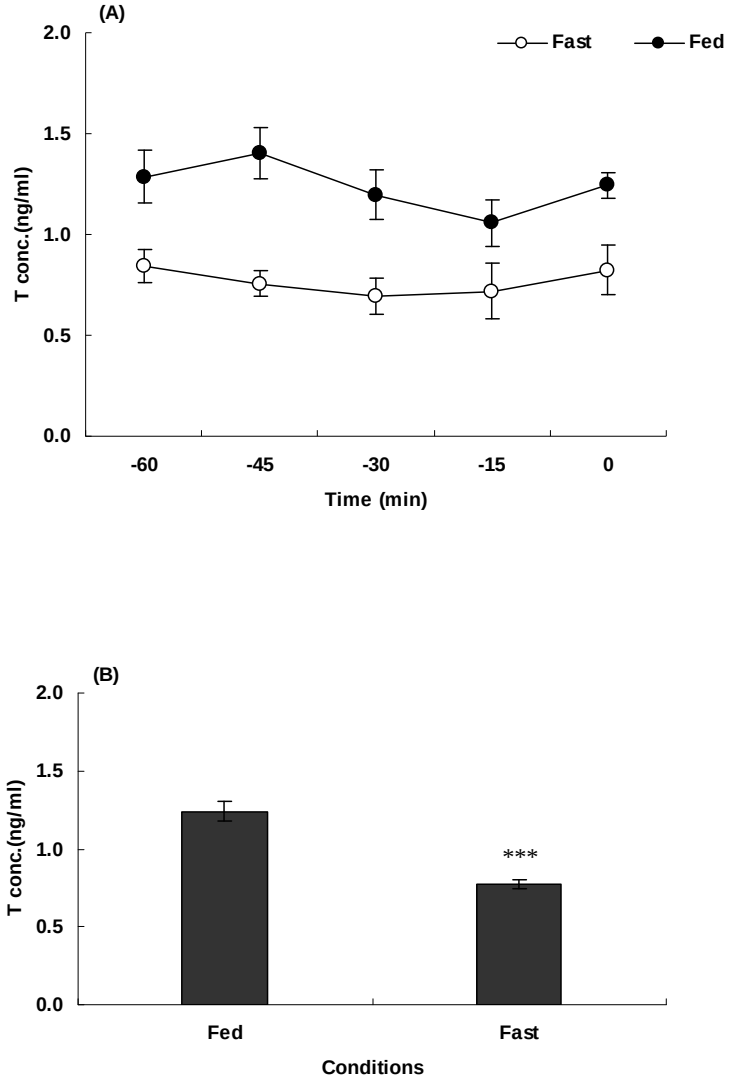


Figure 1.1. (A) Changes in mean (±SEM) basal plasma T concentrations (ng/ml) in 1-hr period in fed and 48-hrs fasting adult male monkeys (**B**) Overall mean (±SEM) basal plasma T concentrations (ng/ml) in 1-hr period in normal fed, and 48-hrs fasting adult male monkeys. ***P<0.001 vs fed (Student's t test).

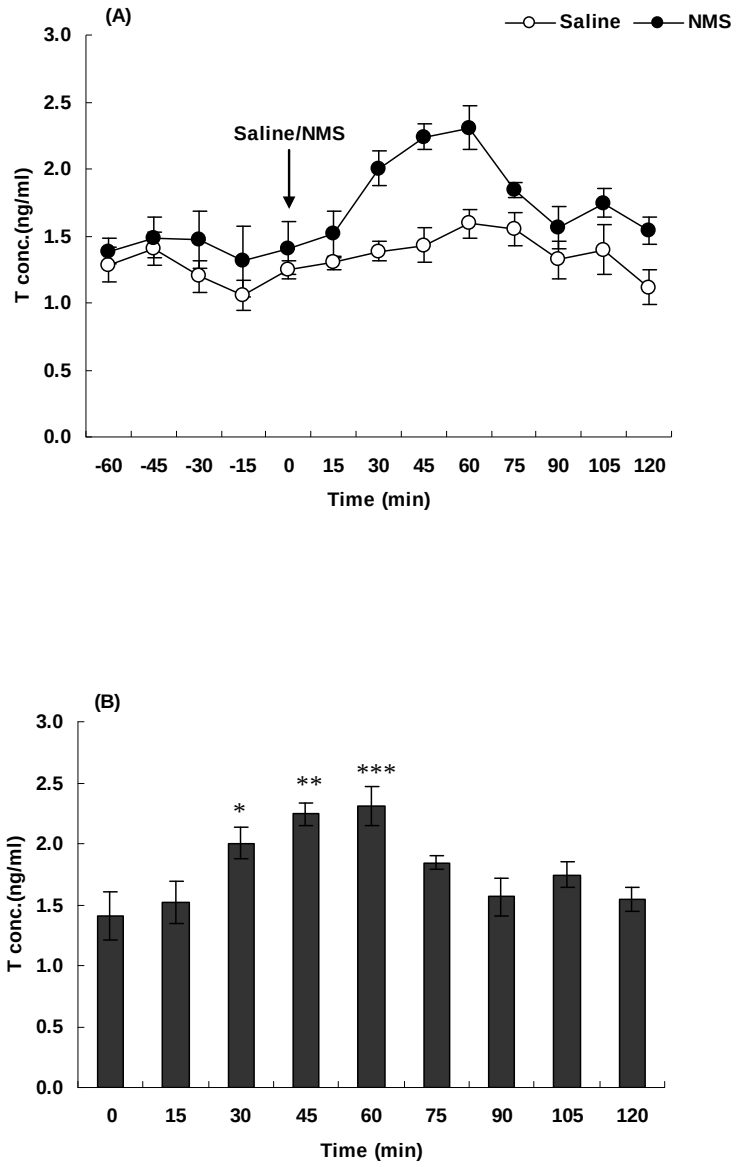


Figure. 1.2. (A) Mean (±SEM) changes in plasma T levels (ng/ml) before and after saline/NMS administration (at 0 min) in normal fed adult male monkeys. **(B)** Comparison of mean (±SEM) T concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in fed monkeys. *P<0.05, **P<0.01, ***P<0.001 vs 0 min (ANOVA followed by post hoc Dunnett's test).

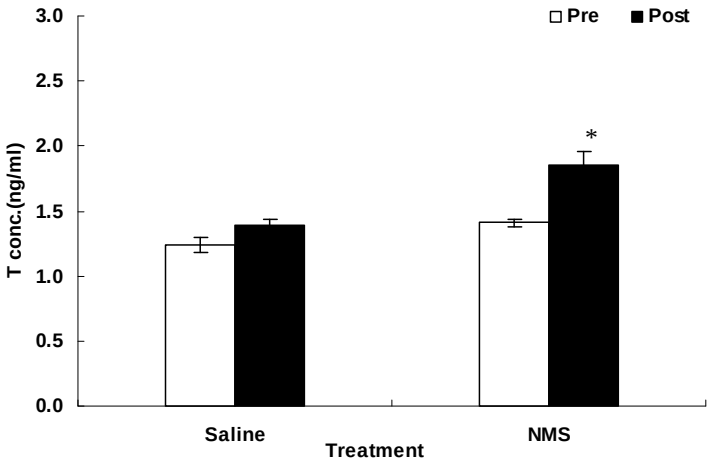


Figure. 1.3. Comparison of mean (±SEM) plasma T concentrations (ng/ml) in 60 min pre- and 120 min post saline/NMS in fed adult male monkeys. *P<0.05 vs pre-treatment (Student's t test)

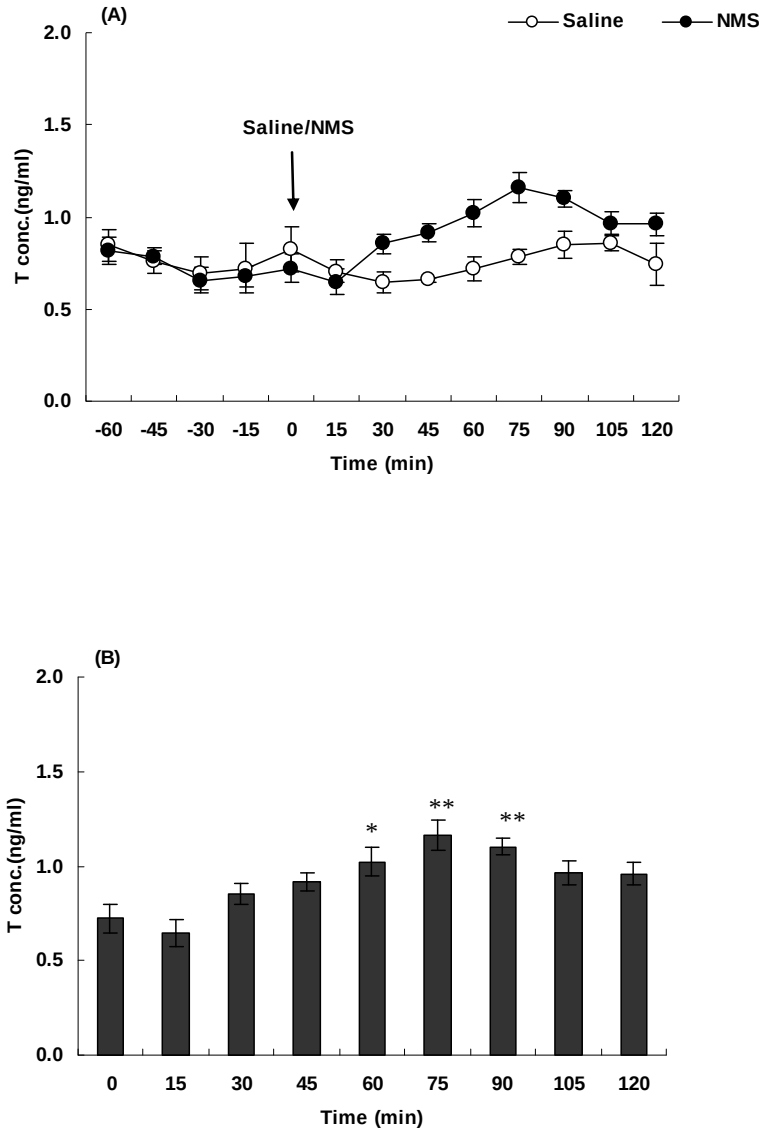


Figure. 1.4. (A) Mean (±SEM) changes in plasma T concentrations (ng/ml) before and after saline/NMS administration (at 0 min) in 48-hrs fasting adult male monkeys. **(B)** Comparison of mean (±SEM) T concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in 48-hrs fasting monkeys. *P<0.05, **P<0.01, ***P<0.001 vs 0 min (ANOVA followed by post hoc Dunnett's test).

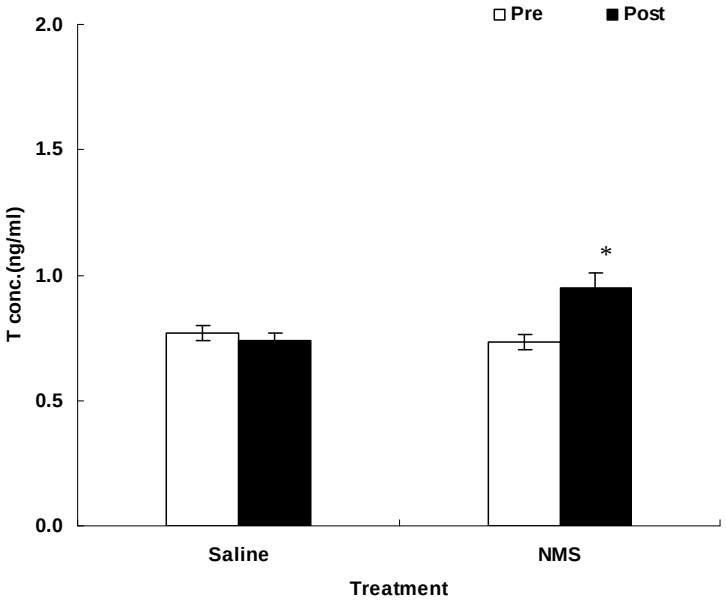


Figure. 1.5. Comparison of mean (±SEM) plasma T levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in 48-hrs fasting adult male monkeys. *P<0.05 vs pre-treatment (Student's t test).

Table 1.5. Individual and mean (±SEM) plasma T concentrations (ng/ml) before and after hCG and saline administration (at 0 min and 60 min respectively) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	1.09	2.61	1.28	1.79	1.69±0.34
-45	1.31	2.40	1.28	2.03	1.76±0.28
-30	1.85	2.34	1.49	2.21	1.97±0.19
-15	1.80	2.18	1.66	2.26	1.98±0.15
0	1.88	2.22	2.03	2.45	2.15±0.12
15	1.95	2.72	2.92	2.69	2.57±0.21
30	4.82	6.98	5.39	5.24	5.61±0.47
45	7.48	7.99	9.34	7.68	8.12±0.42
60	9.25	8.80	9.94	8.98	9.24±0.25
75	10.41	9.18	9.55	10.92	10.02±0.40
90	10.86	10.43	9.85	11.74	10.72±0.40
105	11.19	10.96	9.26	9.94	10.34±0.45
120	9.55	10.42	8.22	8.79	9.25±0.48
135	7.50	7.31	6.13	6.99	6.98±0.30
150	5.13	5.08	4.70	5.79	5.18±0.23
165	4.32	5.17	4.03	4.82	4.59±0.25
180	3.61	4.52	3.98	3.66	3.94±0.21

Table 1.6. Individual and mean (±SEM) plasma testosterone concentrations (ng/ml) before and after hCG and NMS administration (at 0 min and 60 min respectively) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	1.97	1.48	0.92	1.99	1.59±0.25
-45	2.72	2.03	1.26	1.92	1.98±0.30
-30	3.07	2.13	1.02	1.09	1.83±0.49
-15	3.65	2.42	1.31	1.58	2.24±0.53
0	3.47	2.56	1.80	1.52	2.34±0.44
15	3.61	2.85	2.34	1.88	2.67±0.37
30	5.15	6.31	4.94	3.27	4.92±0.63
45	6.78	7.07	5.62	7.36	6.71±0.38
60	8.16	9.63	8.35	9.05	8.80±0.34
75	8.38	10.21	9.86	9.66	9.53±0.40
90	9.02	10.90	8.53	9.83	9.57±0.52
105	8.94	9.38	8.49	9.96	9.19±0.31
120	8.28	9.79	8.78	10.22	9.27±0.45
135	9.02	10.21	8.99	10.77	9.75±0.44
150	7.09	8.76	8.14	8.45	8.11±0.36
165	6.76	8.79	6.82	7.34	7.43±0.47
180	6.65	7.49	6.22	6.58	6.74±0.27

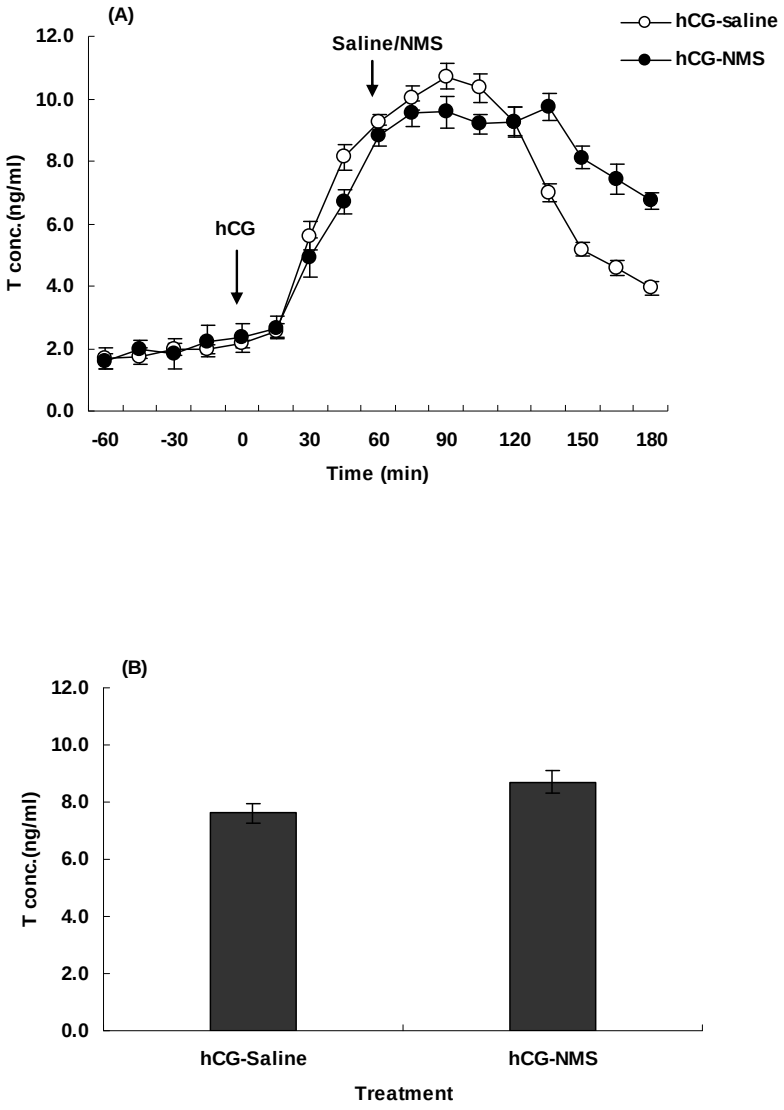


Figure 1.6. (A) Mean (±SEM) changes in hCG (at 0 min) induced plasma T secretion (ng/ml) before and after saline/NMS administration (at 60 min) in intact adult male monkeys. (**B**) Comparison of mean (±SEM) hCG induced plasma T levels (ng/ml) in 120 min after saline/NMS administration. P>0.05 (Student's t test).

Table 1.7. Individual and mean (±SEM) plasma cortisol concentrations (ng/ml) before and after saline administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	374.56	433.94	451.67	401.81	415.50±17.11
-45	359.45	451.21	524.62	389.54	431.21±36.53
-30	391.25	488.35	556.10	414.87	462.64±37.39
-15	358.03	475.71	510.75	431.17	443.92±32.93
0	321.86	502.57	517.33	451.35	448.28±44.45
15	334.16	482.48	551.54	435.85	451.01±45.63
30	376.35	418.93	511.86	443.02	437.54±28.35
45	352.07	487.65	594.24	481.55	478.88±49.56
60	323.69	583.02	520.68	355.11	445.63±62.96
75	382.58	561.40	486.33	372.78	450.77±44.94
90	344.91	512.80	503.18	425.13	446.51±39.14
105	312.53	490.77	468.57	410.34	420.55±39.80
120	391.14	428.91	472.06	387.51	419.91±19.74

Table 1.8. Individual and mean (±SEM) plasma cortisol concentrations (ng/ml) before and after NMS administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	361.51	342.29	423.85	391.35	379.75±17.83
-45	387.32	435.41	369.63	322.63	378.75±23.30
-30	411.23	382.45	363.14	341.27	374.52±14.85
-15	398.12	408.30	370.37	376.18	388.24±8.97
0	383.48	437.99	383.73	366.10	392.83±15.61
15	393.06	455.01	426.97	399.23	418.57±14.21
30	431.14	482.24	494.25	418.93	456.64±18.58
45	485.85	543.85	567.09	527.81	531.15±17.12**
60	502.56	564.47	586.46	591.88	561.34±20.47**
75	527.06	634.31	653.10	560.95	593.86±29.85***
90	563.54	665.20	662.93	537.18	607.21±33.27***
105	618.93	703.91	604.26	515.75	610.71±38.53***
120	523.49	583.10	530.37	479.06	529.01±21.32**

****P<0.01, ***P<0.001 vs 0 min (ANOVA followed by post hoc Dunnett's test).**

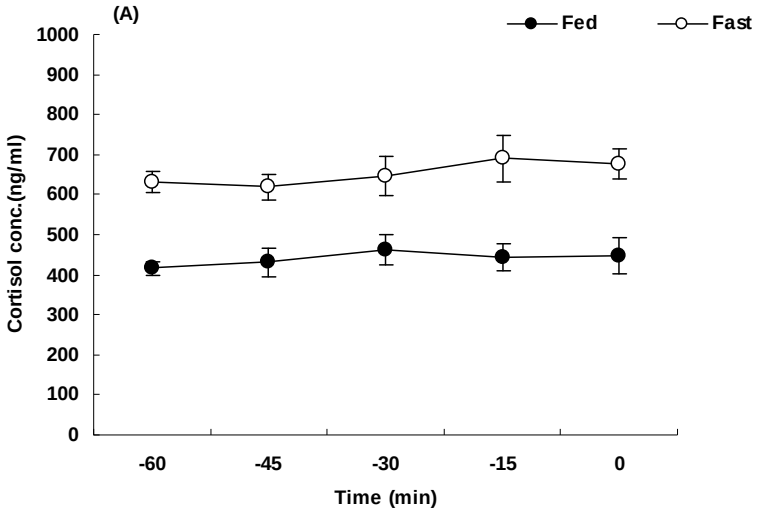
Table 1.9. Individual and mean (±SEM) plasma cortisol concentrations (ng/ml) before and after saline administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	652.59	612.68	572.91	689.46	631.91±25.15
-45	597.69	569.66	599.36	712.83	619.89±31.72
-30	581.55	591.25	619.14	788.96	645.23±48.57
-15	634.11	613.79	653.62	858.91	690.11±56.85
0	688.97	580.92	669.42	762.89	675.55±37.42
15	604.01	492.14	618.37	743.53	614.51±51.43
30	669.09	594.19	572.85	684.15	630.07±27.40
45	690.28	518.24	629.14	607.49	611.29±35.63
60	700.44	668.46	644.68	656.74	667.58±11.98
75	702.69	777.28	652.94	660.96	698.47±28.44
90	730.49	681.21	598.55	736.03	686.57±31.82
105	784.60	630.57	592.12	700.37	676.92±42.31
120	570.19	582.84	570.28	669.67	598.25±23.99

Table 1.10. Individual and mean (±SEM) plasma cortisol concentrations (ng/ml) before and after NMS administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	611.29	512.36	551.78	703.24	594.67±41.51
-45	645.52	534.13	574.23	742.69	624.14±45.74
-30	602.89	521.63	599.30	782.39	626.55±55.22
-15	630.92	580.44	577.52	725.56	628.61±34.56
0	604.38	568.43	550.62	721.18	611.15±38.34
15	635.21	607.16	613.33	732.85	647.14±29.20
30	665.58	638.03	630.74	785.14	679.87±35.88
45	678.98	702.28	676.99	810.95	717.30±31.74
60	714.21	751.92	731.49	926.07	780.92±48.99*
75	754.75	779.13	741.95	954.96	807.70±49.69**
90	805.03	823.65	816.50	977.88	855.77±40.89***
105	799.79	768.25	786.55	845.88	800.12±16.57**
120	738.87	705.60	699.81	842.49	746.69±33.07

***P<0.05, **P<0.01, ***P<0.001 vs 0 min (ANOVA followed by post hoc Dunnett's test).**



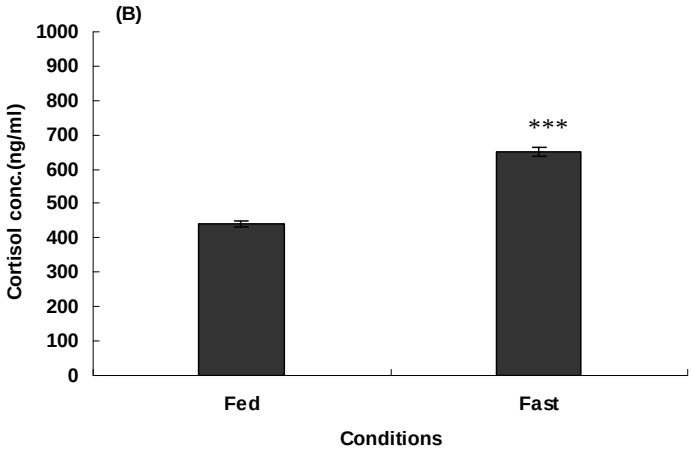


Figure 1.7. (A) Changes in mean (±SEM) basal plasma cortisol concentrations (ng/ml) in 1-hr period in normal fed and 48-hrs fasting monkeys (**B**) Overall mean (±SEM) basal plasma cortisol concentrations (ng/ml) in 1-hr period in normal fed, and 48-hrs fasting adult male monkeys. ***P<0.001 vs fed (Student's t test).

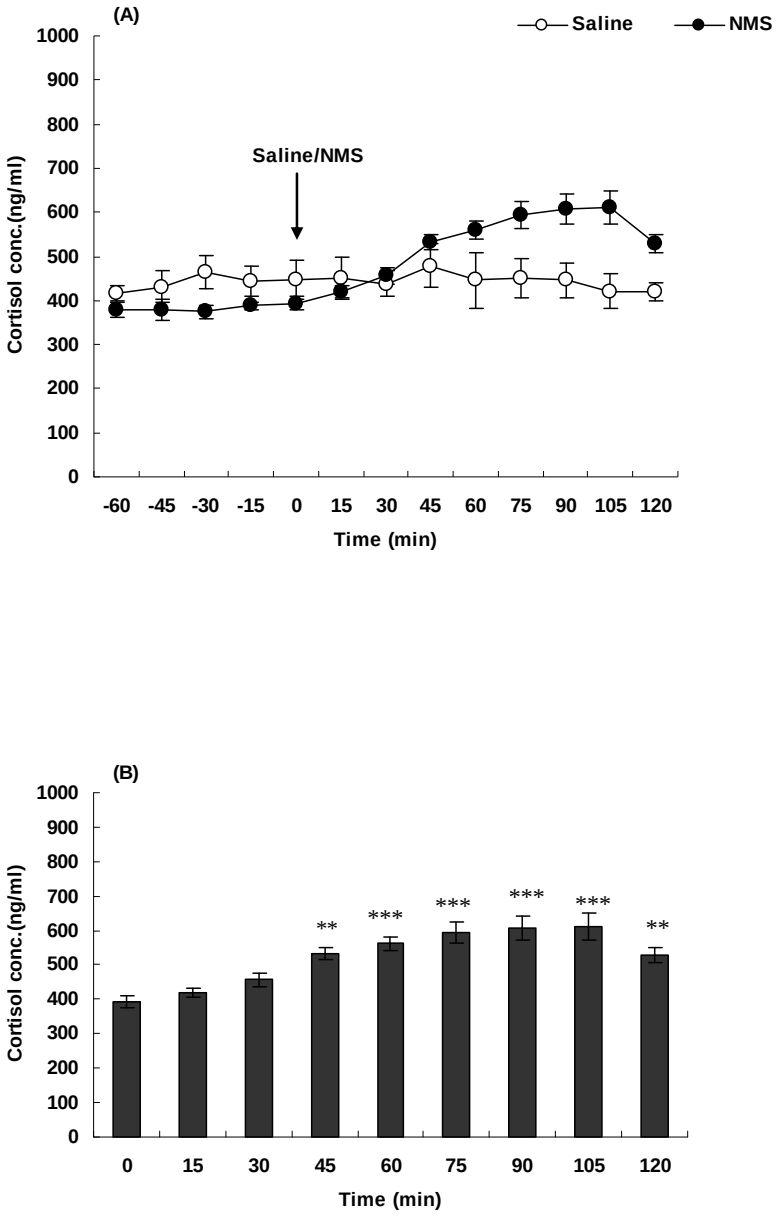


Figure. 1.8. (A) Mean (±SEM) changes in plasma cortisol levels before and after saline/NMS administration (at 0 min) in normal fed adult male monkeys. **(B)** Comparison of mean (±SEM) cortisol concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in fed monkeys. **P<0.01, ***P<0.001 vs 0 min (ANOVA followed by post hoc Dunnett's test).

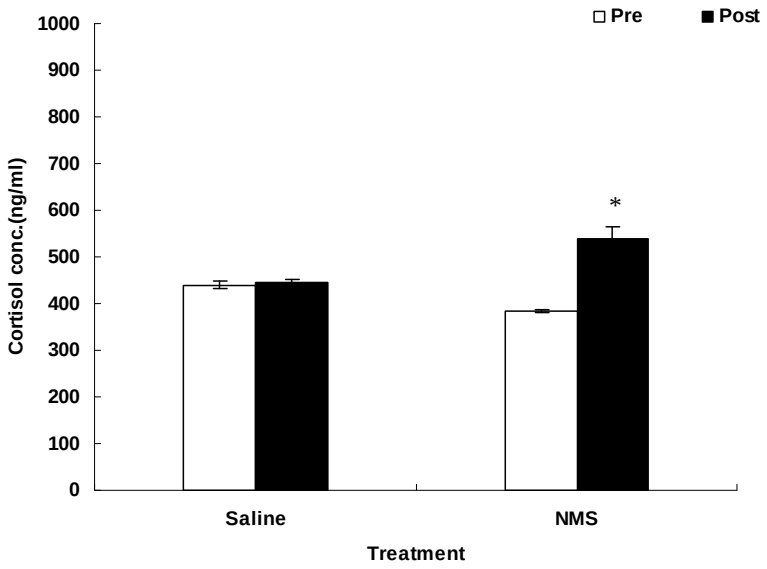


Figure. 1.9. Comparison of mean (±SEM) plasma cortisol levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in normal fed adult male monkeys. *P<0.05 vs pre-treatment (Student's t test).

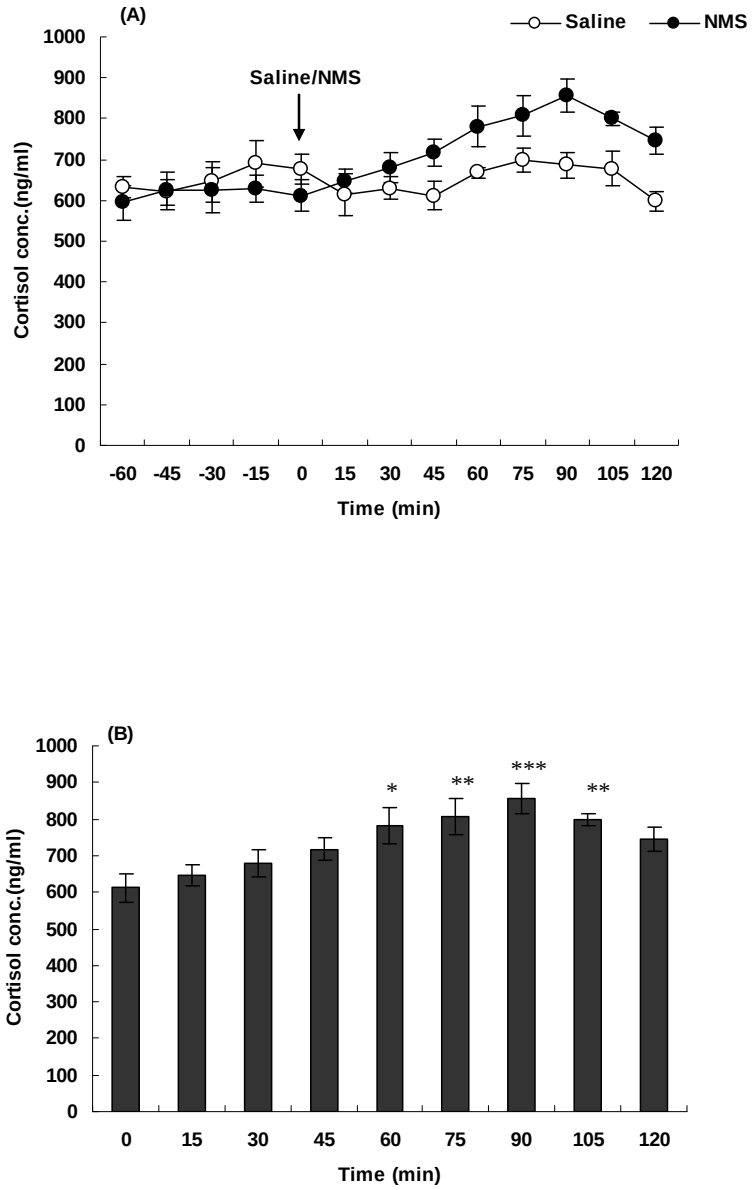


Figure. 1.10. (A) Mean (±SEM) changes in plasma cortisol levels (ng/ml) before and after saline/NMS administration (at 0 min) in 48-hrs fasting adult male monkeys. **(B)** Comparison of mean (±SEM) cortisol concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in 48-hrs fasting monkeys. *P<0.05, **P<0.01, ***P<0.001 vs 0 min (ANOVA followed by post hoc Dunnett's test).

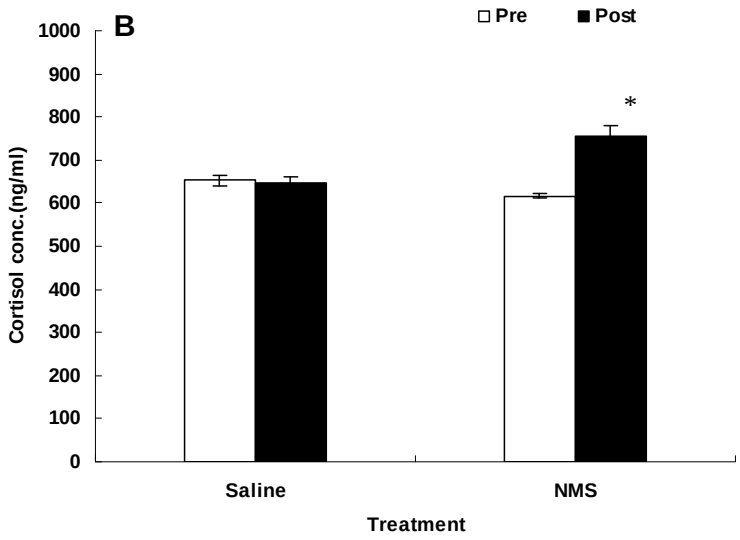


Figure. 1.11. Comparison of mean (±SEM) plasma cortisol levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in 48-hrs fasting adult male monkeys. *P<0.05 vs pre-treatment (Student's t test).

Discussion

In present study it was observed that 48-hrs fasting suppressed basal plasma T levels ($P<0.001$) suggesting that short term fasting has inhibitory effect on HPT axis in monkeys. This inhibition was possibly due to suppressed GnRH secretion, as it was evident in previous findings that inhibitory effect of short term fasting on HPT axis in monkeys is due to inhibition of GnRH secretion (Wahab *et al*., 2008) and not by changes in pituitary response to GnRH or changes in testicular response to LH (Cameron and Nosbisch, 1991). The fasting condition attained in this study was also evident by increase in plasma cortisol levels in metabolically stressed animals.

In our study single peripheral injection of NMS (50 nmol) significantly increased ($P<0.05$) T secretion in both normally fed and 48-hrs fasting animals. On the basis of these results it may be concluded that NMS has ability to overcome the fasting suppressed inactivity of HPG axis. Our results are in accordance with the findings of a previous study where *iv* administration of NMS significantly induced T secretion in dose dependent manner in rhesus monkeys (Jahan *et al*., 2011). This increase in T secretion is more likely due to increase in LH from pituitary and GnRH from hypothalamus. In female rats central administration of NMS significantly elicited LH secretion at estrus and metabolically stressed conditions (Vigo *et al*., 2007) suggesting that NMS might have been playing very important role in regulation of female gonadal axis. Our results suggested that more likely NMS is also a potent regulator of male gonadal axis in monkeys. The most important finding of our study was that in fasting conditions, the T response to NMS administration was delayed compared to normal fed monkeys.

The positive role of NMS on gonadotropin release was not unpredicted as NMU, which acts through the same receptor, influenced LH secretion in OVX female rats when centrally injected (Quan *et al*., 2003, 2004). However unexpectedly the effects of NMU on LH release were inhibitory, which are otherwise stimulatory by satiety signaling factors like leptin (Casanueva, 1999; Cunningham *et al*., 1999). In contrast to these findings Vigo and his colleagues found stimulatory role of NMS on LH secretion in female rats (Vigo *et al*., 2007). Our data also suggest that increase in T secretion after NMS administration might be due its stimulatory effect on LH

release.

The exact mechanism of this stimulatory response of NMS on LH secretion is yet not clear. However possibly NMS modulates expression of neuropeptides in ARC (Ida *et al*., 2005). ARC is the main site with abundant expression of NMU2R (Mori *et al*., 2005), involved in control of reproduction and energy balance. So it may be concluded that this stimulatory role of NMS in HPG axis is due to activation of ARC pathways. Kisspeptin and galanin like peptides, which have abundant expression in ARC, are most suitable candidates for this intermediatory action (Gottsch *et al*., 2004; Tena-Sempere, 2006). NMS also induced LH secretion in fasting female rats at diestrus. Similar response was noticed in underfed animals with different stimuli e.g. kisspeptin and galanin like peptide (Castellano *et al*., 2005, 2006). These observations are clear evidence that NMS has ability to counteract the inhibitory effect of metabolic stress on the gonadotropic axis and potentiate its role in regulation of energy balance and reproduction. In the present study, effect of NMS on hCG induced T secretion was not markedly different from saline treated animals. In this case hCG over activity might have masked the NMS individual effect on T secretion.

In our study, short term fasting (48-hrs) significantly increased ($P<0.001$) basal cortisol concentrations. These findings are in accordance with the study of Wahab *et al*, (2008). NMS and NMU are the potent activators of HPA axis (Hanada *et al*., 2001; Jaszberenyi *et al*., 2007). SCN and PVN expressions of NMS are important in this regard as both SCN and PVN play very important role in CRH release and the regulation of HPA axis (Cascio *et al*., 1987; Ozaki *et al*., 2002; Wren *et al*., 2002; Brighton *et al*., 2004; Ida *et al*., 2005; Jaszberenyi *et al*., 2007). In our study, peripheral administration of NMS stimulated cortisol secretion significantly ($P<0.05$) in both fed and short term fasting monkeys. This increase was more prominent in fasting monkeys compared to normal fed. Similar results were obtained after *icv* administration of NMS and NMU in rats and steers (Jászberényi *et al*., 2007; Yayou *et al*., 2009). It was suggested that this stress response was due to the activation of CRHR1 pathway and not by CRHR2 pathway (Aguilera *et al*., 2001). NMS released from hypothalamus (Mori *et al*., 2005), acts at PVN (Alonso *et al*., 1986) and the amygdala (Wiersma *et al*., 1995), stimulate CRH secretion, which stimulate the secretion of ACTH or dopamine through CRHR1 pathway.

Cortisol is generally considered as negative regulator of HPG axis in males, as in many studies on rats showed that increased levels of cortisol has inhibitory effect on steroidogenesis (Weber *et al*., 2000; Gao *et al*., 2003; Wagner and Claus, 2004: Ge *et al*., 2005a). However it is not clear whether these effects are the part of normal regulative step or detrimental to animal (Wagner and Claus, 2004). On the basis of above observations it was assumed that in fasting conditions the delayed response of T secretion in our study is, due to the increased concentrations of cortisol. However in normal fed monkeys NMS induced cortisol release did not suppress T secretion. So it was not confirmed, whether increased cortisol levels caused suppression of T release or fasting itself has some deleterious effects on NMS expression in hypothalamus. Previously in adult rhesus monkeys no association was found between cortisol and T concentrations and their release was independent of each other (Rose *et al*., 1971; Rasmussen and Suomi, 1989), while in another study positive relationship was found in T and cortisol concentrations (Higley *et al*., 1992). Blockage of glucocorticoid secretion in male savanna baboons did not cause stress induced drop in LH levels (Sapolsky and Krey, 1988). In cotton-top tamarins (*Saguinus* Oedipus) and common marmosets (*Callitrhixjacchus*) irregularities in gonadotropin and GnRH profiles were not associated with elevated cortisol levels (Abbott, 1993) In addition, stress-induced cortisol rise did not suppress LH release in rhesus monkeys (Helmreich *et al*., 1993), and in intact male rhesus monkeys CRH infusion failed to reduce LH levels (Norman, 1993). On the basis of these results it was concluded that in nonhuman primates, the gonadal steroid levels are independent to cortisol concentrations (Bercovitch and Clarke, 1995).

In summary our results suggest that NMS is a modulator of both HPG axis and HPA axis. It induces T secretion in both fed and fasting conditions but its effect was delayed in fasting monkeys compared to NMS treated normal fed. So it is suggested that the suppression of GnRH release by metabolic fuel deficiency might be the result of decrease in NMS receptor signaling to GnRH neurons or the neurons afferent to GnRH neurons. NMS also induced cortisol secretion in both fed and fasting conditions. In fasting conditions it was assumed that elevated cortisol levels might have inhibitory effect on the T secretion. However the earlier studies and the results of present study in fed monkeys exclude this possibility. Further studies are

required to confirm the role of adrenal glucocorticoid in regulation of reproductive processes in adult male rhesus monkeys.

Chapter # 2

Effect of peripheral administration of Neuromedin S on Growth hormone and Prolactin secretion under fed and fasting conditions in adult male rhesus monkeys (*Macaca mulatta*)

Abstract

Background: Growth hormone (GH) and Prolactin (PRL) released from anterior pituitary, directly or indirectly play very important role in male reproduction. In present study the role of peripheral administration of NMS was investigated in the release of GH and PRL in both fed and 48-hrs fasting monkeys.

Materials and Methods: Four intact adult male rhesus monkeys were used in this study. 50 nmol of NMS was injected through a cannula affixed in saphenous vein. Blood samples were collected individually 60 min before and 120 min at 15 min intervals, after NMS/saline administration. The plasma GH and PRL concentrations were determined by using specific Enzyme Immunoassay (EIA) kits.

Results: Short term fasting caused a significant decrease ($P<0.001$) in PRL concentrations but did not cause any significant ($P>0.05$) change in plasma GH levels. NMS injection induced a significant ($P<0.05$) increase in GH concentrations in both normal fed and metabolically stressed conditions compared to saline treated animals. PRL levels in normal fed animals were also significantly ($P<0.05$) increased after NMS administration but no significant change was observed in 48-hrs fasting conditions compared to saline treated animals.

Conclusion: These results suggest that fasting has a negative role on PRL secretion. In conclusion our study suggests that NMS has stimulatory role on the secretion of GH and PRL and both these hormones might play an important intermediate role for NMS in the regulation of reproductive axis. Further studies are needed to explore the importance of this relationship between NMS and these pituitary hormones in the regulation of reproductive functions.

Introduction

In mammals, hormone secretion and needs of the organism are precisely balanced in a particular state. Mainly from the different hypothalamic nuclei, releasing or inhibitory factors define the final concentrations of many pituitary hormones in circulatory system (Schibler & Sassone- Corsi, 2002). Higher brain sites with an integrative system control these nuclei. The afferent inputs to these areas of brain may be of hormonal or neural origin. The neural networks, controlling hormone release include feedback loops in which the released signaling molecule directly or indirectly modifies its pattern of secretion (Schibler & Sassone- Corsi, 2002).

Hypothalamus controls a variety of homeostatic processes, such as metabolic control, reproduction, thermoregulation, lactation, cardiovascular function, feeding, drinking, sleep-wake cycle and hormone secretion. Hypothalamus delivers its secretions through the hypophyseal portal system to the anterior pituitary gland. Important hypothalamic nuclei like PVN, SPOA, ARC and MPOA release their stimulatory and inhibitory factors to pituitary gland which in turn regulate the secretions of other endocrine glands (Everitt and Hokfelt, 1990; Bernardis and Bellinger, 1993; Bernardis and Bellinger, 1998).

A complex network of hormonal system is required for spermatogenesis and steroidogenesis, which are normal testicular functions. Like other glands testes are also controlled by secretion of certain hormones. These hormones are the primary regulators while the local paracrine and autocrine chemicals produced by the cellular parts of testes, work to establish the important microenvironment for sperm development. Steroidogenesis, spermatogenesis and testicular functions are controlled by the complex interaction of autocrine, paracrine and endocrine signals (Heindel and Treinen, 1989; Spiteri-Grech and Nieschlag, 1993; Gnessi *et al*., 1997; Abney, 1999; Hull and Harvey, 2000; Roser, 2001; Welt *et al*., 2002; Huleihel and Lunenfeld, 2004; Holdcraft and Braun, 2004a; Petersen and Soder, 2006).

Growth hormone and prolactin belongs to protein family (Niall *et al*., 1971). GH is secreted from anterior pituitary gland and this secretion is mainly stimulated by ghrelin and growth hormone releasing hormone (GHRH), and repressed by somatostatin. GHRH binds to its receptors (GHRH-R) on the adenohypophyseal cells.

These receptors are coupled to G protein. So GHRH activates adenylate cyclase (AC), concequently accumulation of cyclic adenosine-monophosphate (cAMP) occurs. This elevated cAMP causes phosphorylation and hence activation of the transcription factor CREB via protein kinase A (PKA). CREB targets the gene for a pituitary specific transcription factor named as Pit-1. Pit-1 increases the transcription of GH (Mayo *et al*., 1995).

Growth hormone is required for pubertal maturation and sexual differentiation. It is also involved in gametogenesis, gonadal steroidogenesis, and ovulation. During pregnancy GH is also needed for fetal nutrition, growth, development of mammary gland and lactation. These roles reflect the effect of GH on the secretion and action of FSH and LH (Chandrashekar and Bartke, 1998), directly and indirectly through insulin-like growth factor I production. Moreover, production of GH in mammary and gonadal tissues reflects paracrine or autocrine actions of extrapituitary GH. Experimental studies showed that GH affects gonadal differentiation, steroidogenesis, gonadotrophin secretions and gametogenesis (Zachmann, 1992; Franks, 1998).

Compelling evidences suggest that growth hormone (GH) plays an important role in the reproductive process. The presence of GH receptors has been documented in the ovary (Mathews *et al*., 1989; Lobie *et al*., 1990). In male reproductive system, GH receptors are found ubiquitously including Sertoli and Leydig cells, vas deferens, seminal vesicles and prostate gland (Lobie *et al*., 1990). Deficiency of GH in children led to the delayed onset of pubertal development suggesting its significant role in reproduction (Burns *et al*., 1981). GH also plays very important role in testicular development and growth. GH deficiency in human is associated with abnormally small testes. Similarly, pituitary and testicular GH may affect testicular function, including gametogenesis and steroidogenesis (Spiteri-Grech and Nieschlag, 1992).

Prolactin (PRL) is also very important in male reproduction (Bartke, 2004; Hernandez *et al*., 2006). It plays very important role in steroidogenesis and gametogenesis and affects the reproductive system and sexual behaviour (Bartke, 2004). Specific PRL receptors are located on Leydig cells, accessory reproductive glands and membrane of testes (Roux *et al*., 1985; Hernandez *et al*., 2006). PRL also indirectly regulates testosterone secretion by increasing the number and affinity for

LH receptors on the seminiferous tubules (Lincoln *et al*., 2001; Hair *et al*., 2002) and Leydig cells (Regisford and Katz, 1993; Hondo *et al*., 1995; Jedlinska *et al*., 1995; De Rosa *et al*., 2003). LH secretion regulates Leydig cell function and is very important in testosterone production (Huhtaniemi and Toppari, 1995).

PRL receptor expressions on different tissues suggest that this hormone plays an important role in reproduction through various potential targets. These targets include different groups of neurons in anterior pituitary, hypothalamus, reproductive tracts and different types of somatic cells in the gonads (Posner *et al*., 1974; Werther *et al*., 1989; Kelly *et al*., 1991). PRL, stimulates the release of gonadotropins (Bartke *et al*., 1978; Bartke *et al*., 1986), induces fertility in male mice with PRL deficiency and in male golden hamsters with suppressed testicular activity due to seasonal effects (Bartke *et al*., 1978) and potentiates the sex steroid stimulation of both male and female accessory sex glands (Bartke *et al*., 1978; Prins and Lee, 1982; Freeman *et al*., 2000). In the present study it was hypothesized that NMS is possibly involved in the regulation of HPG axis by affecting the secretion of GH and PRL. For this purpose the effect of NMS was observed on GH and prolactin secretion in normal fed and 48-hrs fasting male monkeys.

Materials and Methods

Animals

The animals used in the concerned study were, four adult normal male monkeys (*Macaca mulatta*) of age and weight ranging from 6-8 years and 7-10 kg respectively. All the animals were kept in specific colony environment of primate facility at Department of Animal Sciences, Quaid-i-Azam University Islamabad, Pakistan. The animals were daily provided with feed comprising of fresh fruits, boiled potatoes, eggs and bread at specific times according to their body weights, and water was available *ad libitum*. Prior to the start of experiment, appetite monitoring was carried out for a month. It was observed that all animals used to finish their food within 10-15 min.

Venous Catheterization

A cathy cannula (Silver surgical complex, Karachi, Pakistan; 0.8 mm O.D/22 G×25mm) was affixed in the sephnous vein after anesthesizing the animals with Ketamine HCl (10 mg/kg BW, *im*), to bring about all the chemical administration and sequential blood sampling. A butterfly tubing (24 G×3/4″ diameter and 300 mm length; JMS Singapore) was attached with free end of the cannula. All the sampling was performed after full recovery of animals from sedation.

Pharmacological Reagents

Pharmacological reagents used in the study are listed below:
Heparin (Sinochem Ningbo, China)
Ketamine HCl (Rotexmedica, Trittau, Germany)
Human Neuromedin S (Anaspec, USA)
All the working solutions were prepared in saline solution (0.9% NaCl).

Blood sampling

Blood sampling (2-3 ml) was conducted, at regular intervals of 15 min, using heparinized syringes. An equivalent quantity of heparinized (5 IU/ml) saline was injected after each sample withdrawal. Samples were collected 60 min before and 120 min after NMS/saline administration. The time of NMS (50 nmol) administration

was considered as 0 min. All blood samples were obtained between 1100-1500 hrs. All experiments were performed in a couple of weeks in order to reduce the alterations in hormonal levels associated with seasonal changes. Samples were centrifuged for 10 min at 3000 rpm, and then plasma was pipetted out and stored at -20˚C until analyzed.

Analysis of hormones

GH and PRL concentrations were quantitatively determined by using EIA kits (Amgenix Inc. USA). The minimum detectable limit for both GH and PRL levels was 0.05 ng/ml; intra-assay and inter-assay coefficients of variation were <8%. All the procedures of EIA were followed as provided with the kits.

Statistical analysis

All the data were presented as mean±SEM. GH and PRL concentrations after NMS and saline administration were compared by one-way ANOVA followed by post hoc Dunnett's multiple comparisons test. Student's t test was employed to compare mean pre- and post-treatment GH and PRL concentrations, under 48-hrs fasting and normal fed conditions.

Statistical significance was set at $P \leq 0.05$. All the data were analyzed by using statistical software GraphPad Prism version 5.

Results

Basal plasma GH concentrations in fed and fasting conditions

Basal plasma concentrations of GH (ng/ml) during 1-hr before saline administration in fed and 48-hrs fasting animals are given in Fig. 2.1A-2.1B. 48-hrs fasting did not cause any significant change ($P>0.05$) in basal plasma GH levels.

Effect of NMS on plasma GH secretion in normal fed adult male monkeys

The individual and mean plasma GH concentrations (ng/ml) before and after saline/NMS administration in normal fed monkeys are given in table 2.1-2.2 and Fig. 2.2A. After 45 min of NMS injection GH concentrations significantly ($P<0.05$) increased compared to 0 min sample. Maximum levels of GH concentrations ($P<0.001$) were observed at 90 min of NMS injection compared to 0 min sample (Fig. 2.2B). Comparison between pre- and post-treatment also showed a significant ($P<0.01$) increase in GH levels after NMS administration (Fig. 2.3).

Effect of NMS on plasma GH secretion in 48-hrs fasting adult male monkeys

The individual and mean plasma GH concentrations (ng/ml) before and after saline/NMS administration in 48-hrs fasting monkeys are given in table 2.3-2.4 and Fig. 2.4A. NMS treatment in 48-hrs fasting monkeys significantly ($P<0.01$) increased GH concentrations after 60 min of NMS injection. Maximum levels of GH concentrations ($P<0.001$) were observed at 90 min of NMS injection compared to 0 min sample (Fig. 2.4B). Comparison between pre- and post-treatment also showed a significant ($P<0.01$) increase in GH levels after NMS administration (Fig. 2.5).

Basal plasma PRL concentrations in fed and fasting conditions

Basal plasma concentrations of PRL (ng/ml) during 1-hr before saline administration in fed and 48-hrs fasting animals are given in Fig. 2.6A-2.6B. Basal PRL concentrations significantly ($P<0.001$) decreased in 48-hrs fasting compared to normal fed adult male monkeys.

Effect of NMS on plasma PRL secretion in normal fed adult male monkeys

The individual and mean plasma PRL concentrations (ng/ml) before and after

saline/NMS administration are given in table 2.5-2.6 and Fig. 2.7A. After 45 min of NMS injection PRL concentrations significantly ($P<0.05$) increased compared to 0 min sample. Maximum levels of PRL concentrations ($P<0.01$) were observed at 60 min of NMS injection compared to 0 min sample (Fig. 2.7B). Comparison between pre- and post-treatment also showed a significant ($P<0.05$) increase in PRL levels after NMS administration (Fig. 2.8).

Effect of NMS on plasma PRL secretion in 48-hrs fasting adult male monkeys

The individual and mean plasma PRL concentrations (ng/ml) before and after saline/NMS administration are given in table 2.7-2.8 and Fig. 2.9A. No significant ($P>0.05$) change was observed after NMS treatment in 48-hrs fasting monkeys compared to 0 min sample (Fig. 2.9B). Comparison between pre- and post-treatment did not show any significant ($P>0.05$) change in PRL levels after NMS administration (Fig. 2.10).

Table 2.1. Individual and mean (±SEM) plasma GH concentrations (ng/ml) before and after saline administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	1.79	1.51	1.99	1.20	1.62±0.17
-45	1.90	1.41	2.08	1.05	1.61±0.23
-30	1.98	1.29	1.96	1.17	1.60±0.22
-15	1.94	1.34	2.04	1.10	1.61±0.23
0	1.88	1.43	2.19	1.23	1.68±0.22
15	2.03	1.37	2.43	1.31	1.79±0.27
30	2.17	1.63	2.52	1.72	2.01±0.21
45	2.46	1.82	2.46	1.83	2.14±0.18
60	2.36	1.88	2.12	1.74	2.03±0.14
75	2.17	2.57	1.84	1.65	2.06±0.20
90	2.11	2.44	2.01	1.47	2.01±0.20
105	2.29	2.74	2.68	1.53	2.31±0.28
120	2.13	2.69	2.01	1.29	2.03±0.29

Table 2.2. Individual and mean (±SEM) plasma GH concentrations (ng/ml) before and after NMS administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	1.19	1.67	1.12	1.69	1.42±0.15
-45	1.29	1.77	1.18	1.65	1.47±0.14
-30	1.21	1.85	1.09	1.76	1.48±0.19
-15	1.30	1.94	1.13	1.82	1.55±0.20
0	1.17	1.85	1.29	1.97	1.57±0.20
15	1.25	2.09	1.81	2.03	1.80±0.19
30	1.51	2.84	2.42	2.80	2.39±0.31
45	2.13	3.21	3.08	3.92	3.09±0.37*
60	2.87	4.15	3.94	5.09	4.01±0.46***
75	3.26	4.67	4.74	5.43	4.53±0.46***
90	4.28	4.96	5.37	6.11	5.18±0.38***
105	4.03	4.89	4.64	5.84	4.85±0.38***
120	4.02	4.53	4.12	4.35	4.26±0.11***

***P<0.05, ***P<0.001 vs 0 min (ANOVA followed by post hoc Dunnett's test).**

Table 2.3. Individual and mean (±SEM) plasma GH concentrations (ng/ml) before and after saline administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	1.41	1.19	1.20	1.62	1.36±0.10
-45	1.37	1.28	1.31	1.72	1.42±0.10
-30	1.32	1.36	1.39	1.79	1.47±0.11
-15	1.44	1.26	1.46	1.87	1.51±0.13
0	1.76	1.44	1.54	2.19	1.73±0.17
15	1.82	1.22	1.48	2.02	1.64±0.18
30	1.96	1.39	1.45	2.22	1.76±0.20
45	2.09	1.52	1.49	2.18	1.82±0.18
60	2.56	1.64	1.62	2.45	2.07±0.25
75	2.59	1.76	1.52	2.73	2.15±0.30
90	2.82	1.88	1.64	2.76	2.28±0.30
105	2.95	1.61	1.88	2.81	2.31±0.33
120	2.26	1.52	1.66	2.08	1.88±0.17

Table 2.4. Individual and mean (±SEM) plasma GH concentrations (ng/ml) before and after NMS administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	1.35	1.75	1.27	1.55	1.48±0.11
-45	1.45	1.72	1.35	1.61	1.53±0.08
-30	1.29	1.59	1.29	1.69	1.47±0.10
-15	1.26	1.68	1.37	1.81	1.53±0.13
0	1.37	1.54	1.51	2.01	1.61±0.14
15	1.49	1.79	1.58	2.88	1.94±0.32
30	2.20	2.75	1.72	3.39	2.52±0.36
45	2.43	2.61	1.95	3.89	2.72±0.41
60	3.14	3.17	2.75	4.26	3.33±0.32**
75	4.05	3.53	4.11	5.23	4.23±0.36***
90	4.33	4.26	4.74	5.29	4.66±0.24***
105	3.25	3.73	4.02	4.46	3.87±0.25***
120	3.13	3.93	3.61	4.19	3.72±0.23***

****P<0.01, ***P<0.001 with 0 min sample (ANOVA followed by post hoc Dunnett's test).**

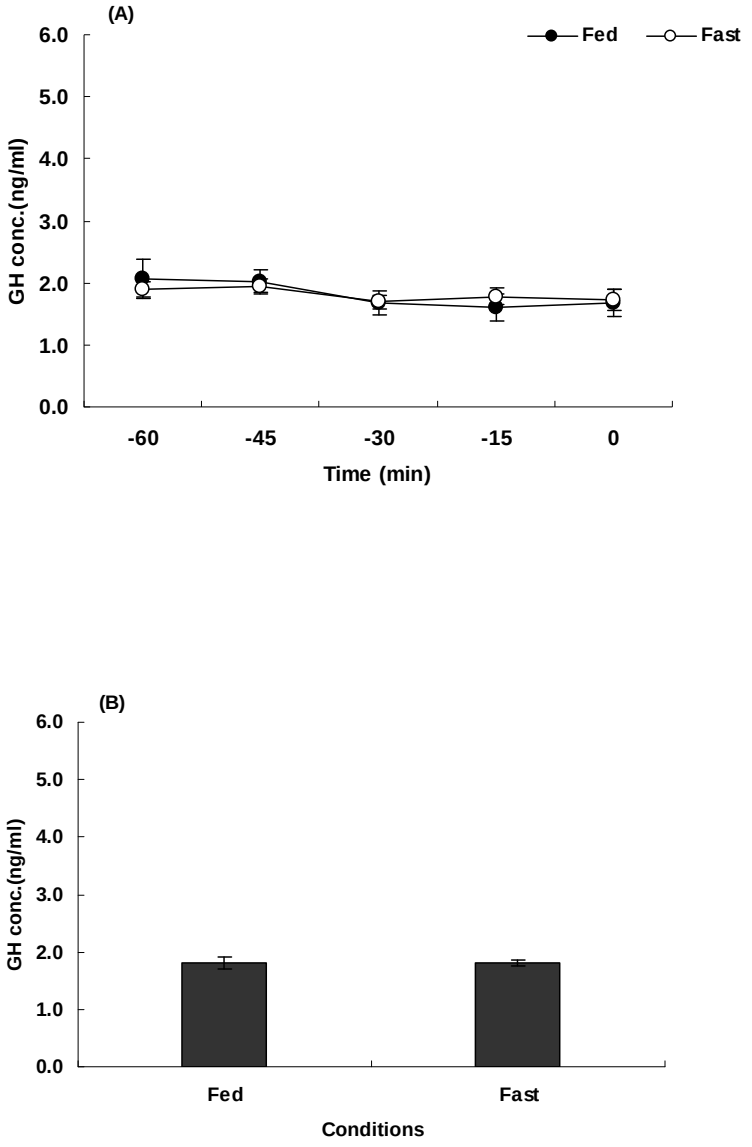


Figure 2.1. (A) Changes in mean (±SEM) basal plasma GH concentrations (ng/ml) in 1-hr period in fed and 48-hrs fasting adult male monkeys (**B**) Overall mean (±SEM) basal plasma GH concentrations (ng/ml) in 1-hr period in normal fed, and 48-hrs fasting adult male monkeys. P>0.05 vs fed (Student's t test).

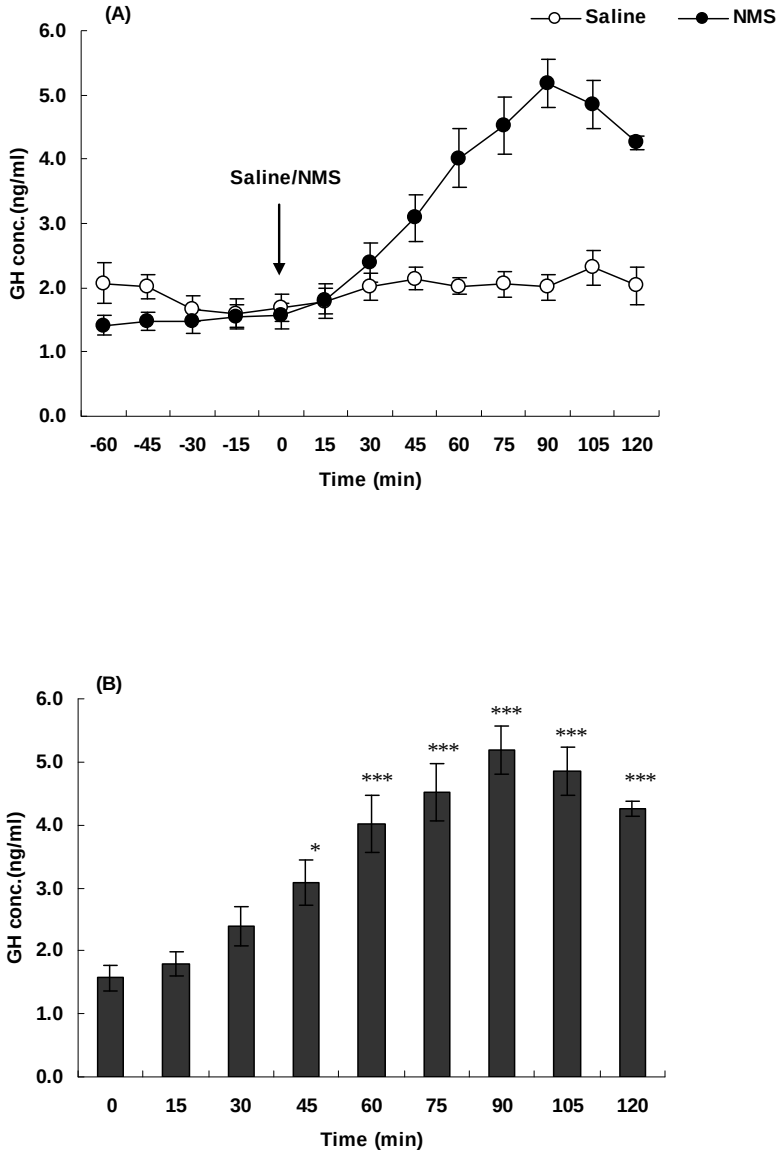


Figure. 2.2. (A) Mean (±SEM) changes in plasma GH levels (ng/ml) before and after saline/NMS administration (at 0 min) in fed adult male monkeys. **(B)** Comparison of mean (±SEM) GH concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in fed monkeys. *P<0.05, ***P<0.001 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).

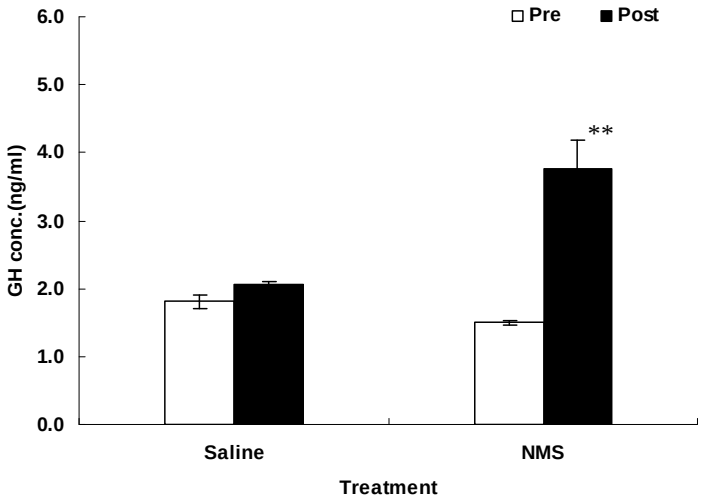


Figure. 2.3. Comparison of mean (±SEM) plasma GH levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in fed adult male monkeys. **P<0.01 vs pre-treatment (Student's t test).

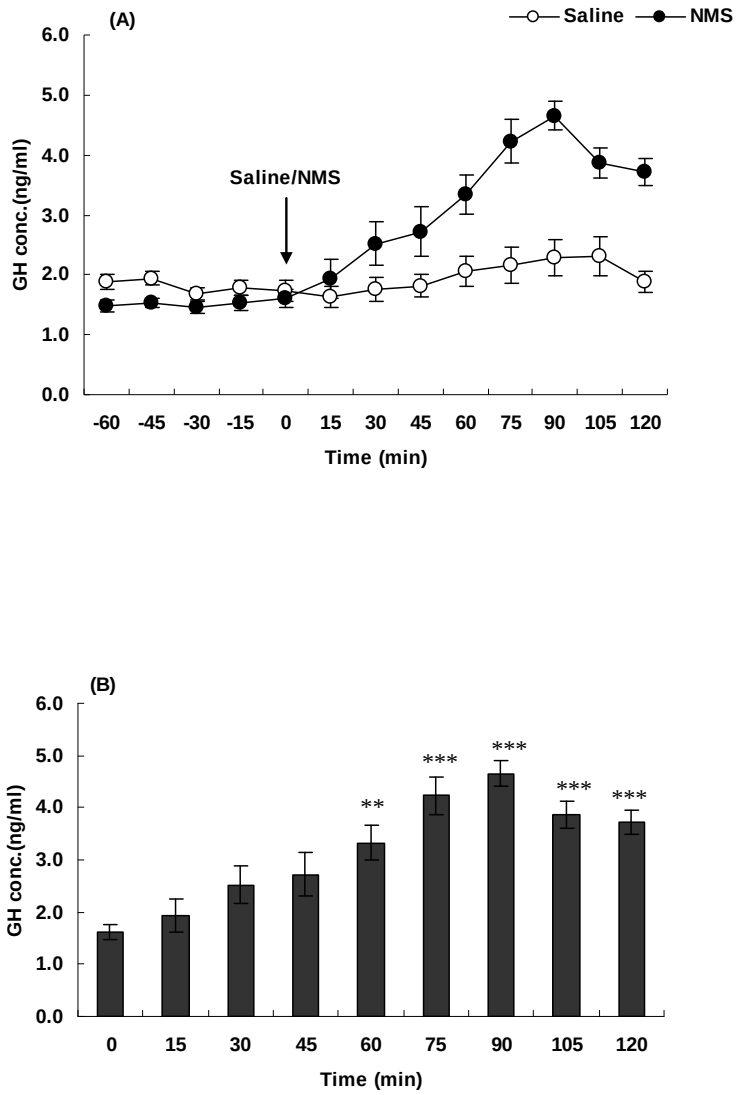


Figure. 2.4. (A) Mean (±SEM) changes in plasma GH levels (ng/ml) before and after saline/NMS administration (at 0 min) in 48-hrs fasting adult male monkeys. **(B)** Comparison of mean (±SEM) GH concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in 48-hrs fasting monkeys. **P<0.01, ***P<0.001 with 0 min sample (ANOVA followed by post hoc Dunnett's test).

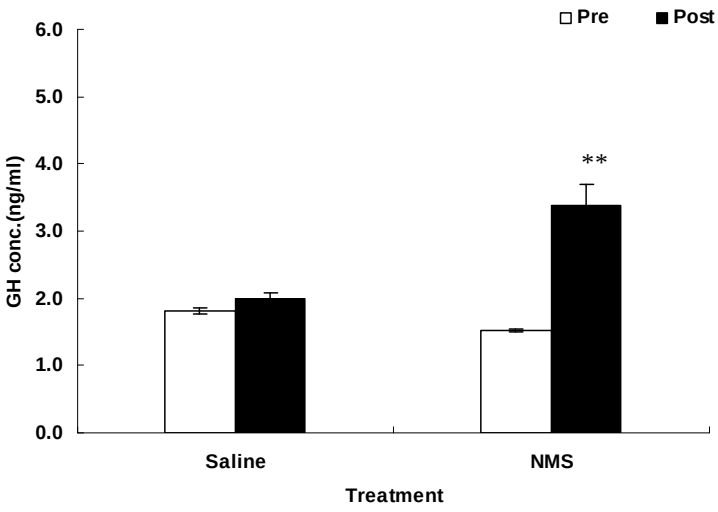


Figure. 2.5. Comparison of mean (±SEM) plasma GH levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in 48-hrs fasting adult male monkeys. **P<0.01 vs pre-treatment (Student's t test).

Table 2.5. Individual and mean (±SEM) plasma PRL concentrations (ng/ml) before and after saline administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	16.39	13.74	15.33	14.32	14.95±0.58
-45	15.43	14.69	15.34	15.09	15.14±0.17
-30	17.39	14.13	14.53	15.78	15.46±0.73
-15	14.83	15.62	15.59	15.54	15.40±0.19
0	15.14	16.18	15.95	16.09	15.84±0.24
15	15.92	14.78	14.99	14.33	15.01±0.33
30	15.73	13.89	15.38	14.92	14.98±0.40
45	16.74	13.34	16.14	15.58	15.45±0.74
60	15.19	13.37	16.79	15.96	15.33±0.73
75	17.38	14.06	16.30	16.52	16.07±0.71
90	16.95	13.48	15.89	15.39	15.43±0.73
105	17.71	13.99	16.02	15.33	15.76±0.77
120	18.21	14.16	16.48	16.08	16.23±0.83

Table 2.6. Individual and mean (±SEM) plasma PRL concentrations (ng/ml) before and after NMS administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	16.06	14.74	15.85	15.93	15.65±0.30
-45	16.92	14.81	16.26	16.29	16.07±0.45
-30	17.89	15.24	17.16	16.25	16.64±0.57
-15	18.19	14.89	18.49	16.92	17.12±0.82
0	18.14	15.77	18.56	17.24	17.43±0.62
15	17.84	15.85	19.10	17.59	17.60±0.67
30	18.93	16.23	20.04	18.95	18.54±0.81
45	19.37	18.43	20.52	20.38	19.68±0.49*
60	21.24	19.93	20.82	18.33	20.08±0.64**
75	18.42	17.05	17.49	16.45	17.35±0.41
90	16.38	15.71	17.16	16.16	16.35±0.30
105	15.63	15.10	16.13	15.37	15.56±0.22
120	16.72	15.87	15.99	15.53	16.03±0.25

***P<0.05, **P<0.001 vs 0 min sample (ANOVA followed by post hoc Dunnett's test)**

Table 2.7. Individual and mean (±SEM) plasma PRL concentrations (ng/ml) before and after saline administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	8.57	9.65	7.49	8.21	8.48±0.45
-45	8.11	9.55	7.12	8.72	8.38±0.51
-30	8.19	9.23	7.18	8.51	8.28±0.43
-15	8.58	9.44	6.99	8.93	8.49±0.53
0	8.33	9.52	7.62	8.69	8.54±0.40
15	8.58	9.03	7.91	8.14	8.42±0.25
30	8.39	8.81	7.65	7.89	8.19±0.26
45	8.65	9.23	7.93	8.16	8.49±0.29
60	8.23	8.87	7.39	8.5	8.25±0.31
75	9.02	9.18	7.98	8.77	8.74±0.27
90	7.97	8.67	7.59	8.53	8.19±0.25
105	8.31	8.54	7.18	7.24	7.82±0.35
120	8.45	9.2	7.32	6.95	7.98±0.52

Table 2.8. Individual and mean (±SEM) plasma PRL concentrations (ng/ml) before and after NMS administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	8.99	9.28	7.1	8.39	8.44±0.48
-45	8.94	9.88	7.43	8.57	8.71±0.51
-30	9.31	9.67	7.89	8.74	8.90±0.39
-15	9.21	8.96	7.61	9.04	8.71±0.37
0	9.71	8.63	7.85	8.49	8.67±0.39
15	9.62	8.12	7.31	8.47	8.38±0.48
30	10.54	9.19	7.62	9.07	9.11±0.60
45	10.74	9.75	7.54	9.36	9.35±0.67
60	10.98	10.04	8.37	9.42	9.70±0.55
75	9.18	9.81	8.23	9.97	9.30±0.39
90	8.73	9.27	7.89	9.29	8.80±0.33
105	8.48	9.09	7.49	8.86	8.48±0.35
120	8.67	9.01	7.18	8.97	8.46±0.43

P>0.05 vs 0 min sample (ANOVA followed by post hoc Dunnett's multiple test)

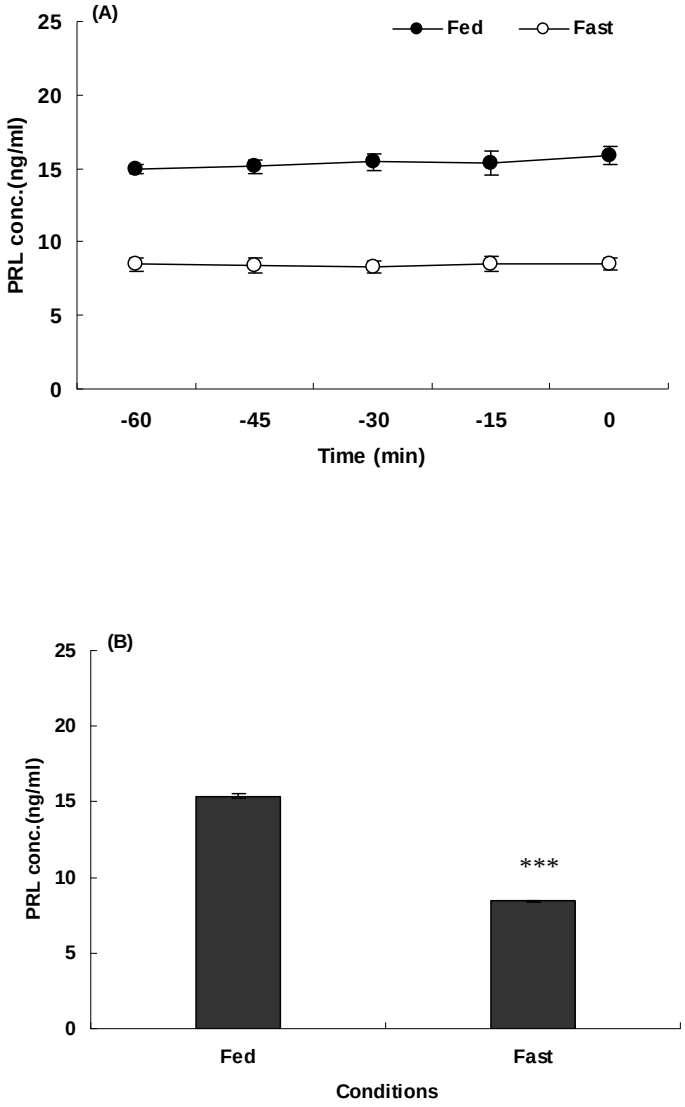


Figure. 2.6. (A) Changes in mean (±SEM) basal plasma PRL concentrations (ng/ml) in 1-hr period in fed and 48-hrs fasting adult male monkeys (**B**) Overall mean (±SEM) basal plasma PRL concentrations in 1-h period in normal fed, and 48 hrs fasting adult male monkeys. ***P< 0.001 vs fed (Student's t test).

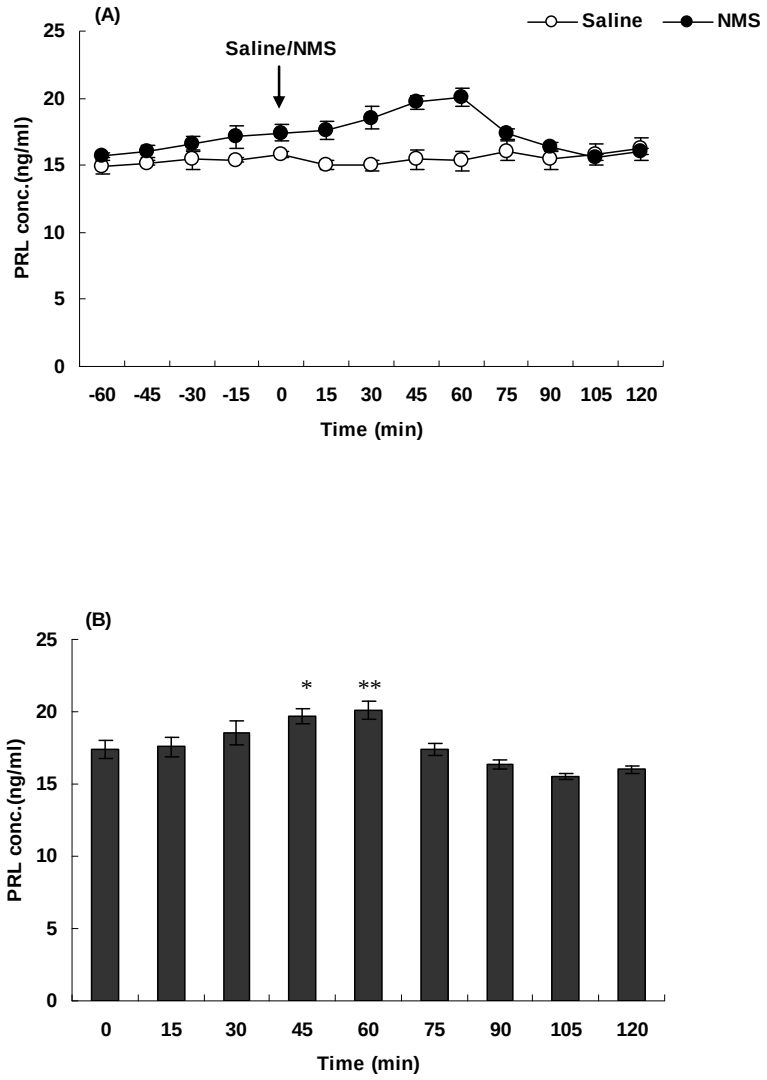


Figure. 2.7. (A) Mean (±SEM) changes in plasma PRL levels (ng/ml) before and after saline/NMS administration (at 0 min) in normal fed adult male monkeys. **(B)** Comparison of mean (±SEM) PRL concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in fed monkeys. *P<0.05, **P<0.01 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).

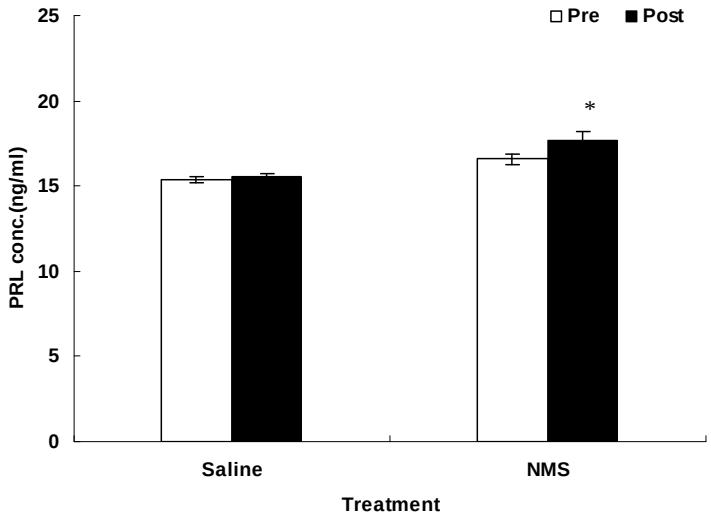


Figure. 2.8. Comparison of mean (±SEM) plasma PRL levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in normal fed adult male monkeys. *P<0.01 vs pre-treatment (Student's t test).

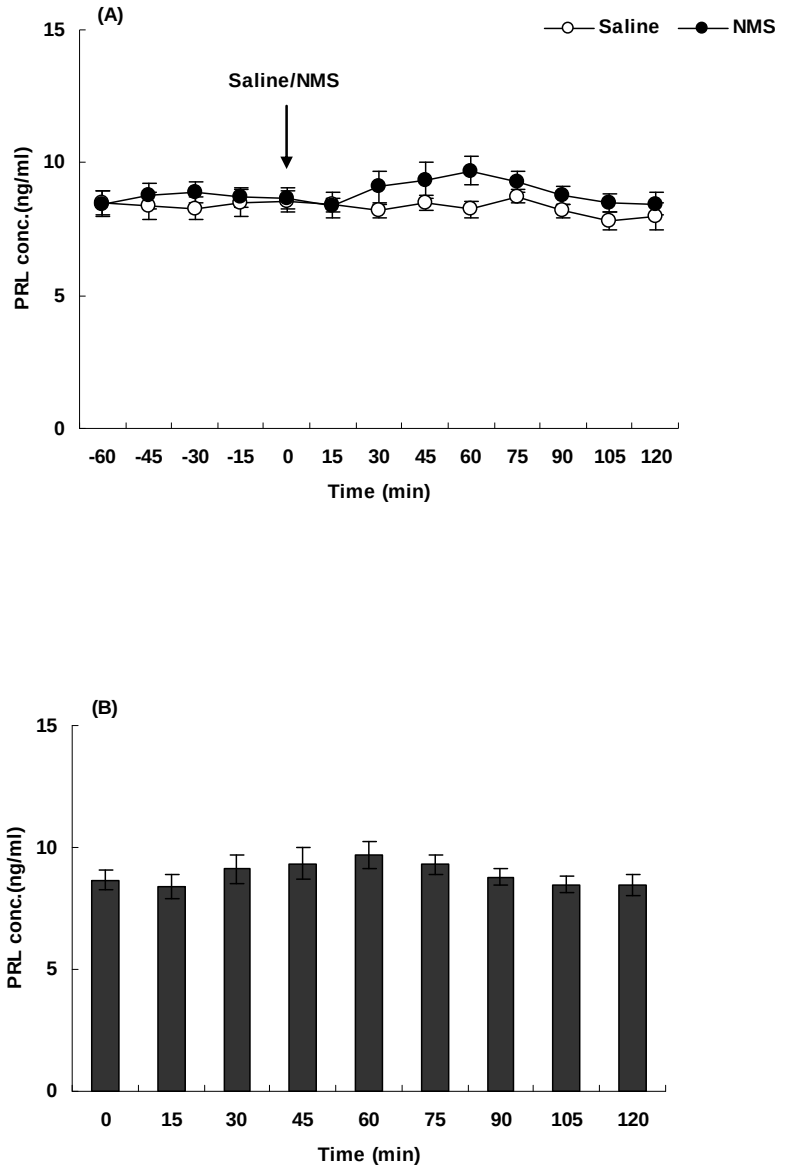


Figure. 2.9. (A) Mean (±SEM) changes in plasma PRL levels (ng/ml) before and after saline/NMS administration (at 0 min) in 48-hrs fasting adult male monkeys. **(B)** Comparison of mean (±SEM) PRL concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in 48-hrs fasting monkeys. P>0.05 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).

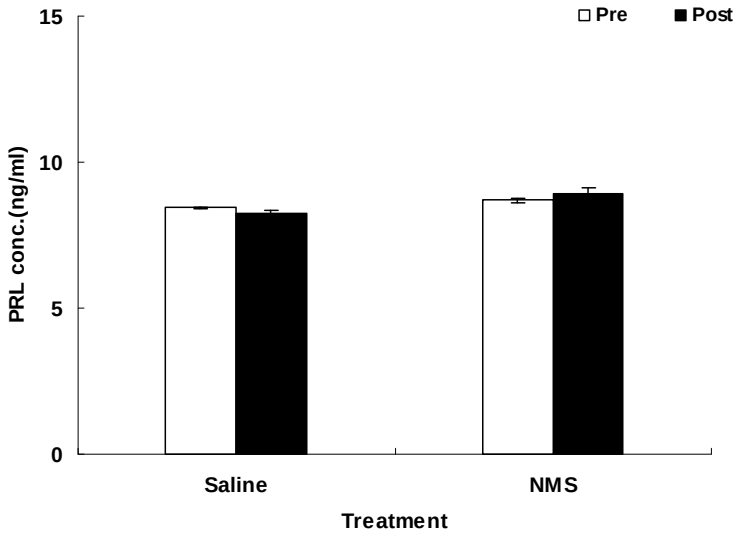


Figure. 2.10. Comparison of mean (±SEM) plasma PRL levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in 48-hrs fasting adult male monkeys. P>0.05 vs pre-treatment (Student's t test).

Discussion

In the present study, the role of peripheral administration of NMS on two pituitary hormones i.e. GH and PRL was studied. Both these hormones are considered very important in regulation of reproductive functions. It was hypothesized that NMS might be playing its stimulatory role in HPG axis through stimulation of the secretion of these two pituitary hormones. For this purpose effect of peripheral administration of NMS on GH and PRL secretion was investigated in normal fed and 48-hrs fasting monkeys.

Growth hormone (GH) plays very important role in autocrine/paracrine and endocrine regulation of reproduction. It is involved in the control of growth, differentiation, proliferation, apoptosis and the secretory activities of reproductive organs. It also regulates the response of reproductive structures to GnRH and gonadotropins (Sirotkin, 2005). GH and its receptors are present in large number of tissues and cells including pituitary, uterus, mammary gland, placenta, Leydig cells, granulosa cells, theca cells, cumulus cells of oocyte and many other reproductive and non-reproductive tissues (Hull and Harvey, 2000a,b, 2001; Kaiser *et al*., 2001; Marchal *et al*., 2003).

In our study 48-hrs fasting did not cause any significant effect on basal GH concentrations and its levels were remained constant in both fed and metabolically stressed animals. GH plays an important role in regulation of metabolic activities during fasting conditions (Norrelund, 2005; Moller and Jorgensen, 2009) but there are discrepancies in GH release in fasting periods in different animals. Among two groups of healthy human adult males, 24-hrs fasting induced a significant rise in GH levels in one group while in second group GH levels remained same to the initial pre fasting values (Alkén *et al*., 2008). Similar results were also observed in young healthy human females (Beer *et al*., 1989). Several other studies also showed that upto 2.5 days fasting did not cause significant change and the GH levels remained same in adult human females (Bergendahl *et al*., 1999; Norrelund *et al*., 2001; Darzy *et al*., 2006; Sakharova *et al*., 2008). Thissen *et al*, found negative effect of fasting on GH secretion in men (Thissen *et al*., 1994). In rats 24-hrs fasting did not effect GH levels but five days fasting caused significant decrease in GH secretion (Ohashi *et al*., 1995).

In our study 48-hrs fasting caused no effect (P>0.05) on GH secretion in rhesus monkeys. On the basis of these findings it is very difficult to suggest the exact role of fasting on GH secretion but it is more logical to say that species difference and periods of fastings employed, might have contributed in these different responses.

Peripheral admistration of NMS significantly increased (P<0.01) GH concentrations in both fed and 48-hrs fasting adult male monkeys suggesting that irrespective of the metabolic status of animals NMS stimulated GH secretion. The possible mechanism involved in the regulation of GH by NMS, is through the α-MSH and β-END from POMC in ARC. Both α-MSH and β-END are the products of the POMC gene (Smith and Funder, 1988). These POMC products stimulate the release of GHRH from hypothalamus. It was shown by Dupont and colleagues that 2 μg and higher dose of β-END resulted in a significant stimulation of plasma GH release from 6 to 10 and 20 to 30-fold respectively (Dupont *et al*., 1976). Another study (Bricaire *et al*., 1973) showed that α-MSH induced GH release in 18 among 23 normal males. Similarly, a significant rise in GH secretion by α-MSH administration in children suffering from hypopituitarism was observed (Bernasconi *et al.,* 1975). NMS expression at the SCN, PVN within the brain (Mori *et al.*, 2005; Ida *et al.*, 2005) may regulate the POMC mRNA expression at ARC. NMS *icv* administration led to the augmentation of POMC mRNA levels in the ARC and elevated expression of c-Foss in ARC POMC neurons (Mori *et al*., 2005). These outcomes propose the involvement of α-MSH in NMS regulated feeding behaviour and pituitary hormones regulation.

Prolactin (PRL) has a wide range of actions in male reproductive functions. It has been shown to cause the induction of proliferation and differentiation of Leydig cells in prepubertal hypophysectomized rats (Dombrowicz *et al*., 1992) and the maintenance of cell morphology of these cells, potentiation of LH induced steroidogenesis in hypophysectomized rats and upregulation of LHR expression (Zipf *et al.,* 1978; Purvis *et al*., 1979; Dombrowicz *et al*., 1992; Bole-Feysot *et al*., 1998; Manna *et al.,* 2001).

In our study, the plasma PRL levels were significantly (P<0.001) lowered after 48-hrs fasting is in accordance with the preexisting literature, where fasting also put the suppressive effect on PRL secretion in different animals (Sirek *et al.,* 1976;

Campbell *et al.,* 1977; Dyer *et al*., 1985; Bergendahl *et al.,* 1991; Xie, 1991). In the present study, as a novel finding it was found that NMS positively regulates plasma PRL concentration ($P<0.05$) in fed monkeys but did not show such stimulatory effect in 48-hrs fasting monkeys. Hypothalamus might be the possible site where NMS may act to effect the stimulation of PRL release. Increase in PRL releasing hormones like VIP or decrease in release of its inhibitory chemicals like dopamine may effect PRL secretion (Ben-Jonathan *et al*., 1989). As ARC in the hypothalamus shows large number of NMS receptors (Mori *et al.,* 2005) so it may be suggested that certain peptides released by ARC may modulate the PRL secretion (Kalra *et al*., 1999) under the effect of NMS. A large number of neurons are present in ARC nucleus, produce β-END, α-MSH and many other nutrition regulated factors (Kalra *et al*., 1999). Pro-opiomelanocortin (POMC) gene which is responsible for the production of β-END and α-MSH (Smith and Funder, 1988), is cleaved to synthesize many hormones like ACTH, lipotropin, α-MSH, corticotrophin-like intermediate peptide (CLIP) and β-END which may act as important link between the metabolic condition of an individual and its reproductive functions (Bergendahl *et al*., 1992; Schwartz *et al*., 1997; Mizuno *et al.*, 1998; Koegler *et al.*, 2001). It is evident that NMS stimulate the expression of the POMC genes in ARC (Mori *et al*., 2005). NMS administration (*icv*) increased the expression of POMC mRNA in the ARC and stimulated expression of c-Fos in POMC neurons (Miyazato *et al*., 2008). Both α-MSH (Hill *et al*., 1993; Hill *et al*., 1991; Nunez and Frawley, 1998) and β-END (Selmanoff and Gregerson, 1986; Kehoe *et al*., 1993) are considered as excitatory in action on PRL secretion. So it seems convincible that NMS plays stimulatory role in PRL secretion by these two products of POMC gene.

Dopamine (DA) released from hypothalamic neurons inhibits PRL secretion (Freeman *et al.*, 2000). This DA comes from three discrete neuronal populations from the pituitary gland: the tuberohypophyseal dopaminergic (TIDA) neurons that arise from the rostral ARC and project to intermediate and neural lobes of the pituitary (Holzbauer and Racke, 1985), the TIDA neurons that are located in the dorsomedial (DM) portion of arcuate (ARC) nucleus and project to the median eminence (ME) (Kawano and Daikoku, 1987), and the periventricular hypophyseal dopaminergic neurons that arise in the periventricular (Pe) nucleus and innervate exclusively the

intermediate lobe (Goudreau *et al*., 1995). Taken together, this data provide compelling evidence supporting a role for ARC neurons in the regulation of hypothalamic DA and thereby PRL secretion.

Another possible mediator of this NMS stimulated PRL release is oxytocin. It was observed that in ovariectomized (OVX) rats with addition to oxytocin antagonist daily stimulatory rhythm of PRL secretion blocked, suggesting that oxytocin has some stimulatory role on PRL secretion (Arey and Freeman, 1989, 1992). Later on, it was confirmed by further experiments that oxytocin has a definite role in regulation of PRL secretion (Egli *et al.,* 2004; Bertram *et al*., 2006). It was also demonstrated that *icv* injection of NMS increased the plasma oxytocin concentration significantly within 5 min in dose dependent manner. It was established that NMS may mimic the suckling-induced oxytocin release (Sakamoto *et al*., 2008). SCN neurons seem to be affecting the release of both DA and oxytocin. From the SCN, VIP fibers originate and lead to DA neurons in ARC (Gerhold *et al.,* 2001), and a previous study suggested that oxytocin neurons are also innervated by VIP fibers in the PVN (Egli *et al*. 2004).

GnRH was the first candidate for paracrine modulation of PRL release from lactotrophs (Denef and Andries., 1983). GnRH only stimulate prolactin secretion when lactotrophs and gonadotrophs are cocultured (Denef and Andries, 1983), pointing towards the possibility of involvement of other gonadotroph-related products in stimulation of prolactin secretion. GnRH has been reported to release prolactin in monkeys, in vivo (Geisthoevel *et al*., 1988; Olive *et al.,* 1989) and in women during the menstrual cycle (Casper and Yen, 1981, Yen *et al*., 1980). Now it is evident that NMS plays a significant role in stimulation of GnRH so it is more likely to say that NMS induced PRL secretion was due to the stimulation of pituitary by GnRH.

In summary, our results suggested that NMS is a presumptive regulator of pituitary hormones like GH and PRL. So, it is plausible that NMS might play its positive role in HPG regulation through the stimulation of pituitary hormones like GH and PRL. Various pathways may be considered as suitable candidates for this regulation but it is very difficult to confirm the exact pathway of NMS action in this regard. Further studies are required to confirm the exact mechanism of this regulation.

Chapter # 3

A study on the role of Neuromedin S on Adipokines secretion under normal and metabolically stressed conditions in adult male rhesus monkeys (*Macaca mulatta*)

Abstract

Background: Adipokines are known as important adiposity signals and play certain roles in various biological processes. Now their involvement in the regulation of HPG axis has also been established. In the present study, we investigated the role of peripheral administration of NMS on adipokines (adiponectin, leptin and resistin) secretion in 48-hrs fasting and normal fed adult male monkeys. For this purpose after NMS administration plasma adiponectin, leptin and resistin levels were determined in normal fed and 48-hrs fasting monkeys.

Materials and Methods: Four intact adult male rhesus monkeys (6-8 yr Age: 7-10 kg BW) were used in this study. 50 nmol of NMS was injected through a cannula affixed in saphenous vein. Blood samples were collected individually 60 min before and 120 min at 15 min intervals, after NMS/saline administration. The plasma adiponectin, leptin and resistin concentrations were determined by using specific Enzyme Immunoassay (EIA) kits.

Results: 48 hrs fasting significantly increased plasma adiponectin ($P<0.001$), while decreased leptin ($P<0.001$) and resistin ($P<0.01$) concentrations compared to normal fed monkeys. No significant ($P>0.05$) change in adiponectin levels was observed after NMS/saline injection in both normal and metabolically stressed conditions. NMS administration induced a significant ($P<0.01$) increase in resistin levels, while suppressed leptin ($P<0.05$) secretion in both fed and 48-hrs fasting conditions compared to saline treated animals.

Conclusion: In conclusion our study suggested that NMS has a definite role in regulation of adipokines secretion. Its inhibitory effect on leptin and stimulatory effect on resistin shows an important relationship between NMS and adipokines in the regulation of reproductive axis in male rhesus monkeys. To best of our knowledge this is the very first study regarding the role of NMS on adipokines secretion in male monkeys. Therefore, further studies are recommended to confirm the exact mechanism of this regulation and its importance in male reproduction.

Introduction

Adipokines, a group of bioactive peptides, are released from adipose tissue and play an important role in variety of biological processes (Fischer-Posovszky *et al.*, 2007). Resistin, leptin and adiponectin are important regulators of metabolism and energy homeostasis. Leptin acts as a surfeit factor and its concentrations are positively related to body fat mass. Adiponectin is most abundantly present in blood circulation and negatively related with body fat mass. Adiponectin enhances sensitivity of insulin while resistin and leptin reduce it (Ahima and Lazar, 2008). Adipokines play a very important role in regulation of reproductive axis. Adiponectin attenuates while Leptin augments the release of main reproductive hormones (Lado-Abeal *et al.*, 2000; Smith *et al.*, 2006; Fischer-Posovszky *et al.*, 2007; Rodriguez-Pacheco *et al.*, 2007; Caminos *et al.*, 2008) but the effect of resistin in regulation of reproduction is not clearly understood.

Adipokines released from adipose tissue act via a network of endocrine, paracrine and autocrine pathways, and playing very important role in variety of physiological aspects, such as cardiovascular functions, lipid and glucose metabolism, immunity, neuroendocrine function and reproduction (Shankar *et al.*, 2010; Pataky *et al.*, 2010). Number of changes appears in adipokine concentrations due to energy imbalance (Arita *et al.*, 1999; Hotta *et al.*, 2000; Kadowaki and Yamauchi, 2005; Fischer-Posovszky *et al.*, 2007; Ahima and Lazar, 2008; Guevara *et al.*, 2008). Adiponectin levels increase while leptin and resistin levels decrease during short term metabolic deficiencies (Rajala *et al.*, 2004; Kadowaki and Yamauchi, 2005; Guevara *et al.*, 2008).

Adiponectin, is basically involved in regulation of insulin sensitivity (Kershaw, 2004). A large number of adiponectin receptors are expressed on testicular Leydig cells, the major source of the testosterone (T), an important element of male reproductive functions (Caminos *et al.*, 2008). It was observed that testosterone therapy decreased adiponectin concentrations and similarly high adiponectin levels decreased T concentrations in rats (Page *et al.*, 2005). It was also suggested that adiponectin is directly involved in HPA axis, by regulating the secretion of hypothalamic and pituitary hormones (Qi *et al.*, 2004; Rodriguez-Pacheco *et al.*, 2007; Iwama *et al.*, 2009).

Leptin has been considered as a major factor, which links the metabolic status to reproduction (Barash *et al*., 1996). Hypothalamus looks to be the major area of the leptin activity in HPG axis regulation (Lin *et al*., 2001; Williams *et al*., 2002). Leptin receptor mRNA is observed in the hypothalamic region might be playing an important role in regulation of feeding and reproductive functions (Magni *et al*., 2000; Barb and Kraeling, 2004). In metabolically stressed ovariectomized (OVX) ewes central administration of leptin restored LH levels but it was failed to do so in normal fed OVX ewes (Henry *et al*., 1999, 2001). Conversely *icv* administration of leptin did not cause any significant change in LH secretion in food restricted OVX sheep, while in normal fed intact sheep it decreased LH levels in circulation (Blache *et al*., 2000; Morrison *et al*., 2001). In sterile ob/ob mice, the sterility defect was overcome with increase in LH concentrations after leptin infusions (Barash *et al*., 1996; Chehab *et al*., 1996; Mounzih *et al*., 1997; Cleary *et al*., 2001). In rat hCG treated Leydig (Caprio *et al*., 1999) cells and testicular slices (Tena-Sempere *et al*., 1999, 2000) leptin suppressed testosterone levels indicating that it plays an inhibitory role in androgen secretion. In contrast to these findings leptin did not affect steroidogenesis in mice and primates (Banks *et al*., 1999; Lado-Abeal *et al*., 1999).

Resistin, expressing in rat adipose tissue, was considered to be a key factor in impairment of insulin sensitivity (Steppan *et al*., 2001a). Barrett-Connor, (1992) suggested that low plasma T levels are frequently associated with insulin resistance although exact mechanism of this alteration is unclear but as this effect is reversed after losing weight, signaling a link to dysfunctioning of adipocytes (Kopelman, 1992). In rat testes 48-hrs fasting significantly reduced resistin mRNA expressions (Nogueiras *et al*., 2004). In an in vitro study in rat testes, different doses of resistin were seen to enhance T concentrations in both basal and hCG induced conditions (Nogueiras *et al*., 2004).

NMS an anorexigenic neuropeptide expressing in SCN of hypothalamus is involved in the regulation of HPA axis (Jászberényi *et al*., 2007) and HPG axis (Vigo *et al*., 2007). On the basis of above evidences that adipokines play a very important role in the regulation of HPG axis, in this study it was hypothesized that NMS might be involved in controlling the secretion of adipokines from adipocytes. For this purpose in the present study, the effect of peripheral NMS injection was investigated on the secretion of leptin, resistin and adiponectin in normal fed and 48-hrs fasting adult

male rhesus monkeys. To best of our knowledge it was the first study regarding NMS role on adipokines secretion.

Materials and Methods

Animals

The animals used in the concerned study were, four adult normal male monkeys (*Macaca mulatta*) of age and weight ranging from 6-8 years and 7-10 kg respectively. All the animals were kept in specific colony environment of primate facility at Department of Animal Sciences, Quaid-i-Azam University Islamabad, Pakistan. The animals were daily provided with feed comprising of fresh fruits, boiled potatoes, eggs and bread at specific times according to their body weights, and water was available *ad libitum*. Prior to the start of experiment, appetite monitoring was carried out for a month. It was observed that all animals used to finish their food within 10-15 min.

Venous Catheterization

A cathy cannula (Silver surgical complex, Karachi, Pakistan; 0.8 mm O.D/22 G×25mm) was affixed in the sephnous vein after anesthesizing the animals with Ketamine HCl (10 mg/kg BW, *im*), to bring about all the chemical administration and sequential blood sampling. A butterfly tubing (24 G×3/4″ diameter and 300 mm length; JMS Singapore) was attached with free end of the cannula. All the sampling was performed after full recovery of animals from sedation.

Pharmacological Reagents

Pharmacological reagents used in the study are listed below:
Heparin (Sinochem Ningbo, China)
Ketamine HCl (Rotexmedica, Trittau, Germany)
Human Neuromedin S (Anaspec, USA)
All the working solutions were prepared in saline solution (0.9% NaCl).

Blood sampling

Blood sampling (2-3 ml) was conducted, at regular intervals of 15 min, using heparinized syringes. An equivalent quantity of heparinized (5 IU/ml) saline was injected after each sample withdrawal. Samples were collected 60 min before and 120 min after NMS/saline administration. The time of NMS (50 nmol)

administration was considered as 0 min. All blood samples were obtained between 1100-1500 hrs. All experiments were performed in a couple of weeks in order to reduce the alterations in hormonal levels associated with seasonal changes. Samples were centrifuged for 10 min at 3000 rpm, and then plasma was pipetted out and stored at -20˚C until analyzed.

Analysis of hormones

Leptin, adiponectin and resistin concentrations were quantitatively determined by using EIA kits (AssayMax Human ELISA; Assaypro 41 Triad south drive St. Charles, USA). The minimum limit of detectable level of leptin was upto 0.12 ng/ml; intra-assay and inter-assay coefficients of variation were 4.5% and 7.2% respectively. The minimum limit of detectable adiponectin levels was upto 0.5 ng/ml; intra-assay and inter-assay coefficients of variation were 4.2% and 7.3% respectively. In case of resistin the minimum detectable level was upto 0.2 ng/ml; intra-assay and inter-assay coefficients of variation were 4.2% and 7.3% respectively. All the procedures of EIA were followed as provided with the kits.

Statistical analysis

All the data were presented as mean±SEM. Leptin, adiponectin and resistin concentrations after NMS and saline administration were compared by one-way ANOVA followed by post hoc Dunnett's multiple comparisons test. Student's t test was employed to compare mean pre- and post-treatment leptin, adiponectin and resistin concentrations, under 48-hrs fasting and normal fed conditions.

Statistical significance was set at $P \leq 0.05$. All the data were analyzed by using statistical software GraphPad Prism version 5.

Results

Basal plasma adiponectin concentrations in fed and fasting conditions

Basal plasma concentrations of adiponectin (ng/ml) during 1-hr before saline administration in fed and 48-hrs fasting animals are given in Fig. 3.1A-3.1B. Adiponectin levels significantly ($P<0.001$) increased in 48-hrs fasting compared to normal fed monkeys.

Effect of NMS on plasma adiponectin secretion in normal fed adult male monkeys

The individual and mean plasma adiponectin concentrations (ng/ml) before and after saline/NMS administration are given in table 3.1-3.2 and Fig. 3.2A. After 60 min of NMS injection adiponectin concentrations significantly ($P<0.01$) decreased compared to 0 min sample (Fig. 3.2B). Comparison between pre- and post-treatment did not show any significant ($P>0.05$) change in adiponectin levels after NMS administration (Fig. 3.3).

Effect of NMS on plasma adiponectin secretion in 48-hrs fasting adult male monkeys

The individual and mean plasma adiponectin concentrations (ng/ml) before and after saline/NMS administration are given in table 3.3-3.4 and Fig. 3.4A. Adiponectin levels significantly ($P<0.01$) decreased after 60 min of NMS injection compared to 0 min sample in 48-hrs fasting monkeys. Maximum decrease in adipoectin concentrations ($P<0.001$) was observed at 90 min of NMS injection compared to 0 min sample (Fig. 3.4B). Comparison between pre- and post-treatment showed a non significant ($P<0.05$) decrease in adiponectin levels after NMS administration (Fig. 3.5).

Basal plasma leptin concentrations in fed and fasting conditions

Basal plasma concentrations of leptin (ng/ml) during 1-hr before saline administration in fed and 48-hrs fasting animals are given in Fig. 3.6A-3.6B. 48-hrs fasting caused a significant decrease ($P<0.001$) in basal plasma leptin levels compared to normal fed adult male monkeys.

Effect of NMS on plasma leptin secretion in normal fed adult male monkeys

The individual and mean plasma leptin concentrations (ng/ml) before and after saline/NMS administration in normal fed monkeys are given in table 3.5-3.6 and Fig. 3.7A. After 45 min of NMS injection leptin concentrations significantly ($P<0.05$) decreased compared to 0 min sample. Maximum decrease in leptin concentrations ($P<0.001$) was observed at 105 min of NMS injection compared to 0 min sample (Fig. 3.7B). Comparison between pre- and post-treatment also showed a significant ($P<0.01$) decrease in leptin levels after NMS administration (Fig. 3.8).

Effect of NMS on plasma leptin secretion in 48-hrs fasting adult male monkeys

The individual and mean plasma leptin concentrations (ng/ml) before and after saline/NMS administration in 48-hrs fasting monkeys are given in table 3.7-3.8 and Fig. 3.9A. NMS treatment in 48-hrs fasting monkeys caused a significant ($P<0.01$) decrease in leptin concentrations after 75 min of NMS injection. The most significant decrease ($P<0.01$) in leptin concentrations ($P<0.001$) was observed at 90 min and 105 min of NMS injection compared to 0 min sample (Fig. 3.9B). Comparison between pre- and post-treatment showed a significant ($P<0.01$) decrease in leptin levels after NMS administration (Fig. 3.10).

Basal plasma resistin concentrations in fed and fasting conditions

Basal plasma concentrations of resistin (ng/ml) during 1-hr before saline administration in normal fed and 48-hrs fasting animals are given in Fig. 3.11A-3.11B. Basal resistin levels significantly ($P<0.01$) decreased in 48-hrs fasting compared to normal fed monkeys.

Effect of NMS on plasma resistin secretion in normal fed adult male monkeys

The individual and mean plasma resistin concentrations (ng/ml) before and after saline/NMS administration in normal fed monkeys are given in table 3.9-3.10 and Fig. 3.12A. After 60 min of NMS injection resistin concentrations significantly ($P<0.05$) increased compared to 0 min sample (Fig. 3.12B). Comparison between pre- and post-treatment showed a significant ($P<0.01$) increase in resistin levels after NMS administration (Fig. 3.13).

Effect of NMS on plasma resistin secretion in 48-hrs fasting adult male monkeys

The individual and mean plasma resistin concentrations (ng/ml) before and after saline/NMS administration in 48-hrs fasting monkeys are given in table 3.11-3.12 and Fig. 3.14A. NMS treatment in 48-hrs fasting monkeys induced a significant ($P<0.05$) increase in resistin concentrations after 45 min of NMS injection. Maximum levels of resistin concentrations ($P<0.01$) were observed at 75 min of NMS injection compared to 0 min sample (Fig. 3.14B). Comparison between pre- and post-treatment also showed a significant ($P<0.01$) increase in resistin levels after NMS administration (Fig. 3.15).

Table 3.1. Individual and mean (±SEM) plasma adiponectin concentrations (ng/ml) before and after saline administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	19.21	21.16	25.91	14.11	20.10±2.44
-45	17.02	19.22	23.14	13.61	18.25±2.00
-30	17.12	15.41	23.65	18.35	18.63±1.78
-15	14.21	16.46	19.33	15.81	16.45±1.07
0	21.33	19.82	23.71	18.16	20.76±1.18
15	17.39	19.46	21.97	16.75	18.89±1.18
30	20.81	25.11	22.48	18.59	21.75±1.38
45	22.45	20.31	18.49	15.82	19.27±1.41
60	21.25	19.99	16.81	15.03	18.27±1.43
75	24.13	20.52	19.16	21.14	21.24±1.05
90	19.44	17.92	17.61	16.60	17.89±0.59
105	18.92	16.27	21.76	19.19	19.04±1.12
120	21.64	17.59	24.14	20.49	20.97±1.36

Table 3.2. Individual and mean (±SEM) plasma adiponectin concentrations (ng/ml) before and after NMS administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	21.34	15.22	13.42	18.23	17.05±1.74
-45	19.55	13.94	12.34	15.99	15.46±1.56
-30	19.92	14.98	14.21	24.18	18.32±2.33
-15	17.64	12.08	12.45	20.24	15.60±2.00
0	19.24	23.3	17.94	21.22	20.43±1.17
15	16.40	20.25	13.09	19.64	17.35±1.65
30	16.90	18.31	13.93	16.88	16.51±0.92
45	14.66	17.92	11.63	15.25	14.87±1.29
60	12.85	16.23	11.33	12.45	13.22±1.06**
75	17.42	19.75	15.97	12.54	16.42±1.51
90	18.15	21.64	13.88	14.95	17.16±1.75
105	22.86	19.55	15.46	15.21	18.27±1.82
120	17.71	18.22	14.94	19.56	17.61±0.97

****P<0.01 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).**

Table 3.3. Individual and mean (±SEM) plasma adiponectin concentrations (ng/ml) before and after saline administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	49.06	45.82	39.44	53.54	46.97±2.97
-45	42.15	39.36	35.12	51.45	42.02±3.46
-30	44.46	48.31	39.74	55.86	47.09±3.41
-15	40.78	41.16	35.87	49.02	41.71±2.72
0	43.19	54.43	34.95	49.76	45.58±4.23
15	43.93	49.91	36.31	46.89	44.26±2.92
30	46.11	56.51	39.96	49.52	48.03±3.45
45	41.85	53.11	36.71	47.46	44.78±3.54
60	42.04	54.62	39.83	55.96	48.11±4.18
75	43.34	49.91	41.37	57.45	48.02±3.64
90	43.83	52.99	45.05	52.09	48.49±2.36
105	44.16	55.65	47.88	53.26	50.24±2.60
120	45.09	52.78	53.91	48.14	49.98±2.05

Table 3.4. Individual and mean (±SEM) plasma adiponectin concentrations (ng/ml) before and after NMS administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	50.21	41.33	45.1	34.02	42.67±3.41
-45	46.59	36.25	37.38	30.76	37.75±3.28
-30	49.33	43.52	39.54	39.24	42.91±2.35
-15	43.42	38.58	40.22	34.25	39.12±1.91
0	45.25	33.59	43.9	38.62	40.34±2.67
15	49.77	41.23	44.82	48.29	46.03±1.91
30	41.29	40.27	39.71	42.13	40.85±0.54
45	38.51	41.09	34.75	37.29	37.91±1.32
60	34.55	31.61	25.77	33.55	31.37±1.96**
75	33.07	29.88	28.95	34.62	31.63±1.33*
90	30.13	29.08	25.55	27.36	28.03±1.00***
105	31.66	36.13	26.94	28.05	30.70±2.07**
120	37.11	39.36	29.14	33.25	34.72±2.25

***p<0.05, **P<0.01, ***P<0.001 with 0 min sample (ANOVA followed by post hoc Dunnett's test).**

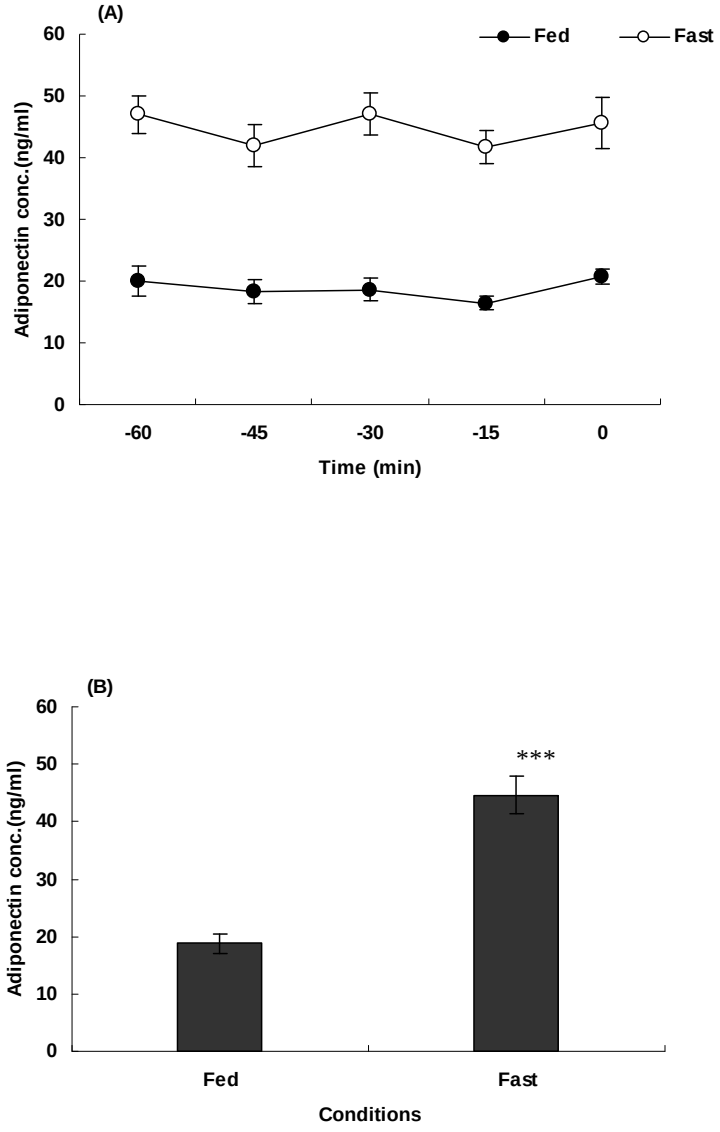


Figure. 3.1. (A) Changes in mean (±SEM) basal plasma adiponectin concentrations (ng/ml) in 1-hr period in fed and 48-hrs fasting adult male monkeys (**B**) Overall mean (±SEM) basal plasma adiponectin concentrations (ng/ml) in 1-hr period in normal fed, and 48-hrs fasting adult male monkeys. ***P<0.001 vs fed (Student's t test).

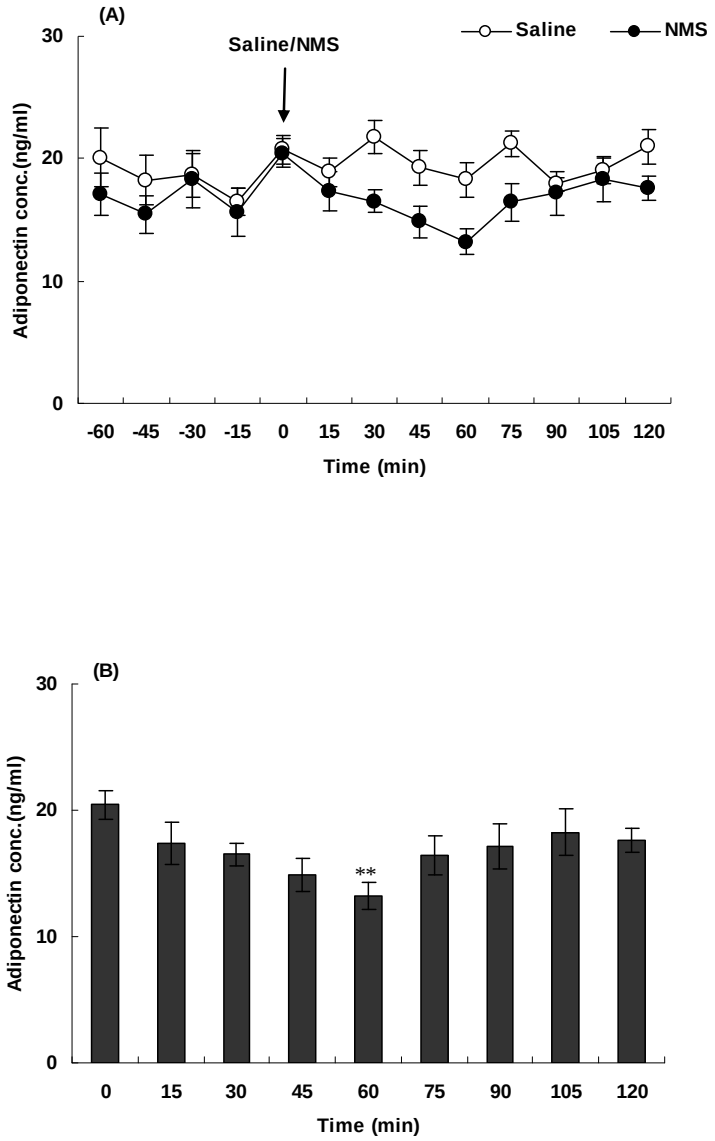


Figure. 3.2. (A) Mean (±SEM) changes in plasma adiponectin levels (ng/ml) before and after saline/NMS administration (at 0 min) in normal fed adult male monkeys. **(B)** Comparison of mean (±SEM) adiponectin concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in fed monkeys. **P<0.01 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).

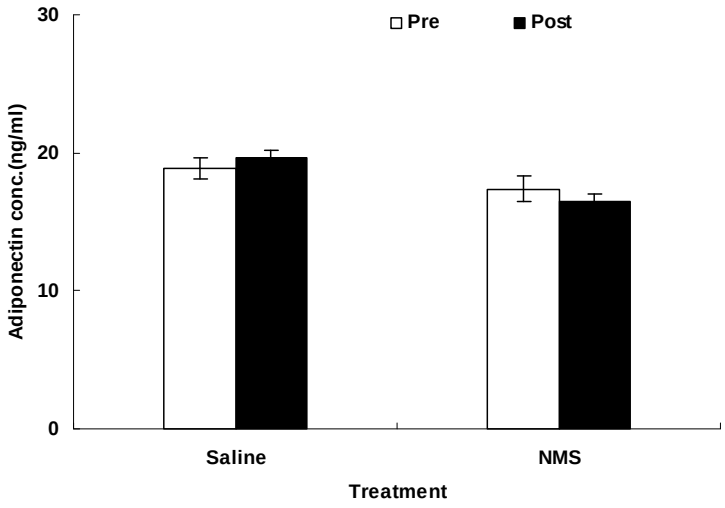


Figure. 3.3. Comparison of mean (±SEM) plasma adiponectin levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in normal fed adult male monkeys. P>0.05 vs pre-treatment (Student's t test).

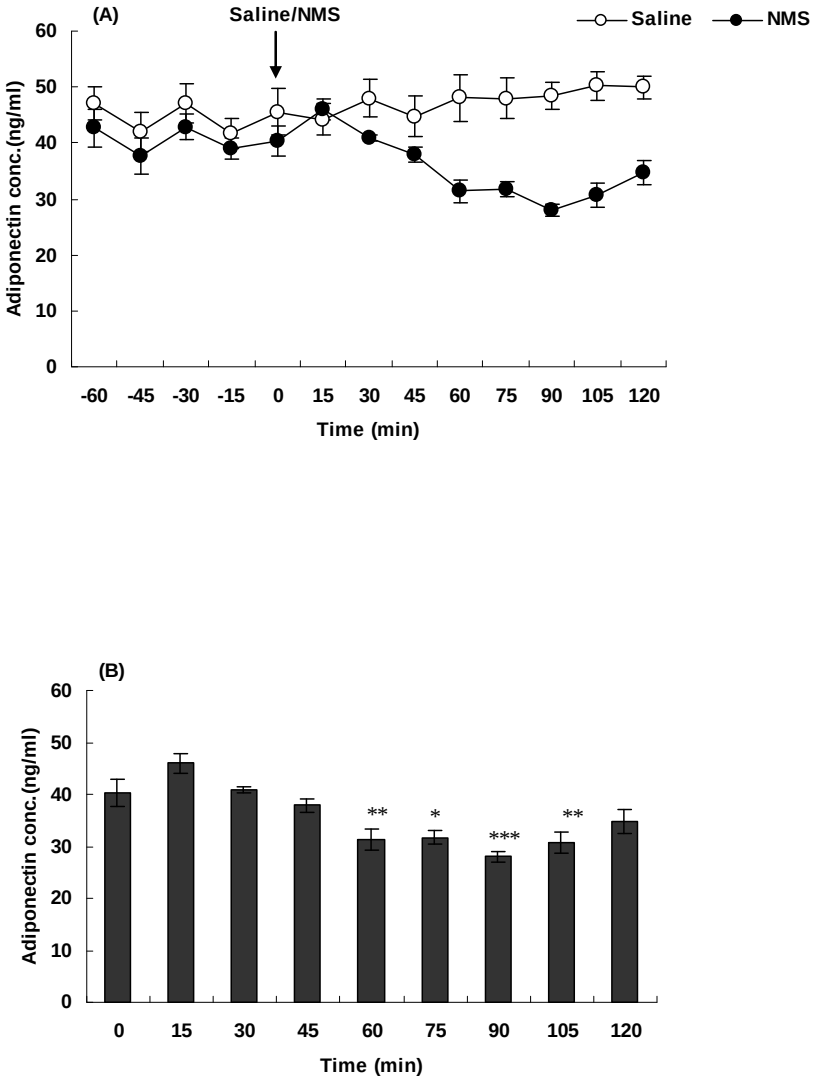


Figure. 3.4. (A) Mean (±SEM) changes in plasma adiponectin levels (ng/ml) before and after saline/NMS administration (at 0 min) in 48-hrs fasting adult male monkeys. **(B)** Comparison of mean (±SEM) adiponectin concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in 48-hrs fasting monkeys. *p<0.05, **P<0.01, ***P<0.001 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).

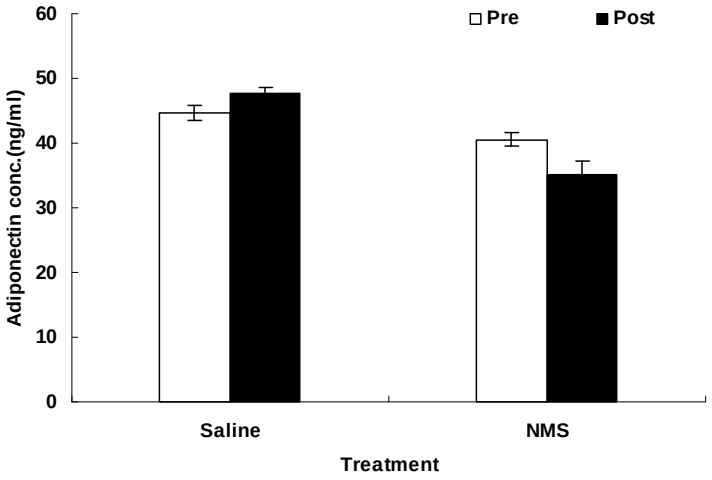


Figure. 3.5. Comparison of mean (±SEM) plasma adiponectin levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in 48-hrs fasting adult male monkeys. P>0.05 vs pre-treatment (Student's t test).

Table 3.5. Individual and mean (±SEM) plasma leptin concentrations (ng/ml) before and after saline administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	2.92	3.99	3.61	3.05	3.39±0.25
-45	3.11	3.61	3.71	3.16	3.40±0.15
-30	3.22	3.78	3.92	3.22	3.54±0.18
-15	3.17	3.51	3.69	3.15	3.38±0.13
0	3.61	3.81	4.04	3.75	3.80±0.09
15	3.51	3.94	4.13	3.40	3.75±0.17
30	3.19	4.06	4.41	3.98	3.91±0.26
45	3.41	3.89	3.97	4.06	3.83±0.15
60	3.59	3.41	3.72	3.82	3.64±0.09
75	3.38	3.88	3.59	3.74	3.65±0.11
90	3.65	3.71	2.99	3.20	3.39±0.17
105	3.21	3.39	3.04	2.96	3.15±0.10
120	2.98	3.41	3.34	3.14	3.22±0.10

Table 3.6. Individual and mean (±SEM) plasma leptin concentrations (ng/ml) before and after NMS administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	3.03	4.13	4.11	3.10	3.59±0.30
-45	3.52	3.77	4.69	3.26	3.81±0.31
-30	2.97	3.79	3.82	3.48	3.52±0.20
-15	3.17	3.51	4.66	4.21	3.89±0.34
0	3.19	3.42	4.41	3.62	3.66±0.26
15	3.35	3.51	3.82	3.98	3.67±0.14
30	2.99	2.56	2.93	3.72	3.05±0.24
45	2.54	2.39	2.41	3.49	2.71±0.26*
60	2.99	2.79	2.31	3.08	2.79±0.17*
75	2.08	2.21	2.52	2.71	2.38±0.14***
90	1.77	1.62	1.91	2.19	1.87±0.12***
105	1.55	1.41	1.99	1.92	1.72±0.14***
120	1.72	2.01	1.89	2.71	2.08±0.22***

***P<0.05, ***P<0.001 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).**

Table 3.7. Individual and mean (±SEM) plasma leptin concentrations (ng/ml) before and after saline administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	0.81	1.2	1.42	0.78	1.05±0.16
-45	0.97	1.04	1.13	0.81	0.99±0.07
-30	0.88	0.92	0.99	1.11	0.98±0.05
-15	1.02	0.99	1.06	1.19	1.07±0.04
0	1.00	0.82	1.11	1.09	1.01±0.07
15	0.85	0.91	1.05	1.17	1.00±0.07
30	0.93	1.01	1.59	0.89	1.11±0.16
45	1.16	0.93	1.04	0.86	1.00±0.07
60	0.99	0.88	1.15	0.71	0.93±0.09
75	1.08	0.95	1.11	0.83	0.99±0.06
90	0.85	1.05	0.82	0.96	0.92±0.05
105	0.89	1.21	0.95	0.75	0.95±0.10
120	1.04	1.13	0.98	0.92	1.02±0.04

Table 3.8. Individual and mean (±SEM) plasma leptin concentrations (ng/ml) before and after NMS administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	1.03	0.84	1.51	1.12	1.13±0.14
-45	0.96	1.16	1.18	1.27	1.14±0.07
-30	1.26	1.02	1.24	1.44	1.24±0.09
-15	0.94	1.01	0.99	1.10	1.01±0.03
0	0.73	1.15	1.25	0.86	1.00±0.12
15	0.91	1.14	1.06	1.05	1.04±0.05
30	0.73	0.96	0.96	1.01	0.92±0.06
45	0.68	0.79	0.85	0.91	0.81±0.05
60	0.77	0.64	1.01	0.84	0.82±0.08
75	0.62	0.55	0.81	0.79	0.69±0.06*
90	0.61	0.49	0.72	0.67	0.62±0.05**
105	0.47	0.53	0.69	0.78	0.62±0.07**
120	0.52	0.72	0.62	0.68	0.64±0.04**

***p<0.05, **P<0.01 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).**

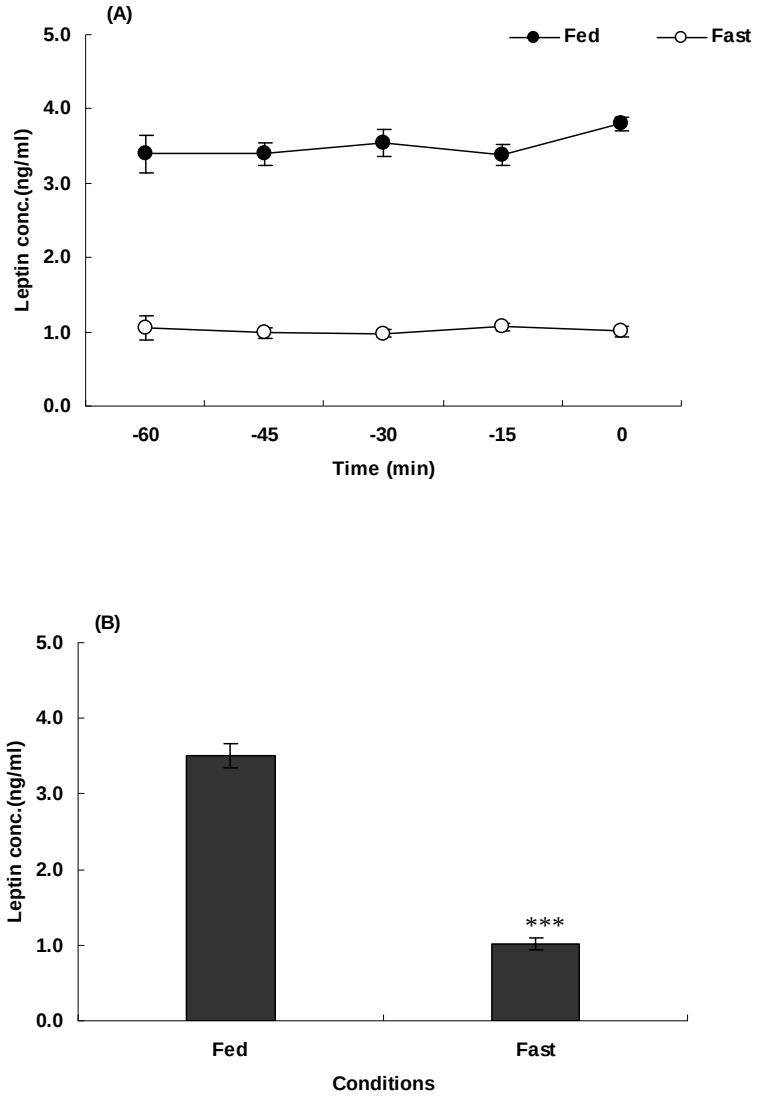


Figure. 3.6. (A) Changes in mean (±SEM) basal plasma leptin concentrations (ng/ml) in 1-hr period in fed and 48-hrs fasting adult male monkeys (**B**) Overall mean (±SEM) basal plasma leptin concentrations (ng/ml) in 1-hr period in normal fed, and 48-hrs fasting adult male monkeys. ***P<0.001 vs fed (Student's t test).

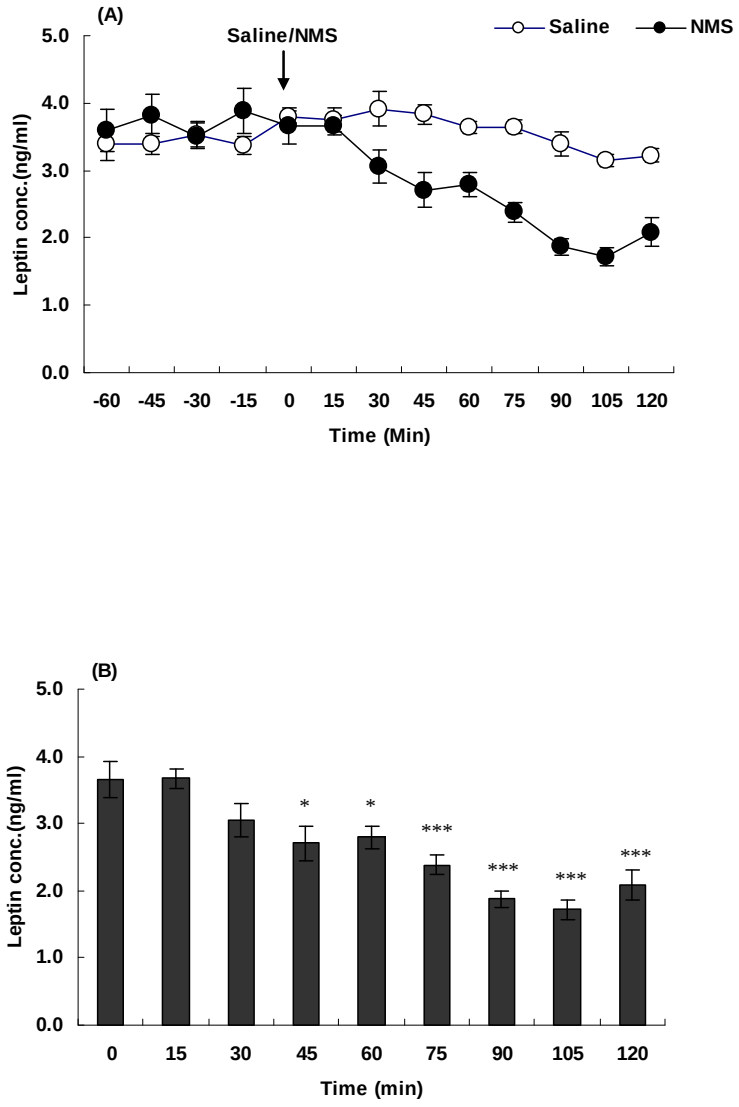


Figure. 3.7. (A) Mean (±SEM) changes in plasma leptin levels (ng/ml) before and after saline/NMS administration (at 0 min) in normal fed adult male monkeys. **(B)** Comparison of mean (±SEM) leptin concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in fed monkeys. *P<0.05, ***P<0.001 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).

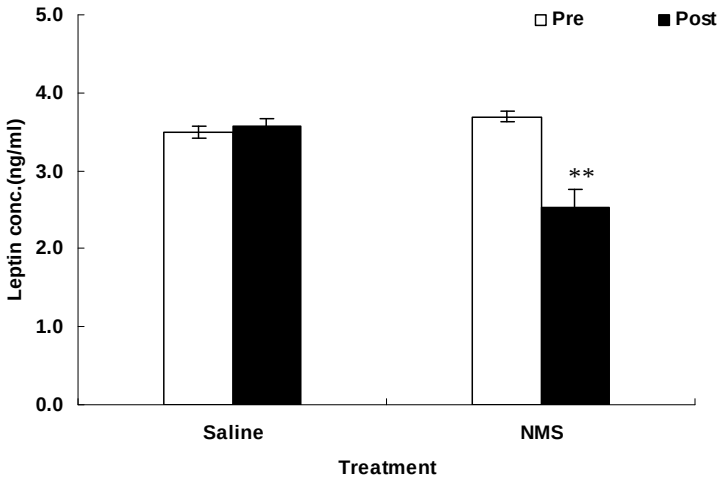


Figure. 3.8. Comparison of mean (±SEM) plasma leptin levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in normal fed adult male monkeys. **P<0.01 vs pre-treatment (Student's t test).

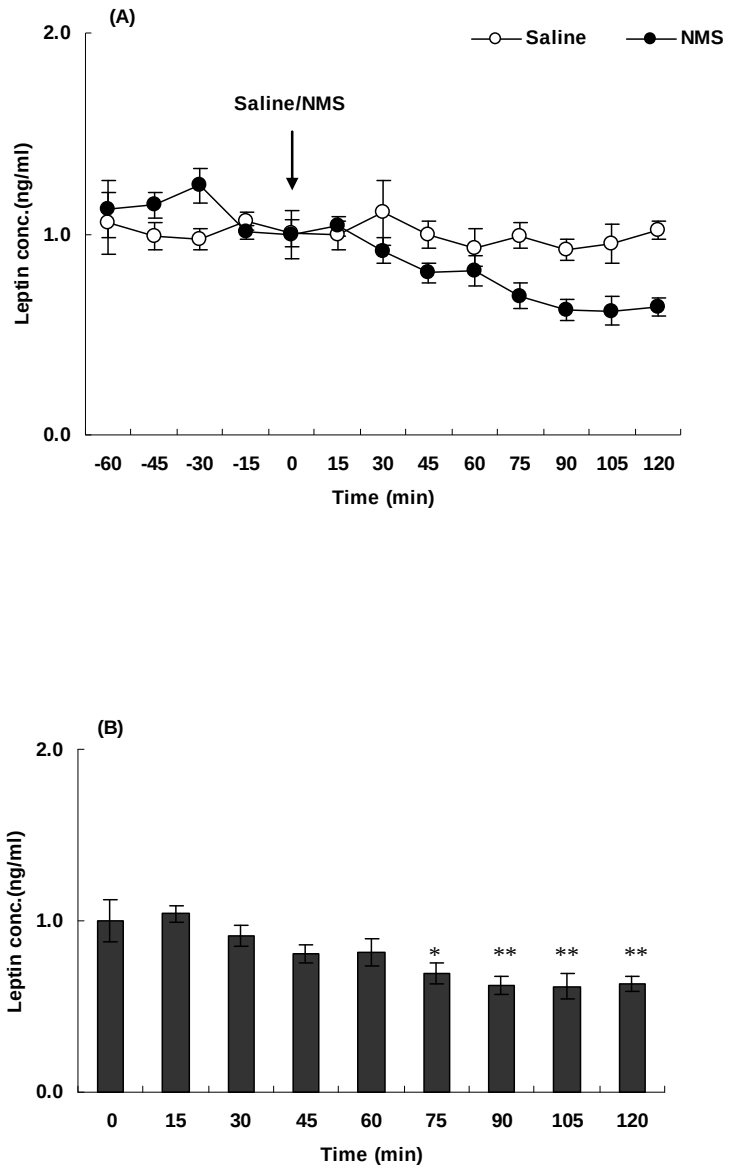


Figure. 3.9. (A) Mean (±SEM) changes in plasma leptin levels (ng/ml) before and after saline/NMS administration (at 0 min) in 48-hrs fasting adult male monkeys. **(B)** Comparison of mean (±SEM) leptin concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in 48-hrs monkeys. *p<0.05, **P<0.01 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).

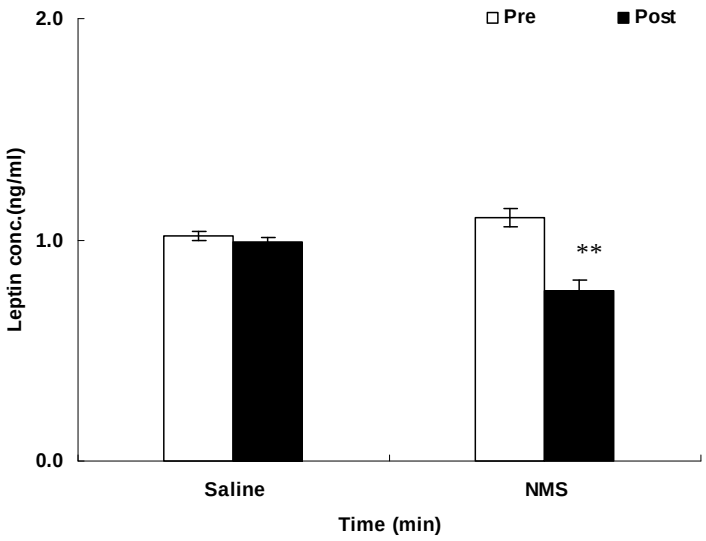


Figure. 3.10. Comparison of mean (±SEM) plasma leptin levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in 48-hrs fasting adult male monkeys. **P<0.01 vs pre-treatment (Student's t test).

Table 3.9. Individual and mean (±SEM) plasma resistin concentrations (ng/ml) before and after saline administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	15.05	12.23	17.91	17.46	15.66±1.31
-45	15.36	13.75	15.84	16.24	15.30±0.55
-30	14.69	12.47	14.99	16.08	14.56±0.76
-15	15.24	12.79	15.73	17.11	15.22±0.90
0	14.68	13.94	14.07	16.46	14.79±0.58
15	15.01	11.02	14.62	18.19	14.71±1.47
30	15.24	12.81	16.98	18.64	15.92±1.25
45	14.92	13.15	16.81	17.70	15.65±1.01
60	14.43	14.21	15.97	17.26	15.47±0.71
75	16.28	14.52	15.21	15.96	15.49±0.39
90	16.21	15.61	16.39	16.76	16.24±0.24
105	16.06	13.29	17.10	16.21	15.67±0.82
120	15.96	11.6	16.91	16.06	15.13±1.20

Table 3.10. Individual and mean (±SEM) plasma resistin concentrations (ng/ml) before and after NMS administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	15.31	13.51	13.09	18.05	14.99±1.13
-45	14.24	11.23	15.99	17.65	14.78±1.37
-30	17.17	12.77	16.78	16.28	15.75±1.01
-15	18.27	14.53	15.07	15.56	15.86±0.83
0	15.20	16.18	16.89	17.21	16.37±0.45
15	16.39	17.44	14.51	18.68	16.76±0.88
30	18.96	17.97	16.68	19.75	18.34±0.66
45	18.41	18.54	17.67	19.90	18.63±0.46
60	19.53	18.08	17.94	19.57	18.78±0.45*
75	17.13	17.69	17.98	19.26	18.02±0.45
90	14.65	17.02	16.61	18.09	16.59±0.72
105	15.26	16.68	17.10	17.06	16.53±0.43
120	16.31	15.81	17.42	17.26	16.70±0.38

***P<0.05 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).**

Table 3.11. Individual and mean (±SEM) plasma resistin concentrations (ng/ml) before and after saline administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	13.41	12.19	10.71	10.40	11.68±0.70
-45	13.06	11.49	10.45	11.55	11.64±0.54
-30	13.52	12.95	11.86	12.75	12.77±0.34
-15	12.93	10.99	12.17	11.08	11.79±0.46
0	12.81	10.61	11.26	12.46	11.79±0.51
15	13.22	11.13	11.52	12.99	12.22±0.52
30	13.02	10.82	12.85	11.76	12.11±0.51
45	13.34	11.95	12.68	12.81	12.70±0.29
60	12.95	11.09	11.07	12.45	11.89±0.48
75	12.09	12.83	11.43	11.76	12.03±0.30
90	13.24	11.14	10.83	10.98	11.55±0.57
105	12.92	11.95	10.51	10.59	11.49±0.58
120	13.51	11.83	9.86	10.02	11.31±0.86

Table 3.12. Individual and mean (±SEM) plasma resistin concentrations (ng/ml) before and after NMS administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	10.84	12.52	12.73	16.40	13.12±1.17
-45	10.99	12.30	13.05	16.86	13.30±1.26
-30	10.85	12.05	12.18	14.61	12.42±0.79
-15	9.66	13.76	12.89	15.99	13.08±1.31
0	9.55	11.01	12.61	15.88	12.26±1.36
15	10.09	12.33	12.15	16.09	12.67±1.25
30	11.28	14.30	13.11	17.27	13.99±1.26
45	15.69	16.08	15.33	19.65	16.69±1.00*
60	16.21	17.33	16.99	19.79	17.58±0.77**
75	16.99	17.23	18.24	20.23	18.17±0.74**
90	14.65	17.49	15.38	18.11	16.41±0.83
105	12.64	17.07	15.69	17.89	15.82±1.15
120	13.01	16.65	13.22	15.44	14.58±0.88

***p<0.05, **P<0.01 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).**

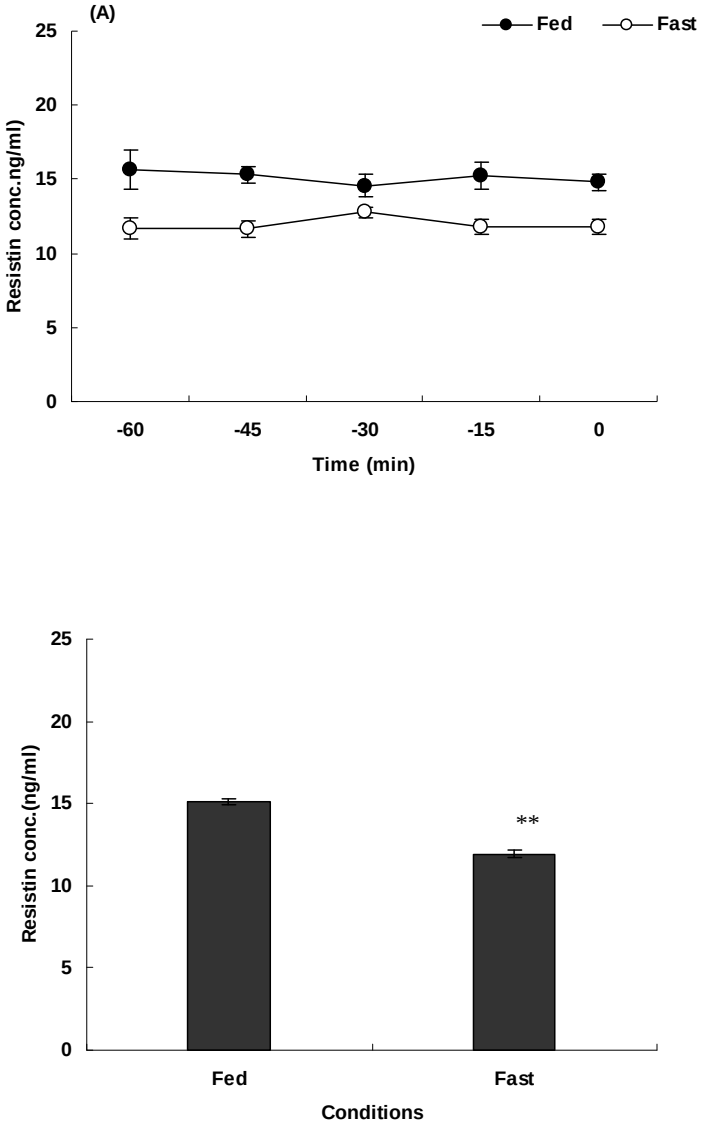


Figure. 3.11. (A) Changes in mean (±SEM) basal plasma resistin concentrations (ng/ml) in 1-hr period in fed and 48-hrs fasting adult male monkeys (**B**) Overall mean (±SEM) basal plasma resistin concentrations (ng/ml) in 1-hr period in normal fed, and 48-hrs fasting adult male monkeys. **P<0.001 vs fed (Student's t test).

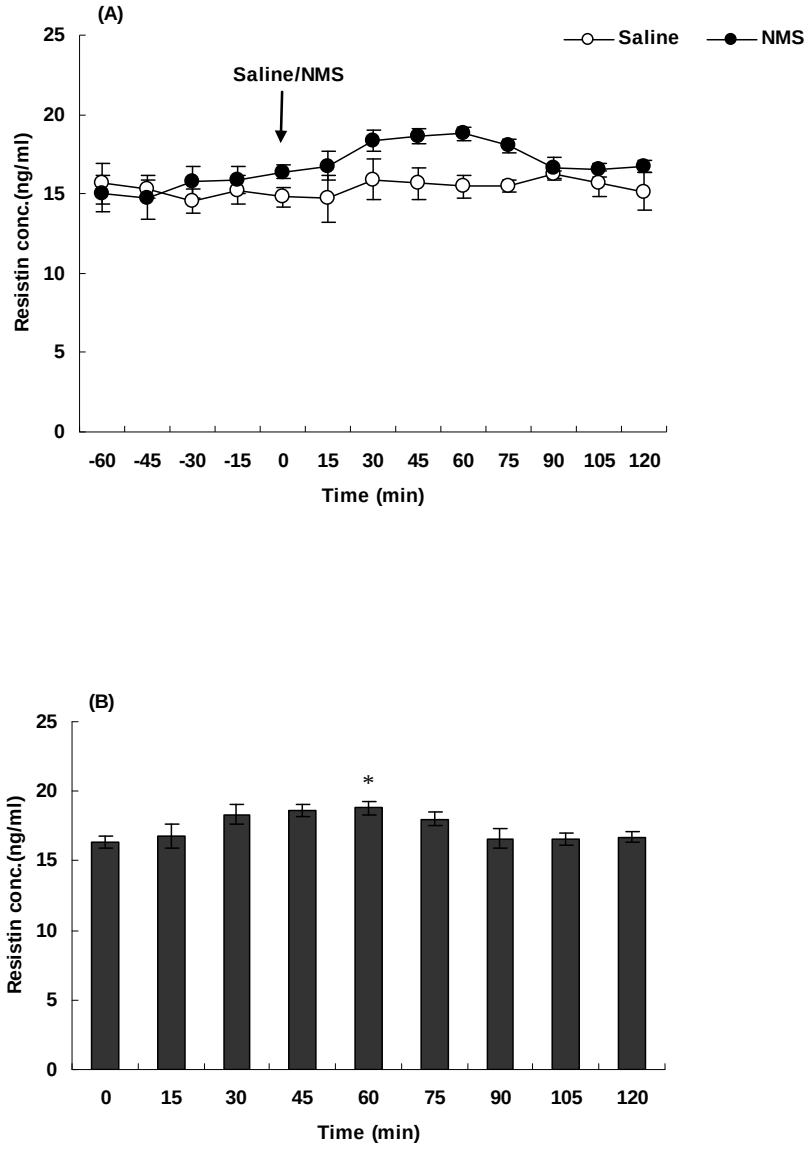


Figure. 3.12. (A) Mean (±SEM) changes in plasma resistin levels (ng/ml) before and after saline/NMS administration (at 0 min) in normal fed adult male monkeys. **(B)** Comparison of mean (±SEM) resistin concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in fed monkeys. *P<0.05 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).

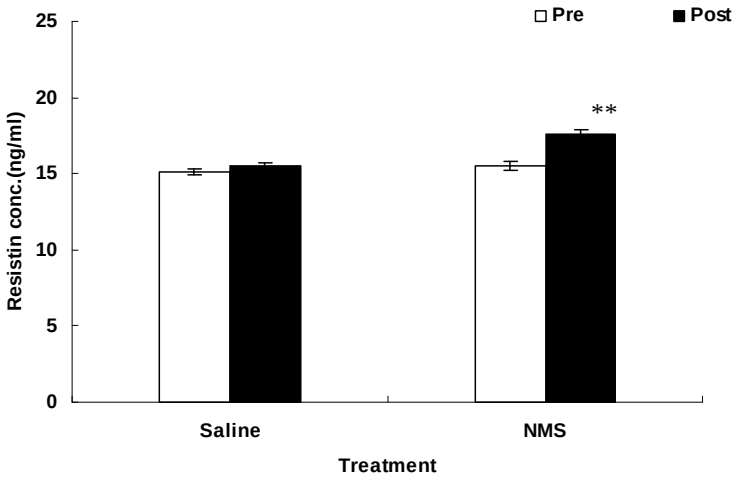


Figure. 3.13. Comparison of mean (±SEM) plasma resistin levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in normal fed adult male monkeys. **P<0.01 vs pre-treatment (Student's t test).

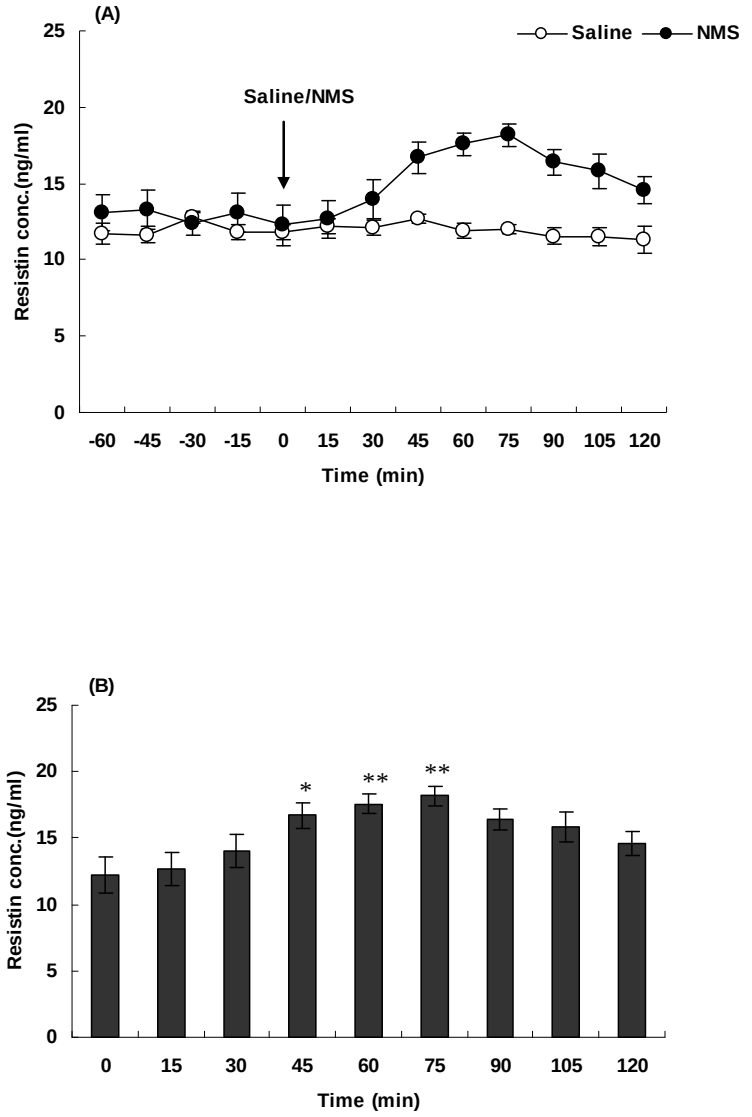


Figure. 3.14. (A) Mean (±SEM) changes in plasma resistin levels (ng/ml) before and after saline/NMS administration (at 0 min) in 48-hrs fasting adult male monkeys. **(B)** Comparison of mean (±SEM) resistin concentrations (ng/ml) between post NMS (15-120 min) and pre NMS (at 0 min) in 48-hrs fasting monkeys. *p<0.05, **P<0.01 vs 0 min sample (ANOVA followed by post hoc Dunnett's test).

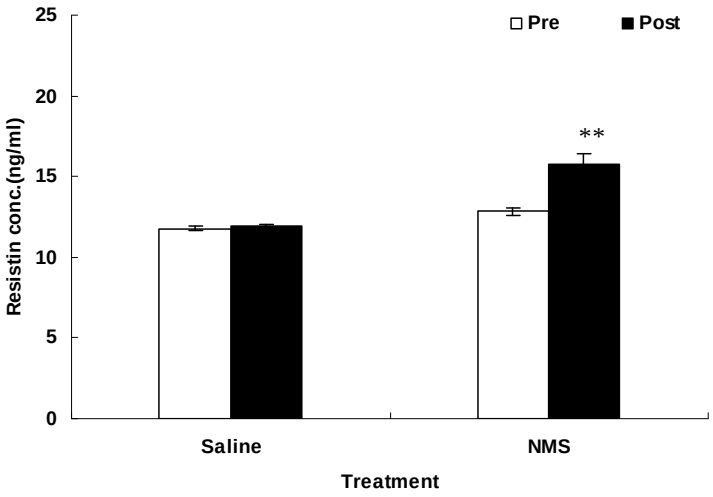


Figure. 3.15. Comparison of mean (±SEM) plasma resistin levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in 48-hrs fasting adult male monkeys. **P<0.01 vs pre-treatment (Student's t test).

Discussion

In the present study, we investigated the role of peripheral administration of NMS on adipokines (leptin, adiponectin and resistin) secretion in 48-hrs fasting and normal fed adult male monkeys. We hypothesized that NMS being a food regulatory peptide might have some effects on adipokines secretions, which may possibly modulate its regulatory effect on energy metabolism and reproductive functions. No data is available in this regard and we are the pioneer to investigate the role of NMS on adipokines regulation in non human primates. Adipokines released from adipose tissue act via a network of endocrine, paracrine and autocrine pathways, and playing very important role in variety of physiological aspects, such as cardiovascular functions, lipid and glucose metabolism, immunity, neuroendocrine function and reproduction (Shankar *et al*., 2010; Pataky *et al*., 2010).

In our study, 48-hrs fasting significantly increased ($P<0.001$) basal adiponectin levels compared to normal fed conditions suggesting that fasting might have stimulatory effect on adiponectin secretion. This effect may be due to the fasting induced suppression of HPG axis and hence T secretion. Elevated levels of androgens were observed to decrease adiponectin concentrations while in androgen receptor null mice, adiponectin levels were reasonably increased (Combs *et al*., 2003; Bottner *et al*., 2004; Fan *et al*., 2005; Xu *et al*., 2005). Some other studies in rats also indicated that LH and T secretions are inhibited by adiponectin (Rodriguez-Pacheco *et al*., 2007; Caminos *et al*., 2008). Other possibility is that during fasting, expression of certain peptides and their receptors, like kisspeptin may contribute to this elevated adiponectin response (Brown *et al*., 2008; Wahab *et al*., 2010). In the present study, after NMS administration, overall adiponectin levels were non significantly ($P>0.05$) decreased compared to pre-treated NMS, although some individual values showed significant decrease. These findings suggest that NMS might have little or no effect on adiponectin secretion from adipocytes. However considering that this dose of NMS might have no effect on adiponectn secretion, the different doses of NMS may be applied in future, to investigate its exact role on adiponectin secretion.

In this study, leptin levels were significantly decreased ($P<0.001$) in case of fasting monkeys compared to normal fed, suggesting that fasting has suppressive

effect on leptin secretion. These results are in accordance with various studies where fasting caused decreased leptin concentrations in rodents, pigs and humans (Ahima *et al*., 1996; Kolaczynski *et al.,* 1996; Barb *et al*., 2001b). In the cow and ewe, 48-hrs fasting resulted in decreased leptin as well as LH levels (Amstalden *et al*., 2000; Henry *et al*., 2001; Morrison *et al*., 2001). Similarly in OVX gilts fasting for 7 days also reduced serum leptin and LH secretion (Whisnant and Harrell, 2002). All these data suggest that fasting negatively affects HPG axis via inhibiting leptin and LH secretion (Wahab *et al*., 2010). In the present study we demonstrated that NMS (50 nmol) administration significantly decreased ($P<0.01$) leptin levels in both normal fed and 48-hrs fasting conditions. The leptin regulatory pathways include large number of neuropeptides and several intracellular complex pathways (Kuo *et al*., 2005). Our results showed that NMS is playing a significant role in leptin suppression in monkeys. The exact mechanism that how NMS suppresses the leptin levels and what pathway it uses is still under question. However it is proposed that NMS induced HPG axis regulation might not involve leptin stimulation in non human primates.

Resistin is known as a novel adipokine having a potential role in the regulation of adipocyte differentiation and insulin sensitivity (Kim *et al*., 2001; Steppan *et al*., 2001a). Resistin gene and its mRNA expression in testes suggests that like ghrelin and leptin, it acts as an endocrine mediator in regulation of reproduction and energy homeostasis (Nogueiras *et al*., 2004). Role of resistin in reproduction is least understood among all the adipokines. In our study, 48-hrs fasting suppressed ($P<0.01$) resistin levels suggesting that fasting has an inhibitory effect on resistin secretion. Similarly fasting and leptin administration (*icv*) signifintly reduced testicular resistin mRNA levels (Nogueiras *et al*., 2004). In the present study, both in 48-hrs fasting and normal fed conditions, peripheral administration of NMS significantly increased ($P<0.01$) circulating resistin levels. This stimulatory response was independent of the metabolic status of animals. It was shown that in rat testes, both FSH and LH participate in tuning of resistin expression (Nogueiras *et al*., 2004). Under the control of gonadotropins, the testicular resistin expression was assumed to playing very important role in development and function of testes (Tena-Sempere and Huhtaniemi, 2003). Further evidence suggested that resistin has also ability to significantly increase basal and hCG induced T levels in vitro (Nogueiras *et al*., 2004). Our results

proposed that NMS affects the stimulation of resistin which might be playing, some contributory role in T secretion and regulation of HPG axis.

In summary, NMS plays very interesting role in regulation of adipokines. Role of NMS on adiponectin is not clearly understood. However NMS play a definite role in regulation of leptin and resistin. It inhibits leptin secretion but on the other hand stimulates resistin levels in both fed and metabolically stressed conditions. The exact mechanism that how NMS regulates the adipokine secretion on the basis of this single study is very difficult to prove so further studies are required to explore the pathways, involved in this regulation.

Chapter # 4

Effect of peripheral Neuromedin S administration on basal insulin secretion under fed and fasting conditions in adult male rhesus monkeys (*Macaca mulatta*)

Abstract

Background: Insulin is an adiposity signal, believed to be involved in long time food intake regulator and storage of energy. It was assumed that NMS and insulin are playing a synergistic role in food regulation and energy metabolism, so NMS might have some stimulatory effect on insulin secretion. For this purpose the effect of *iv* administration of NMS was investigated on the basal insulin secretion in normal fed and 48-hrs fasting male rhesus monkeys.

Materials and Methods: Four intact adult male rhesus monkeys (6-8 yr Age: 7-10 kg BW) were used in this study. 50 nmol of NMS was injected through a cannula affixed in saphenous vein. Blood samples were collected individually 60 min before and 120 min at 15 min intervals, after NMS/saline administration. The plasma insulin concentrations were determined by using specific Enzyme Immunoassay (EIA) kits.

Results: Insulin levels were significantly ($P<0.001$) decreased in 48-hrs fasting monkeys compared to normal fed but NMS infusion did not induce any significant change ($P>0.05$) in insulin concentrations in both fed and fasting conditions.

Conclusion: Our results suggest that although both NMS and insulin act as anorexigenic peptides and utilize same hypothalamic neuronal system in food regulation and energy metabolism but exogenous NMS action in this regard looks independent of insulin involvement. On the basis of this single study, it is very difficult to confirm the exact mechanism and relationship between NMS and insulin therefore, further studies must be planned to explore the exact mechanism and pathways for NMS in metabolic and reproductive processes, and to confirm the relationship between NMS and insulin.

Introduction

Hypothalamus plays a major role in controlling glucose metabolism of the body. It is believed that hypothalamus regulate this activity by production and storage of glucose in liver (Shimazu, 1987; Uyama *et al*., 2004), utilizing glucose by muscles (Burcelin *et al*., 2000), and regulating insulin secretion (Magnan *et al*., 1999). Now it has become very clear that the gut-brain axis is a key factor in controlling metabolic status and regulation of glucose metabolism in the body (Delaere *et al*., 2010).

Insulin was discovered as presumptive adiposity signal. It is believed that insulin might be involved in long time food intake regulator and storage of energy. Central infusion of insulin significantly reduced body weight and food intake (Sipols *et al*., 1995; Volk *et al*., 1999). In 24-hrs, insulin response to food intake, overall insulin concentrations and fasting insulin concentrations are correlated with fat, stored in the body (Porte, 1981). It is suggested that insulin is transported from serum to cerebrospinal fluid (CSF) through a well regulated mechanism and binds to specific receptors to perform its actions. Many areas of the brain, especially ARC inhabit insulin receptors, which are involved in energy regulation and controlling feeding behaviours. Insulin and leptin in combination are involved in activation of α–MSH secretion and inhibition of agouti related peptide (AgRP) and neuropeptide Y (NPY) from hypothalamus (Benoit *et al*., 2000). In mice, the absence of central insulin receptor, caused hyperphagia and increased body fat (Bruning *et al*., 2000; Obici *et al*., 2002). These results confirmed that insulin play very important role in regulation of energy balance. Insulin can be considered as important adiposity signal as, in addition to inhibition of food intake it also restores the set point of body weight by increasing energy utilization. At cellular level the interaction of leptin and insulin signal is most interesting. In ARC both increase POMC expression and inhibit NPY gene expression. In knock out mice for both leptin and insulin receptors, resulted in heavier body weight and increased food intake (Bruning *et al*., 2000).

Insulin is secreted by pancreas and plays an important role in energy metabolism. Pancreas has receptor expressions for some neuropeptides like kisspeptin, which may be involved in regulation of insulin secretion and energy homeostasis (Hauge-Evans *et al.,* 2006; Suckale and Solimena, 2008; Bowe *et al.,*

2009. Presence of insulin receptors in ARC regions (Bruning *et al*., 2000) suggests its role in regulation of reproductive axis.

NMS is an anorexigenic neuropeptide expressed in SCN region of hypothalamus play a stimulatory role in HPG axis in both rodents (Vigo *et al*., 2007) and primates (Jahan *et al*., 2011). To date, not a single study is available on the effect of NMS on insulin secretion. In the present study it was hypothesized that NMS may play its role in energy metabolism by regulating the insulin signaling. For this purpose the effect of NMS in normal fed and 48-hrs fasting monkeys in insulin secretion was investigated to explain the exact pathway of NMS signaling in metabolic regulation and reproduction.

Materials and Methods

Animals

The animals used in the concerned study were, four adult normal male monkeys (*Macaca mulatta*) of age and weight ranging from 6-8 years and 7-10 kg respectively. All the animals were kept in specific colony environment of primate facility at Department of Animal Sciences, Quaid-i-Azam University Islamabad, Pakistan. The animals were daily provided with feed comprising of fresh fruits, boiled potatoes, eggs and bread at specific times according to their body weights, and water was freely available through out the day. Prior to the start of experiment, appetite monitoring was carried out for a month. It was observed that all animals used to finish their food within 10-15 min.

Venous Catheterization

A cathy cannula (Silver surgical complex, Karachi, Pakistan; 0.8 mm O.D/22 G×25mm) was affixed in the sephnous vein after anesthesizing the animals with Ketamine HCl (10 mg/kg BW, *im*), to bring about all the chemical administration and sequential blood sampling. A butterfly tubing (24 G×3/4″ diameter and 300 mm length; JMS Singapore) was attached with free end of the cannula. All the sampling was performed after full recovery of animals from sedation.

Pharmacological Reagents

Pharmacological reagents used in the study are listed below:
Heparin (Sinochem Ningbo, China)
Ketamine HCl (Rotexmedica, Trittau, Germany)
Human Neuromedin S (Anaspec, USA)
All the working solutions were prepared in saline solution (0.9% NaCl).

Blood sampling

Blood sampling (2-3 ml) was conducted, at regular intervals of 15 min, using heparinized syringes. An equivalent quantity of heparinized (5 IU/ml) saline was injected after each sample withdrawal. Samples were collected 60 min before and 120 min after NMS/saline administration. The time of NMS (50 nmol) administration

was considered as 0 min. All blood samples were obtained between 1100-1500 hrs. All experiments were performed in a couple of weeks in order to reduce the alterations in hormonal levels associated with seasonal changes. Samples were centrifuged for 10 min at 3000 rpm, and then plasma was pipetted out and stored at -20˚C until analyzed.

Analysis of hormones

Plasma insulin concentrations were quantitatively determined by using EIA kits (Calbiotech Inc. CA). The minimum limit of detectable insulin levels was upto 1.47 µIU/ml; Intra-assy and inter-assay coefficients of insulin were 8.1% and 8.5% respectively. All the procedures of EIA were followed as provided with the kits.

Statistical analysis

All the data were presented as mean±SEM. Insulin concentrations after NMS and saline administration were compared by one-way ANOVA followed by post hoc Dunnett's multiple comparisons test. Student's t test was employed to compare mean pre- and post-treatment insulin concentrations, under 48-hrs fasting and normal fed conditions.

Statistical significance was set at $P \leq 0.05$. All the data were analyzed by using statistical software GraphPad Prism version 5.

Results

Basal plasma insulin concentrations in fed and fasting conditions

Basal plasma concentrations of insulin (µIU/ml) during 1-hr before saline administration in fed and 48-hrs fasting animals are given in Fig. 4.1A-4.1B. Insulin levels significantly ($P<0.001$) decreased in 48-hrs fasting compared to normal fed adult male monkeys.

Effect of NMS on plasma insulin secretion in normal fed adult male monkeys

The individual and mean plasma insulin concentrations (µIU/ml) before and after saline/NMS administration in normal fed monkeys are given in table 4.1-4.2 and Fig. 4.2A. Saline/NMS administration did not cause any significant change in insulin concentrations compared to 0 min sample. Comparison between pre- and post-treatment did not show any significant ($P>0.05$) change in insulin levels both in NMS and saline treated monkeys (Fig. 4.2B).

Effect of NMS on plasma insulin secretion in 48-hrs fasting adult male monkeys

The individual and mean plasma insulin concentrations (µIU/ml) before and after saline/NMS administration in 48-hrs fasting monkeys are given in table 4.3-4.4 and Fig. 4.3A. NMS administration in 48-hrs fasting monkeys did not cause any significant ($P>0.05$) change in insulin concentrations compared to 0 min sample. Comparison between pre- and post-treatment did not show any significant ($P>0.05$) change in insulin levels after NMS administration (Fig. 4.3B).

Table 4.1. Individual and mean (±SEM) plasma insulin concentrations (µIU/ml) before and after saline administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	16.50	16.17	19.23	21.27	18.29±1.21
-45	21.26	25.29	18.69	24.33	22.39±1.50
-30	23.02	19.18	22.31	21.26	21.44±0.84
-15	24.92	25.47	19.78	17.29	21.87±1.99
0	18.28	19.08	14.52	22.14	18.51±1.57
15	27.21	22.69	18.54	25.71	23.54±1.91
30	19.25	24.41	23.97	22.19	22.46±1.17
45	23.51	29.89	21.97	27.15	25.63±1.79
60	19.29	23.88	19.72	21.81	21.18±1.06
75	22.17	18.92	24.29	23.12	22.13±1.15
90	18.87	13.31	17.27	21.53	17.75±1.72
105	16.89	18.29	21.66	17.69	18.63±1.05
120	19.14	21.71	20.17	22.19	20.80±0.70

Table 4.2. Individual and mean (±SEM) plasma insulin concentrations (µIU/ml) before and after NMS administration (at 0 min) in normal fed adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	15.21	19.70	21.41	19.75	19.02±1.33
-45	19.03	24.31	27.11	24.59	23.76±1.70
-30	20.70	22.18	18.95	23.79	21.41±1.03
-15	24.45	27.95	23.61	27.99	26.00±1.15
0	18.28	19.59	26.07	31.43	23.84±3.05
15	22.36	23.51	29.35	27.13	25.59±1.61
30	17.06	21.24	23.97	28.77	22.76±2.46
45	25.41	24.15	19.21	21.89	22.67±1.36
60	16.48	18.08	21.21	30.66	21.61±3.17
75	19.32	16.91	14.23	22.96	18.36±1.85
90	23.10	13.31	19.67	25.28	20.34±2.61
105	17.55	18.23	22.53	19.57	19.47±1.10
120	14.80	24.07	15.10	28.29	20.57±3.35

P>0.05 vs 0 min sample ((ANOVA followed by post hoc Dunnett's test).

Table 4.3. Individual and mean (±SEM) plasma insulin concentrations (µIU/ml) before and after saline administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	2.87	2.02	1.76	1.90	2.14±0.25
-45	2.69	2.34	1.89	2.05	2.24±0.18
-30	1.98	2.14	2.41	2.49	2.26±0.12
-15	2.22	1.91	1.71	2.39	2.06±0.15
0	1.94	2.23	2.24	2.53	2.24±0.12
15	2.26	2.51	2.72	1.95	2.36±0.17
30	2.75	2.02	2.18	1.77	2.18±0.21
45	1.99	1.82	2.07	2.16	2.01±0.07
60	1.72	2.10	1.92	2.40	2.04±0.14
75	1.84	2.19	1.81	2.12	1.99±0.10
90	1.64	1.80	1.93	1.88	1.81±0.06
105	1.92	1.69	2.11	2.09	1.95±0.10
120	1.43	1.52	1.92	2.15	1.76±0.17

Table 4.4. Individual and mean (±SEM) plasma insulin concentrations (µIU/ml) before and after NMS administration (at 0 min) in 48-hrs fasting adult male monkeys (n=4).

Time (Min)	Animal Numbers				Mean±SEM
	201	202	203	204	
-60	2.29	2.50	2.12	1.89	2.20±0.13
-45	2.51	2.47	2.01	1.98	2.24±0.14
-30	2.57	2.3	2.19	1.71	2.19±0.18
-15	2.44	2.36	2.11	1.63	2.14±0.18
0	2.37	2.13	2.02	1.88	2.10±0.10
15	2.21	2.42	2.19	2.01	2.21±0.08
30	2.34	2.12	2.64	2.33	2.36±0.11
45	2.10	2.31	2.19	1.99	2.15±0.07
60	1.93	1.81	1.61	1.62	1.74±0.08
75	2.06	1.89	1.76	1.66	1.84±0.09
90	1.97	2.27	2.16	1.73	2.03±0.12
105	1.73	2.27	1.68	2.25	1.98±0.16
120	1.79	2.29	2.36	1.95	2.10±0.14

P>0.05 vs 0 min sample ((ANOVA followed by post hoc Dunnett's test).

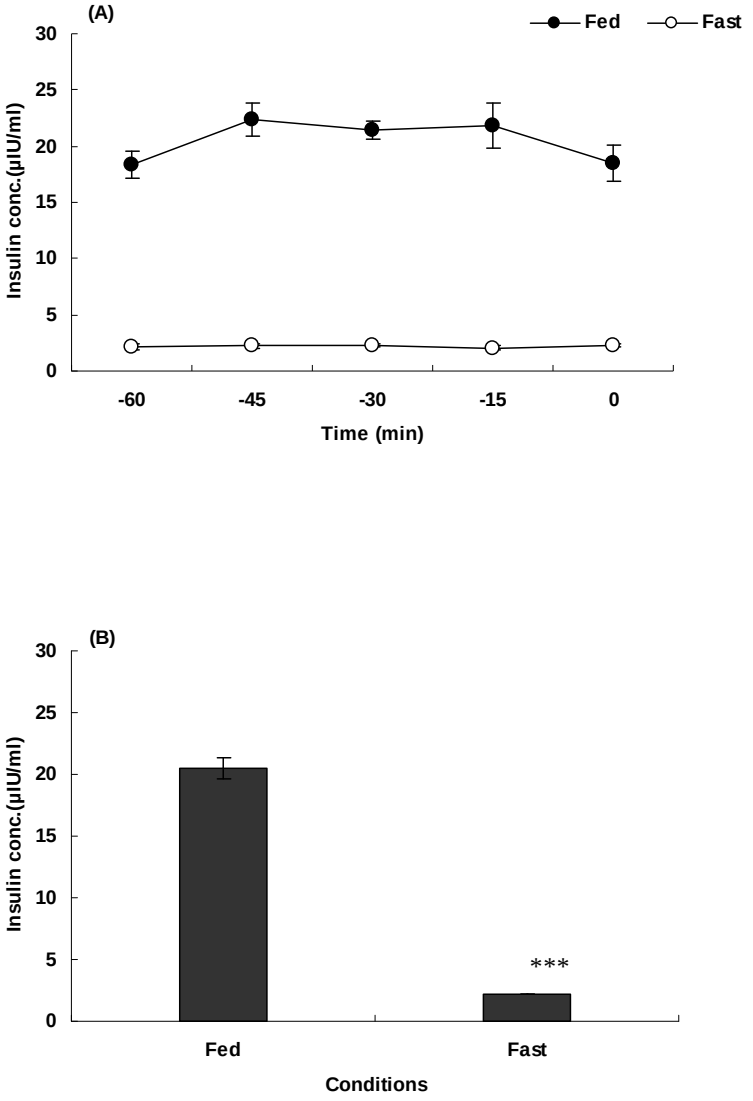


Figure 4.1. (A) Changes in mean (±SEM) basal plasma insulin concentrations (µIU/ml) in 1-hr period in normal fed, and 48-hrs fasting adult male monkeys. (**B**) Overall mean (±SEM) basal plasma insulin concentrations (µIU/ml) in 1-hr period in normal fed, and 48-hrs fasting adult male monkeys. ***P<0.001 vs fed (Student's t test).

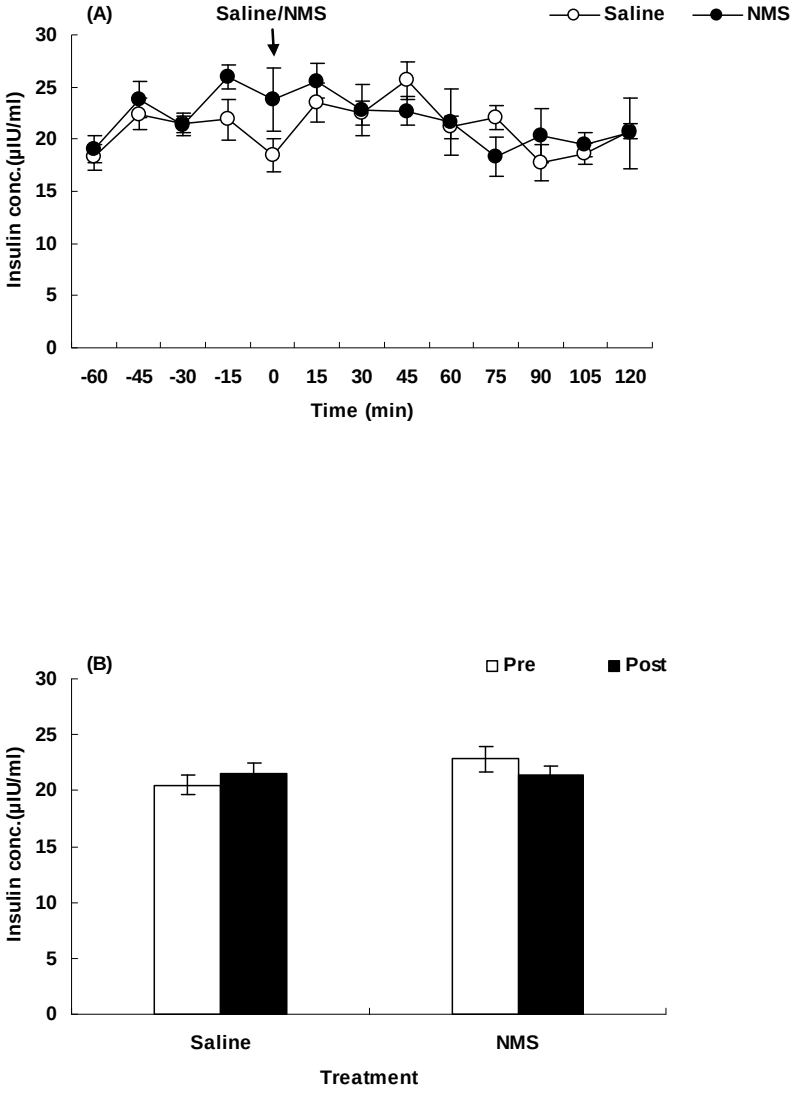


Figure. 4.2. (A) Mean (±SEM) changes in plasma insulin levels (µIU/ml) before and after saline/NMS administration (at 0 min) in normal fed adult male monkeys. **(B)** Comparison of mean (±SEM) plasma insulin levels (µIU/ml) in 60 min pre- and 120 min post saline/NMS in fed adult male monkeys. P>0.05 vs pre-treatment (Student's t test).

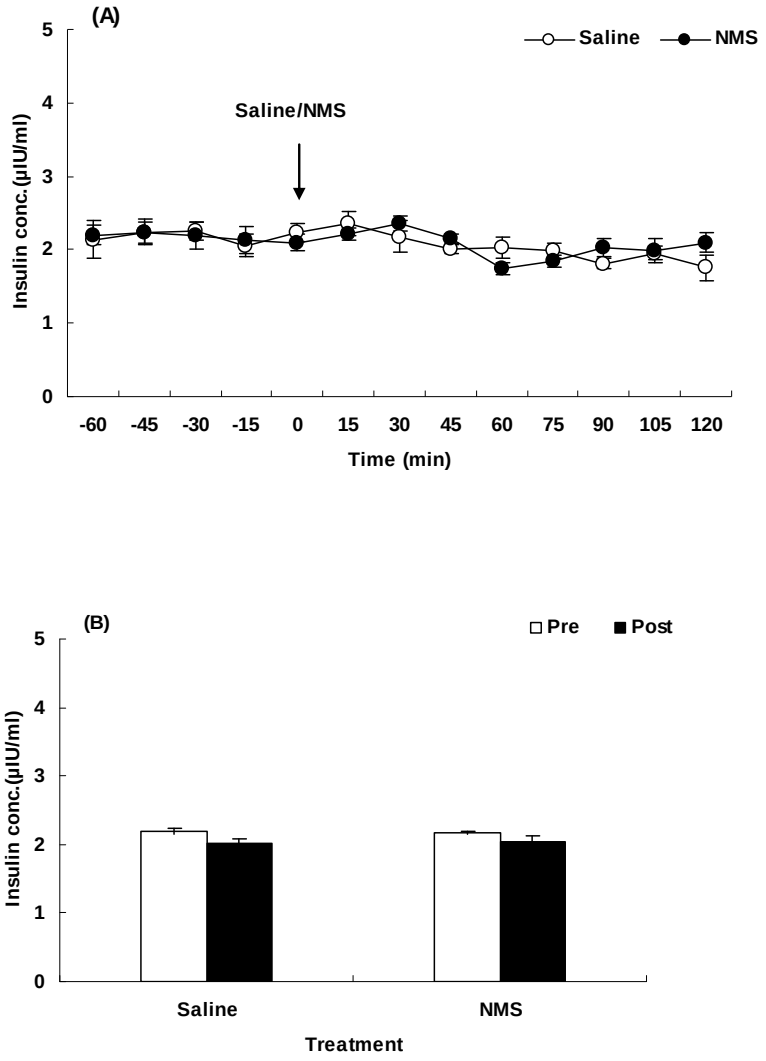


Figure. 4.3. (A) Mean (±SEM) changes in plasma insulin levels (µIU/ml) before and after saline/NMS administration (at 0 min) in 48-hrs fasting adult male monkeys. **(B)** Comparison of mean (±SEM) plasma insulin levels (µIU/ml) in 60 min pre- and 120 min post saline/NMS in 48-hrs fasting adult male monkeys. P>0.05 vs pre-treatment (Student's t test).

Discussion

In this study, the peripheral effect of NMS on insulin regulation in normal fed and nutritionally suppressed adult male rhesus monkeys was investigated. Insulin is very important adiposity signal. It is believed that insulin might be involved in long time food intake regulator and storage of energy. Insulin and its receptors were identified in rat brain, including ARC in hypothalamus, olfactory bulbs and hippocampus (DeFronzo, 1988). Similar findings were also obtained for the human brain (Tanaka *et al*., 1998). Presence of insulin receptors in these brain areas suggesting its role in food intake, reproductive functions, glucoregulation, learning and memory (Stockhorst *et al*., 2004). The increased insulin levels at ARC region in the hypothalamus, reduced food intake and body weight. It was suggested that insulin stimulates POMC gene expression at POMC neurons and regulates its response on feeding behaviour. NMS was also considered to induce its anorexigenic effect through this neuronal system (Ida et al., 2005). So it was hypothesized that NMS and insulin might have some synergistic role in food regulation or NMS have some stimulatory effect on insulin secretion.

In our study, 48-hrs fasting significantly reduced ($P<0.001$) plasma insulin levels compared to normal fed monkeys. This was expected as insulin levels are related to glucose circulation in blood. Short term fasting reduced blood glucose levels, which rendered the decline in insulin secretion. To our dismay, exogenous NMS administration did not cause any stimulatory or inhibitory effect on insulin secretion in both normal fed and 48-hrs fasted monkeys. These results indicate the absence of NMU2R receptors on pancreatic cells in rhesus monkeys. It further suggests that NMS pathway in metabolic regulation and reproduction is independent of insulin secretion.

In summary, we conclude that although both NMS and insulin act as anorexigenic peptides and utilize same hypothalamic neuronal system in food regulation and energy metabolism but exogenous NMS action in this regard looks independent of insulin involvement. On the basis of this single study, it is very difficult to confirm as we don’t know the relationship between endogenous NMS and insulin in regulation of energy metabolism. So further studies are required to explore

the exact mechanism and pathways for NMS in metabolic and reproductive processes, and to investigate the relationship between NMS and other metabolic hormones.

References

- **Abbott DH** 1993 Social conflict and reproductive suppression in marmoset and tamarin monkeys. In: Mason WA, Mendoza SP (eds). Primate social conflict. Albany: *SUNY Press*. pp: 331-373.
- **Abney TO** 1999 The potential roles of estrogens in regulating Leydig cell development and function. *Steroids.* 64: 610-617.
- **Aguilera G, Rabadan-Diehl C and Nikodemova M** 2001 Regulation of pituitary corticotropin releasing hormone receptors. *Peptides*. 22: 69-74.
- **Ahima RS and Lazar MA** 2008 Adipokines and the peripheral and neural control of energy balance. *Mol Endocrinol.* 22: 1023-1031.
- **Ahima RS, Prabakaran D, Mantzoros C, Qu DQ, Lowell B, Maratos-Flier E and Flier JS** 1996 Role of leptin in the neuroendocrine response to fasting. *Nature.* 382: 250-252.
- **Alkén J, Petriczko E and Marcus C** 2008 Effect of fasting on young adultswho have symptoms of hypoglycemia in the absence of frequent meals. *Eur J Clin Nutr*. 62: 721-726.
- **Alois JA, Bergendahl M, Iranmanesh A and Veldhuis JD** 1997 Pulsatile intravenous gonadotropin-releasing hormone administration averts fasting-induced hypogonadotropism and hypoandrogenemia in healthy, normal weight men. *J Clin Endocrinol Metab.* 82: 1543-1548.
- **Alonso G, Szafarczyk A, Balmefrezol M and Assenmacher I** 1986 Immunocytochemical evidence for stimulatory control by the ventral noradrenergic bundle of parvocellular neurons of the paraventricular nucleus secreting corticotropin releasing hormone and vasopressin in rats. *Brain Res.* 397: 297-307.
- **Amstalden M, Garcia MR, Williams SW, Stanko RL, Nizielski SE, Morrison CD, Keisler DH and Williams GL** 2000 Leptin gene expression, circulating leptin, and luteinizing hormone pulsatility are acutely responsive

to short-term fasting in prepubertal heifers: relationships to circulating insulin and insulin-like growth factor I. *Biol Reprod*. 63: 127-133.

- **Antoni FA, Palkovits M, Makara GB, Linton EA, Lowry PJ and Kiss JZ** 1983 Immunoreactive corticotropin-releasing hormone in the hypothalamoinfundibular tract. *Neuroendocrinology.* 36: 415-423.

- **Arey BJ and Freeman ME** 1989 Hypothalamic factors involved in the endogenous stimulatory rhythm regulating prolactin secretion. *Endocrinology.* 124: 878-883.

- **Arey BJ and Freeman ME** 1992 Activity of oxytocinergic neurons in the paraventricular nucleus mirrors the periodicity of the endogenous stimulatory rhythm regulating prolactin secretion. *Endocrinology.* 130: 126-132.

- **Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T and Matsuzawa Y** 1999 Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun.* 257: 79-83.

- **Banks WA, McLay RN, Kastin AJ, Sarmiento U and Scully S** 1999 Passage of leptin across the blood-testis barrier. *Am J Physiol.* 276: 1099-1104.

- **Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, Clifton DK and Steiner RA** 1996 Leptin is a metabolic signal to the reproductive system. *Endocrinology.* 137: 3144-3147.

- **Barb CR and Kraeling RR** 2004 Role of leptin in the regulation of gonadotropin secretion in farm animals. *Anim Reprod Sci*. 82–83: 155-167.

- **Barb CR, Barrett JB, Kraeling RR and Rampacek GB** 2001b Serum leptin concentrations, luteinizing hormone and growth hormone secretion during feed and metabolic fuel restriction in the prepuberal gilt. *Domest Anim Endocrinol.* 20: 47-63.

- **Barb CR, Kraeling RR and Rampacek GB** 2002 Metabolic regulation of the neuroendocrine axis in pigs. *Reprod Suppl.* 59: 203-217.
- **Barrett-Connor E** 1992 Lower endogenous androgen levels and dyslipidemia in men with non-insulin-dependent diabetes mellitus. *Ann Intern Med.* 117: 807-811.
- **Bartke A** 2004 Prolactin in the male: 25 years later. *J Androl.* 25: 661-666.
- **Bartke A, Hafiez AA, Bex FJ and Dalterio S** 1978 Hormonal interactions in regulation of androgen secretion. *Biol Reprod*. 18: 44-54.
- **Bartke A, Klemcke H and Matt K** 1986 Effects of physiological and abnormally elevated prolactin levels on the pituitary–testicular axis. *Med Biol.* 63: 264-272.
- **Beer SF, Bircham PM, Bloom SR, Clark PM, Hales CN, Hughes CM, Jones CT, Marsh DR, Raggatt PR and Findlay AL** 1989 The effect of a 72-h fast on plasma levels of pituitary, adrenal, thyroid, pancreatic and gastrointestinal hormones in healthy men and women. *J Endocrinol*. 120: 337-350.
- **Ben-Jonathan N, Arbogast LA and Hyde JF** 1989 Neuroendocrine regulation of prolactin release. *Prog Neurobiol*. 33: 399-447.
- **Benoit S, Schwartz M, Baskin D, Woods SC and Seeley RJ** 2000 CNS melanocortin system involvement in the regulation of food intake. *Horm Behav*. 37: 299-305.
- **Bercovitch FB and Clarke AS** 1995 Dominance Rank, Cortisol Concentrations, and Reproductive Maturation Male Rhesus Macaques. *Physiol Behav*. 58: 215-221.
- **Bergendahl M, Evans WS, Pastor C, Patel A, Iranmanesh A and Veldhuis JD** 1999 Short-term fasting suppresses leptin and (conversely) activates disorderly growth hormone secretion in midluteal phase women-a clinical research center study. *J Clin Endocrinol Metab*. 84: 883-894.

- **Bergendahl M, Perheentupa A and Huhtainemi I** 1991 Starvation-induced suppression of pituitary-testicular function in rats is reversed by pulsatile gonadotropin-releasing hormone substitution. *Biol Reprod.* 44: 413-419.
- **Bergendahl M, Wiemann J, Clifton DK, Huhtaniemi I and Steiner R** 1992 Short-term starvation decreases POMC mRNA but does not alter GnRH mRNA in the brain of adult male rats. *Neuroendocrinology.* 56: 913-920.
- **Berk ML and Finkelstein JA** 1981 An autoradiographic determination of the efferent projections of the suprachiasmatic nucleus of the hypothalamus. *Brain Res.* 226: 1-13.
- **Bernardis LL and Bellinger LL** 1993 The lateral hypothalamic area revisited: neuroanatomy. Body weight regulation, neuroendocrinology and metabolism. *Neurosci Biobehav Rev.* 17: 141-193.
- **Bernardis LL and Bellinger LL** 1998 The dorsomedial hypothalamic nucleus revisited: 1998 update. *Proc Soc Exp Biol Med.* 218: 284-306.
- **Bernasconi S, Torresani T and Illig R** 1975 The Effect of α-MSH on Plasma Growth Hormone, Cortisol and TSH in Children. *J Clin Endocrinol Metab.* 40: 759-763.
- **Bertram R, Egli M, Toporikova N and Freeman ME** 2006 A mathematical model for the mating-induced prolactin rhythm of female rats. *Am J Physiol*. 290: 573-582.
- **Biederman J, Baldessarini RJ, Harmatz JS, Rivinus TM, Arana GW, Herzog DB and Schildkraut U** 1986 Heterogeneity in anorexia nervosa. *Biol Psychiatry*. 21: 213-216.
- **Blache D, Celi P, Blackberry MA, Dynes RA and Martin GB** 2000 Decrease in voluntary feed intake and pulsatile luteinizing hormone secretion after ICV infusion of recombinant bovine leptin immature male sheep. *Reprod Fertil Develop*. 12: 373-381.
- **Blache D, Chagas LM, Blackberry MA, Vercoe PE and Martin GB** 2000

Metabolic factors affecting the reproductive axis in male sheep. *J Reprod Fertil.* 120: 1-11.

- **Bole-Feysot C, Goffin V, Edery M, Binart N and Kelly PA** 1998 Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev.* 19: 225-268.

- **Bottner A, Kratzsch J, Muller G, Kapellen TM, Bluher S, Keller E, Bluher M and Kiess W** 2004 Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. *J Clin Endocrinol Metab.* 89: 4053-4061.

- **Bowe JE, King AJ, Kinsey-Jones JS, Foot VL, Li XF, O'Byrne KT, Persaud SJ and Jones PM** 2009 Kisspeptin stimulation of insulin secretion: mechanisms of action in mouse islets and rats. *Diabetologia* 52: 855-862.

- **Bray GA** 1997 Obesity and reproduction. *Hum Reprod.* 12: 26-32.

- **Bricaire H, Strauch G, Girault D and Rifaï M** 1973 Alpha-MSH stimulation of growth hormone release. *J Clin Endocrinol Metab*. 37: 990-993.

- **Brighton PJ, Szekeres PG and Willars GB** 2004 Neuromedin U and its receptors: structure, function, and physiological roles. *Pharmacol Rev*. 56: 231-248.

- **Bronson FH** 1985 Mammalian reproduction: an ecological perspective. *Biol Reprod.* 32: 1-26.

- **Bronson FH and Manning JM** 1991 The energetic regulation of ovulation: a realistic role for body fat. *Biol Reprod.* 44: 945-950.

- **Brown RE, Imran SA, Ur E and Wilkinson M** 2008 KiSS-1 mRNA in adipose tissue is regulated by sex hormones and food intake. *Mol Cell Endocrinol.* 281: 64-72.

- **Bruning JC, Gautam D and Burks DJ** 2000 Role of brain insulin receptor in control of body weight and reproduction. *Science*. 289: 2122-2125.

- **Bucholtz DC, Vidwans NM, Herbosa CG, Schillo KK and Foster DL** 1996

Metabolic interfaces between growth and reproduction. Pulsatile luteinizing hormone secretion is dependent on glucose availability. *Endocrinology.* 137: 601-607.

- **Buijs RM, Markman M, Nunes-Cardoso B, Hou YX and Shinn S** 1993 Projections of the suprachiasmatic nucleus to stress-related areas in the rat hypothalamus: a light and electron microscopic study. *J Comp Neurol*. 335: 42-54.

- **Buijs RM, van Eden CG, Goncharuk VD and Kalsbeek A** 2003 The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system. *J Endocrinol.* 177: 17-26.

- **Buijs RM, Wortel J, Van Heerikhuize JJ, Feenstra MG, Ter Horst GJ, Romijn HJ and Kalsbeek A** 1999 Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus adrenal (cortex) pathway. *Eur J Neurosci*. 11: 1535-1544.

- **Burcelin R, Dolci W and Thorens B** 2000 Portal glucose infusion in the mouse induces hypoglycemia: evidence that the hepatoportal glucose sensor stimulates glucose utilization. *Diabetes.*49:1635-1642.

- **Cameron JL** 1996 Regulation of reproductive hormone secretion in primates by short-term changes in nutrition. *Rev Reprod.* 1: 117-26.

- **Cameron JL, Helmreich DL and Schreihofer DA** 1993 Modulation of reproductive hormone secretion by nutritional intake: stress signals versus metabolic signals. *Hum Reprod.* 8: 162-167.

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

- **Caminos JE, Nogueiras R, Gaytán F, Pineda R, González CR, Barreiro ML, Castaño JP, Malagón MM, Pinilla L, Toppari J, Diéguez C and Tena-Sempere M** 2008 Novel expression and direct effects of adiponectin in the rat testis. *Endocrinology.* 149: 3390-3402.

- **Campbell CA, Kurcz M, Marshall S and Meites J** 1977 Effects of starvation in rats on serum levels of follicle stimulating hormone, luteinizing hormone, thyrotropin, growth hormone and prolactin; response to LH-releasing hormone and thyrotropin-releasing hormone. *Endocrinology.* 100: 580-587.

- **Caprio M, Isidori AM, Carta AR, Moretti C, Dufau ML and Fabbri A** 1999 Expression of functional leptin receptors in rodent Leydig cells. *Endocrinology*. 140: 4939-47.

- **Casanueva FF and Dieguez C** 1999 Neuroendocrine regulation and actions of leptin. *Front Neuroendocrinol*. 20: 317-363.

- **Cascio CS, Shinsako J and Dallman MF** 1987 The suprachiasmatic nuclei stimulate evening ACTH secretion in the rat. *Brain Res*. 423: 173-178.

- **Casper RF And Yen SS** 1981 Simultaneous pulsatile release of prolactin and luteinizing hormone induced by luteinizing hormone-releasing factor agonist. *J Clin Endocrinol Metab.* 52: 934-936.

- **Castellano JM, Navarro VM, Fernandez-Fernandez R, Nogueiras R, Tovar S, Roa J, Vazquez MJ, Vigo E, Casanueva FF, Aguilar E, Pinilla L, Dieguez C and Tena-Sempere M** 2005 Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. *Endocrinology.* 146:3917-3925.

- **Castellano JM, Navarro VM, Fernandez-Fernandez RR, Roa J, Vigo E, PinedaR, Steiner RA, Aguilar E, Pinilla L and Tena-Sempere M** 2006 Effects of galanin like peptide on luteinizing hormone secretion in the rat: Sexually dimorphic responses and enhanced sensitivity at male puberty. *Am J Physiol Endocrinol Metab.* 291: 1281-1289.

- **Chakravarty I, Sreedhar R, Gosh KK and Bulusu S** 1982 Circulating gonadotropin profiles in severe cases of protein-calorie malnutrition. *Fertil Steril.* 37: 650-654.

- **Chandrashekar V and Bartke A** 1998 The role of growth hormone in the

control of gonadotropin secretion in adult male rats. *Endocrinology.* 139: 1067-1074.

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

- **Chu C, Jin Q, Kunitake T, Kato K, Nabekura T, Nakazato M, Kangawa K and Kannan H** 2002 Cardiovascular actions of central neuromedin U in conscious rats. *Regul Pept*. 105: 29-34.

- **Cleary MP, Bergstrom HM, Dodge TL, Getzin SC, Jacobson MK and Phillips FC** 2001 Restoration of fertility in young obese (Lep (ob) Lep (ob)) male mice with low dose recombinant mouse leptin treatment. *Int J Obes Relat Metab Disord.* 25: 95-97.

- **Combs TP, Berg AH, Rajala MW, Klebanov S, Iyengar P, Jimenez-Chillaron JC, Patti ME, Klein SL, Weinstein RS and Scherer PE** 2003 Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. *Diabetes.* 52: 268-276.

- **Cotman CW and Iversen LL** 1987 Excitatory amino acids in the brain. Focus on NMDA receptors. *Trend Neurosci.* 10: 263-265.

- **Crewal T, Miekelsen D and Hafs HD** 1971 Androgen secretion and spermatogenesis in rats following semi-starvation. *Proc Soc Exp Biol Med.* 138: 723-727.

- **Cunningham MJ, Clifton DK and Steiner RA** 1999 Leptin's actions on the reproductive axis: perspectives and mechanisms. *Biol Reprod*. 60:616-622.

- **Darzy KH, Murray RD, Gleeson HK, Pezzoli SS, Thorner MO and Shalet SM** 2006 The impact of short-term fasting on the dynamics of 24-hour growth hormone (GH) secretion in patients with severe radiation-induced GH deficiency. *J Clin Endocrinol Metab*. 91: 987-994.

- **De Rosa M, Zarrilli S, Di Sarno A, Milano N, Gaccione M, Boggia B,**

Lombardi G and Colao A 2003 Hyperprolactinemia in men: clinical and biochemical features and response to treatment. *Endocrine.* 20: 75- 82.

- **DeFronzo RA** 1988 The triumvirate: β-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes.* 37: 667-687.

- **Delaere F, Magnan C and Mithieux G** 2010 Hypothalamic integration of portal glucose signals and control of food intake and insulin sensitivity. *Diabetes Metab*. 36: 257-262.

- **Delgado H, Lechtig A, Martorell R, Brineman E and Klein RE** 1978 Nutrition, lactation and postpartum amenorrhea. *Am J Clin Nutr.* 31: 322-327.

- **Denef C and Andries M** 1983 Evidence for paracrine interation between gonadotrophs and lactotrophs in pituitary cell aggregates. *Endocrinology.* 112: 813-822.

- **Dombrowicz D, Sente B, Closset J and Hennen G** 1992 Dose-dependent effects of human prolactin on the immature hypophysectomized rat testis. *Endocrinology.* 130: 695-700.

- **Dubey AK, Cameron JL, Steiner RA and Plant TM** 1986 Inhibition of gonadotropin secretion in castrated male rhesus monkey (Macaca mulatta) induced by dietary restriction. Analogy with the prepubertal hiatus of gonadotropin releae. *Endocrinology.* 118: 518-525.

- **Dubuc I, Nouel D, Coquerel A, Menard JF, Kitabgi P and Costentin J** 1988 Hypothermic effect of neuromedin N in mice and its potentiation by peptidase inhibitors. *Eur J Pharmacol.* 151: 117-121.

- **Dupont A, Cusan L, Garon M, Labrie F and Hao L C** 1976 β-Endorphin: Stimulation of growth hormone release in vivo. *Proc Natl Acad Sci.* 74: 358-359.

- **Dyer RG, Mansfield S, Corbet H and Dean ADP** 1985 Fasting impairs LH secretion in female rats by activating an inhibitory opioid pathway. *J Endocrinol*. 105: 91-97.

- **Easdon MP, Chesworth JM, Aboul-ela MBA and Henderson GD** 1985 The effect of undernutrition of beef cows on blood hormone and metabolite concentrations postpartum. *Reprod Nutr Dev.* 25: 113-126.

- **Ebling FJ** 2005 The neuroendocrine timing of puberty. *Reproduction.* 129: 675-683.

- **Ebling FJP, Wood RI, Karsch FJ, Vannerson LA, Sutties JM, Bucholtz DC, Schall RE and Foster DL** 1990 Metabolic interfaces between growth and reproduction. III central mechanisms controlling pulsatile luteinizing hormone secretion in the nutritionally growth limited female lamb. *Endocrionology.* 126: 2719-2727.

- **Echternkamp SE, Ferrell CL and Rone JD** 1982 Influence of prepartum and postpartum nutrition on luterinizing hormone secretion is suckled postpartum beef heifers. *Theriogenology.* 18: 283-296.

- **Egli M, Bertram R, Sellix MT and Freeman ME** 2004 Rhythmic secretion of prolactin in rats: action of oxytocin coordinated by vasoactive intestinal polypeptide of suprachiasmatic nucleus origin. *Endocrinology.* 145: 3386-3394.

- **Eisenberg E** 1981 Toward an understanding of reproductive function in anorexia nervosa. *Fertil Steril.* 36: 543-550.

- **Engeland WC and Arnhold MM** 2005 Neural circuitry in the regulation of adrenal corticosterone rhythmicity. *Endocrinology*. 28: 325-332.

- **Everitt BJ and Hokfelt T** 1990 Neuroendocrine anatomy of the hypothalamus. *Acta Neurochir*. 47: 1-15.

- **Fan W, Yanase T, Nomura M, Okabe T, Goto K, Sato T, Kawano H, Kato S and Nawata H** 2005 Androgen receptor null male mice develop late-onset obesity caused by decreased energy expenditure and lipolytic activity but show normal insulin sensitivity with high adiponectin secretion. *Diabetes.* 54: 1000-1008.

- **Fischer-Posovszky P, Wabitsch M and Hochberg Z** 2007 Endocrinology of

adipose tissue - an update. *Horm Metab Res.* 39: 314-321.

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

- **Foster DL, Ebling FJ, Micka AF, Vennerson LA, Bucholtz DC, Wood RI, Suttie JM and Fenner DE** 1989 Metabolic interfaces between growth and reproduction. Nutritional modulation of gonadotropin, prolactin and growth hormone secretion in the growth-limited female lamb. *Endocrinology.* 125: 342-350.

- **Foster DL, Nagatani S, Buchlotz DC, Tsukamura H, Tanaka T and Maeda** K 1998 Metabolic links between nutrition and reproduction: signals, sensors and pathways controlling GnRH secretion. In: Hansel W, Gray GA, Ryan DH (eds). Nutrition and Reproduction. Baton Rouge, LA: *Penington Biomedical Research Foundation, Lousiana state University Press.* pp: 59-80.

- **Franks S** 1998 Growth hormone and ovarian function. *Baillieres Clin Endocrinol Metab.* 12: 331-340.

- **Freeman ME, Kanyicska B, Lerant A and Nagy G** 2000 Prolactin: structure, function, and regulation of secretion. *Physiol Rev.* 80: 1523-1631.

- **Fujii R, Hosoya M, Fukusumi S, Kawamata Y, Habata Y, Hinuma S, Onda H, Nishimura O and Fujino M** 2000 Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3. *J Biol Chem*. 275: 21068-21074.

- **Geisthoevel F, Arana JB, Balmaceda JP, Rojas FJ and Hasch R** 1988 Prolactin and gonadotrophin dynamics in response to antagonists of LHRH and dopamine in ovariectomized rhesus monkeys: a dissection of their common secretion. *Hum Reprod.* 3: 591-596.

- **Gerhold LM, Horvath TL and Freeman ME** 2001 Vasoactive intestinal peptide fibers innervate neuroendocrine dopaminergic neurons. *Brain Res*. 919: 48-56.

- **Gill CJ and Rissmann EF** 1997 Female sexual behavior is inhibited by short- and long-term food restriction. *Physiol Behav.* 61: 387-394.
- **Gnessi L, Fabbri A and Spera G** 1997 Gonadal peptides as mediators of development and functional control of the testis: an integrated system with hormones and local environment. *Endocr Rev.* 18: 541-609.
- **Gonzalez RR, Simon C, Caballero-Campo P, Norman R, Chardonnens D, Devoto L and Bischof P** 2000 Leptin and reproduction. *Hum Reprod Update.* 6: 290-300.
- **Gottsch ML, Clifton DK and Steiner RA** 2004 Galanin-like peptide as a link in the integration of metabolism and reproduction. *Trends Endocrinol Metabol.* 15: 215-221.
- **Goudreau JL, Falls WM, Lookingland KJ and Moore KE** 1995 Periventricular- hypophysial dopaminergic neurons innervate the intermediate but not the neural lobe of the rat pituitary gland. *Neuroendocrinology.* 62: 147-154.
- **Guan XM, Yu H, Jiang Q, Van Der Ploeg LH and Liu Q** 2001 Distribution of neuromedin U receptor subtype 2 mRNA in the rat brain. *Brain Res Gene Expr Patterns*. 1: 1-4.
- **Guevara R, Valle A, Gianotti M, Roca P and Oliver J** 2008 Gender-dependent differences in serum profiles of insulin and leptin in caloric restricted rats. *Horm Metab Res.* 40: 38-43.
- **Hair WM, Gubbay O, Jabbour HN and Lincoln GA** 2002 Prolactin receptor expression in human testis and accessory tissues: localization and function. *Mol Hum Reprod*. 8: 606-611.
- **Hamilton GD and Bronson FH** 1985 Food restriction and reproductive development in wild house mice. *Biol Reprod.* 32: 773-778.
- **Hamilton GD and Bronson FH** 1986 Food restriction and reproductive development: male and female mice and male rats. *Am J Physiol.* 250: 370-376.

- **Hanada R, Nakazato M, Murakami N, Sakihara S, Yoshimatsu H, Toshinai K, Hanada T, Suda T, Kangawa K, Matsukura S and Sakata T** 2001 A role for neuromedin U in stress response. *Biochem Biophys Res Commun*. 289: 225-228.

- **Hanada T, Date Y, Shimbara T, Sakihara S, Murakami N, Hayashi Y, Kanai Y, Suda T, Kangawa K and Nakazato M** 2003 Central actions of neuromedin U via corticotropin-releasing hormone. *Biochem Biophys Res Commun*. 311: 954-958.

- **Hauge-Evans AC, Richardson CC, Milne HM, Christie MR, Persaud SJ and Jones PM** 2006 A role for kisspeptin in islet function. *Diabetologia* 49: 2131-2135.

- **Heindel JJ and Treinen KA** 1989 Physiology of the male reproductive system: endocrine, paracrine and autocrine regulation. *Toxicol Pathol*. 17: 411-445.

- **Helke CJ, Krause JE, Mantyh PW, Couture R and Bannon MJ** 1990 Diversity in mammalian tachykinin peptidergic neurons: multiple peptides, receptors, and regulatory mechanisms. *FASEB J.* 4: 1606-15.

- **Helmreich DL and Cameron JL** 1992 Suppression of luteinizing hormone secretion during food restriction in male rhesus monkey (*Macaca mulatta*): Failure of naloxone to restore normal pulsatility. *Neuroendocrinology.* 54: 464-473.

- **Helmreich DL, Mattern LG and Cameron JL** 1993 Lack of a role of the hypothalamic-pituitary-adrenal axis in the fasting-induced suppression of luteinizing hormone secretion in adult male rhesus monkeys (*Macaca mulatta*). *Endocrinology.* 132: 2427-2437.

- **Henry BA, Goding JW, Alexander WS, Tilbrook AJ, Canny BJ, Dunshea F, Rao A, Mansell a and Clarke IJ** 1999 Central administration of leptin to ovariectomized ewes inhibits food intake without affecting the secretion of hormones from pituitary gland: evidence for a dissociation of effects on

appetite and neuroendocrine function. *Endocrinology.* 140: 1175-1182.

- **Henry BA, Goding JW, Tilbrook AJ, Dunshea F and Clarke IJ** 2001 Intracerebroventricular infusion of leptin elevates the secretion of luteinizing hormone without affecting food intake in long-term food-restricted sheep, but increases growth hormone irrespective of bodyweight. *J Endocrinol*. 168: 67-77.

- **Henry BA, Goding JW, Tilbrook AJ, Dunshea FR and Clarke IJ** 2001 Intracerebroventricular infusion of leptin elevates the secretion of luteinising hormone without affecting food intake in long-term food-restricted sheep, but increases growth hormone irrespective of bodyweight. *J Endocrinol*. 168: 67-77.

- **Hernandez ME, Soto-Cid A, Rojas F, Pascual LI, Aranda-Abreu GE, Toledo R, Garcia LI, Quintanar-Stephano A and Manzo J** 2006 Prostate response to prolactin in sexually active male rats. *Reprod Biol Endocrinol*. 4: 1-12.

- **Higley JD, Mehlman PT, Taub DM, Higley SB, Suomi SL, Linnoila M and Vickers JH** 1992 Cerebrospinal fluid monoamine and adrenal correlates of aggression in free-ranging rhesus monkeys. *Arch Gen Psych*. 49: 436-441.

- **Hill JB, Lacy ER, Nagy GM, Go¨rcs TJ and Frawley LS** 1993 Does alpha-melanocyte- stimulating hormone from the pars intermedia regulate suckling-induced prolactin release? Supportive evidence from morphological and functional studies. *Endocrinology.* 133: 2991-2997.

- **Hill JB, Nagy GM and Frawley LS** 1991 Suckling unmasks stimulatory effect of dopamine on prolactin release: possible role for a-melanocyte-stimulating hormone as a mammotrope responsiveness factor. *Endocrinology.* 129: 843-847.

- **Hoffer LJ, Beitins IZ, Kyung NH and Bistrian BR** 1986 Effects of severe dietary restriction on male reproductive hormones. *J Clin Endocrinol Metab.* 62: 288-292.

- **Holdcraft RW and Braun RE** 2004a Hormonal regulation of spermatogenesis. *Int J Androl*. 27: 335-342.
- **Holness MJ, Munns MJ and Sugden MC** 1999 Current concepts concerning the role of leptin in reproductive function. *Mol Cell Endocrinol*. 157: 11-20.
- **Holzbauer M and Racke´ K** 1985 The dopaminergic innervation of the intermediate lobe and of the neural lobe of the pituitary gland. *Med Biol*. 63: 97-116.
- **Honzawa M, Sudoh T, Minamino N, Tohyama M and Matsuo H** 1987 Topographic localization of neuromedin U-like structures in the rat brain: an immunohistochemical study. *Neuroscience*. 23: 1103-1122.
- **Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T and Matsuzawa Y** 2000 Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol.* 20: 1595-1599.
- **Houshyar H, Gomez F, Bhargava A and Akana SF** 2006 From Malthus to motive: how the HPA axis engineers the phenotype, yoking needs to wants. *Prog Neurobiol*. 79: 247-340.
- **Howland BE** 1972 Effect of restricted food intake on LH levels in female rats. *J Anim Sci.* 34: 445-447.
- **Howland BE** 1975 The influence of food restriction and subsequent refeeding on gonadotropin secretion and serum testosterone levels in male rats. *J Reprod Fertil.* 44: 429-436.
- **Howland BE** 1980 Effect of glucoprivation induced by 2-deoxy-D-glucose on serum gonadotropin levels, pituitary response to GnRH and progesterone-induced release of luteinizing hormone in rats. *Horm Metab Res.* 12: 520-523.
- **Huhtaniemi I and Toppari J** 1995 Endocrine, paracrine and autocrine regulation of testicular steroidogenesis. *Adv Exp Med Biol*. 377: 33-54.

- **Huleihel M and Lunenfeld E** 2004 Regulation of spermatogenesis by aracrine/autocrine testicular factors. *Asian J Androl*. 6: 259-268.
- **Hull KL and Harvey S** 2000 Growth hormone: a reproductive endocrine–paracrine regulator*? Rev Reprod*. 5: 175-182.
- **Hull KL and Harvey S** 2000a. Growth hormone: roles in male reproduction. *Endocrine*. 13: 243-250.
- **Hull KL and Harvey S** 2000b Growth hormone: a reproductive endocrine-paracrine regulator. *Rev Reprod*. 5: 175-182.
- **Hull KL and Harvey S** 2001 Growth hormone: roles in female reproduction. *J Endocrinol.* 168: 1-23.
- **Ichimaru T, Mori Y and Okamura H** 2001 A possible role of neuropeptide Y as a mediator of undernutrition to the hypothalamic gonadotropin-releasing hormone pulse generator in goats. *Endocrinology.* 142: 2489-2498.
- **Ida T, Mori K, Miyazato M, Egi Y, Abe S, Nakahara K, Nishihara M, Kangawa K and Murakami N** 2005 Neuromedin S is a novel anorexigenic hormone. *Endocrinology*. 146: 4217-4223.
- **Iwama S, Sugimura Y, Murase T, Hiroi M, Goto M, Hayashi M, Arima H and Oiso Y** 2009 Central adiponectin functions to inhibit arginine vasopressin release in conscious rats. *J Neuroendocrinol*. 21:753-759.
- **Jahan S, Ahmed S, Shah NA and Hizbullah** 2011 Role of Neuromedin S in plasma testosterone and cortisol concentrations in male adult rhesus monkey (*Macaca mulatta*). *Pak J Zoo*. 43: 147-153.
- **Jaszberenyi M, Bagosi Z, Thurzo B, Foldesi I and Telegdy G** 2007 Endocrine and behavioral effects of Neuromedin S. *Endocrinology*. 14: 631-639.
- **Jennes L, Stumpf WE and Tappaz ML** 1983 Anatomical relationships of dopaminergic and GABAergic systems with the GnRH-systems in the septo-hypothalamic area. *Exp Brain Res.* 50: 91-99.

- **Kadowaki T and Yamauchi T** 2005 Adiponectin and adiponectin receptors. *Endocr Rev.* 26: 439-451.

- **Kaiser GG, Sinowatz F and Palma GA** 2001 Effects of growth hormone on female reproductive organs. *Anat Histol Embryol*. 30: 265-271.

- **Kalra SP, Dube MG, Pu S, Xu B, Horvath TL and Kalra PS** 1999 Interacting appetite- regulating pathways in the hypothalamic regulation of body weight. *Endocr Rev*. 20: 68-100.

- **Kalsbeek A, Buijs RM, van Heerikhuize JJ, Arts M and van der Woude TP** 1992 Vasopressin-containing neurons of the suprachiasmatic nuclei inhibit corticosterone release. *Brain Res.* 580: 62-67.

- **Kalsbeek A, van der Vliet J and Buijs RM** 1996a Decrease of endogenous vasopressin release necessary for expression of the circadian rise in plasma corticosterone: a reverse microdialysis study. *J Neuroendocrinol*. 8: 299-307.

- **Kalsbeek A, van Heerikhuize JJ, Wortel J and Buijs RM** 1996b A diurnal rhythm of stimulatory input to the hypothalamo–pituitary–adrenal system as revealed by timed intrahypothalamic administration of the vasopressin V1 antagonist. *J Neurosci*. 16: 5555-5565.

- **Kawakami M, Uemura T and Hayashi R** 1982 Electrophysiological correlates of pulsatile gonadotropin release in rats. *Neuroendocrinology.* 35: 63-67.

- **Kawano H and Daikoku S** 1987 Functional topography of the rat hypothalamic dopamine neuron systems: retrograde tracing and immunohistochemical study. *J Comp Neurol.* 265: 242-253.

- **Kehoe L, Parman R, Janik J and Callahan P** 1993 Opiate receptor subtype involvement in the stimulation of prolactin release by beta-en-dorphin in female rats. *Neuroendocrinology.* 57: 875-883.

- **Kelly PA, Djiane J, Postel–Vinay MD and Edery M** 1991 The prolactin/ growth hormone receptor family. *Endocr Rev.*12: 235-51.

- **Kennedy GC and Mitra J** 1963 Body-weight and food intake as initiating factors for puberty in the rat. *J Physiol.* 166: 408-418.
- **Kershaw EEFJ** 2004 Adipose tissue as an endocrine organ. *J Clin Endocrinol Metabol*. 89: 2548-2556.
- **Kim KH, Lee K, Moon YS and Sul HS** 2001 A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. *J Biol Chem.* 276: 11252-11256.
- **Knobil E** 1981 Patterns of hypophysiotropic signals and gonadotropin secretion in the rhesus monkey. *Biol Reprod.* 24: 44-49.
- **Knobil E, Plant TM, Wildt L, Belchetz PE and Marshall G** 1980 Control of the rhesus monkey menstrual cycle: permissive role of hypothalamic gonadotropin-releasing hormone. *Science.* 207: 1371-1373.
- **Knuth UA and Friesen HG** 1983 Starvation induced anoestrous: effect of chronic food restriction on body weight, its influence on oestrous cycle and gonadotropin secretion in rats. *Acta Endocrinol.* 104: 402-409.
- **Koegler F, Grove K, Schiffmacher A, Smith M and Cameron J** 2001 Central melanocortin receptors mediate changes in food intake in the rhesus macaque. *Endocrinology.* 142: 2586-2592.
- **Kolaczynski JW, Considine RV, Ohannesian J, Marco C, Opentanova I, Nyce MR, Myint M and Caro JF** 1996 Responses to leptin in short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. *Diabetes.* 45: 1511-1515.
- **Kopelman P** 1992 Neuroendocrine function in obesity. In: Grossman A (eds) Clinical Endocrinology London, UK: *Blackwell Scientific Publications*. pp: 863-867.
- **Kos K, Harte AL, da Silva NF, Tonchev A, Chaldakov G, James S, Snead DR, Hoggart B, O'Hare JP, McTernan PG and Kumar S** 2007 Adiponectin and resistin in human cerebrospinal fluid and expression of

adiponectin receptors in the human hypothalamus. *J Clin Endocrinol Metab.* 92: 1129-1136.

- **Kreier F, Kalsbeek A, Ruiter M, Yilmaz A, Romijn JA, Sauerwein HP, Fliers E, Buijs RM** 2003 Central nervous determination of food storage-a daily switch from conservation to expenditure: implications for the metabolic syndrome. *Eur J Pharmacol*. 480: 51-65.

- **Krsmanović LZ, Stojilković SS, Merelli F, Dufour SM, Virmani MA and Catt KJ** 1992 Calcium signaling and episodic secretion of gonadotropin-releasing hormone in hypothalamic neurons. *PNAS.* 89: 8462-8466.

- **Kulin HE, Bwibo N, Mutie D and Santner SJ** 1984 Gonadotropin excretion during puberty in malnourished children. *J Pediat.* 105: 325-328.

- **Kuo AY, Cline MA, Werner E, Siegel PB and Denbow DM** 2005 Leptin effects on food and water intake in lines of chickens selected for high or low body weight. *Physiol Behav.* 84: 459-464.

- **Lado-Abeal J, Clapper JA, Chen Zhu B, Hough CM, Syapin PJ and Norman RL** 2002 Hypoglycemia-induced suppression of luteinizing hormone (LH) secretion in intact female rhesus macaques: role of vasopressin and endogenous opioids. *Stress.* 5: 113-119.

- **Lado-Abeal J, Hickox JR, Cheung TL, Veldhuis JD, Hardy DM and Norman RL** 2000 Neuroendocrine consequences of fasting in adult male macaques: effects of recombinant rhesus macaque leptin infusion. *Neuroendocrinology.* 71: 196-208.

- **Lado-Abeal J, Lukyanenko YO, Swamy S, Hermida RC, Hutson JC and Norman RL** 1999 Short-term leptin infusion does not affect circulating levels of LH, testosterone or cortisol in food-restricted pubertal male rhesus macaques. *Clin Endocrinol.* 51:41-51.

- **Leonhardt S, Shahab M, Luft H, Wuttke W and Jarry H** 1999 Reduction of luteinzing hormone secretion induced by long-term feed restriction in male rats is associated with increased expression of GABA-synthesizing enzymes

without alterations of GnRH gene expression. *J Neuroendocrinol.* 11: 613-619.

- **Leranth C, Maclusky NJ, Brown TJ, Chen EC, Redmond Jr DE and Naftolin F** 1992 Transmitter content and afferent connections of estrogen-sensitive progestin receptor-containing neurons in the primate hypothalamus. *Neuroendocrinology.* 55: 667-682.

- **Lin J, Barb CR, Kraeling RR and Rampacek GB** 2001 Developmental changes in the long form leptin receptor and related neuropeptide gene expression in the pig brain. *Biol Reprod*. 64: 1614-1618.

- **Lincoln GA and Short RV** 1980 Seosonal breeding: nature's contraceptive. *Recent Prog Horm Res.* 36: 1-52.

- **Lincoln GA, Townsend J and Jabbour HN** 2001 Prolactin actions in the sheep testis: a test of the priming hypothesis. *Biol Reprod*. 65: 936-943.

- **Lobie PE., Breipohl W., Aragon JG and Waters MJ.** 1990. Cellular localization of the growth hormone receptor/binding protein in the male and female reproductive systems. *Endocrinology*. 126: 2214-2221.

- **Lowrey PL and Takahashi JS** 2000 Genetics of the mammalian circadian system: photic entrainment, circadian pacemaker mechanisms, and posttranslational regulation. *Annu Rev Genet*. 34: 533-562.

- **Maeda K, Ohkura S, Uenoyama Y, Wakabayashi Y, Oka Y, Tsukamura H and Okamura H** 2010 Neurobiological mechanisms underlying GnRH pulse generation by the hypothalamus. *Brain Res.* 1364: 103-115.

- **Magnan C, Collins S, Berthault MF, Kassis N, Vincent M, Gilbert M, Pénicaud L, Ktorza A, Assimacopoulos-Jeannet F** 1999 Lipid infusion lowers sympathetic nervous activity and leads to increased beta-cell responsiveness to glucose. *J Clin Invest* 103: 413-419.

- **Magni P, Motta M and Martini L** 2000 Leptin: possible link between food intake, energy expenditure, and reproductive function. *Regul Pept.* 92: 51-56.

- **Mahesh VB and Brann DW** 2005 Regulatory role of excitatory amino acids in reproduction. *Endocrine.* 28: 271-280.
- **Majovski LV, Jacques S, Hartz G and Fogwell LA** 1981 Dopaminergic (DA) systems: their role in pathological neurobehavioral symptoms. *Neurosurgery*. 9: 751-757.
- **Malendowicz LK, Nussdorfer GG, Markowska A, Nowak KW and Torlinski L** 1993 Effects of neuromedin-N on the pituitary– adrenocortical axis of dexamethasone-suppressed rats. *Neuropeptides*. 24: 1-4.
- **Malendowicz LK, Nussdorfer GG, Markowska A, Tortorella C, Nowak M and Warchol JB** 1994 Effects of neuromedin U (NMU)-8 on the rat hypothalamo–pituitary–adrenal axis. Evidence of a direct effect of NMU- 8 on the adrenal gland. *Neuropeptides*. 26: 47-53.
- **Manna PR, El-Hefnawy T, Kero J and Huhtaniemi IT** 2001 Biphasic action of prolactin in the regulation of murine Leydig tumor cell functions. *Endocrinology.* 142: 308-318.
- **Manning JM and Bronson FH** 1989 Effects of prolonged exercise on puberty and luteinizing hormone secretion in female rats. *Am J Physiol.* 257: 1359-1364.
- **Marchal R, Caillaud M, Martoriati A, Gerard N, Mermillod P and Goudet G** 2003. Effect of growth hormone (GH) on in vitro nuclear and cytoplasmic oocyte maturation, cumulus expansion, hyaluronan synthases, and connexins 32 and 43 expression, and GH receptor messenger Rna expression in equine and porcine species. *Biol Reprod*. 69: 1013-1022.
- **Marstellar FA and Lynch CB** 1983 Reproductive consequences of food restriction at low temperature in lines of mice divergently selected for thermoregulatory nesting. *Behav Genet.* 13: 397-410.
- **Mathews LS, Enberg B, and Norstedt G** 1989 Regulation of rat growth hormone receptor gene expression. *J Biol Chem*. 264: 9905-9910.

- **Mayo KE, Godfrey PA, Suhr ST, Kulik DJ and Rahal JO** 1995 Growth hormonereleasing hormone: synthesis and signaling. *Recent Prog Horm Res*. 50: 35-73.

- **Menzaghi F, Heinrichs SC, Merlo-Pich E, Tilders FJ and Koob GF** 1994 Involvement of hypothalamic corticotropin-releasing factor neurons in behavioral responses to novelty in rats. *Neurosci Lett*. 168: 139-142.

- **Merry MJ and Holechan AM** 1979 Onset of puberty and duration of fertility in rats fed on restricted diet. *J Reprod Fertil.* 57: 253-259.

- **Minamino N, Kangawa K and Matsuo H** 1983 Neuromedin B: a novel bombesin-like peptide identified in porcine spinal cord. *Biochem Biophys Res Commun*. 114: 541-548.

- **Minamino N, Sudoh T, Kangawa K and Matsuo H** 1985 Neuromedins: novel smooth-muscle stimulating peptides identified in porcine spinal cord. *Peptides*. 6: 245-248.

- **Miyazato M, Mori K, Ida T, Kojima M, Murakami N and Kangawa K** 2008 Identification and functional analysis of a novel ligand for G protein-coupled receptor, Neuromedin S. *Regul Pept.* 145: 37-41.

- **Mizuno T, Kleopoulos S, Bergen H, Roberts JL, Priest C and Mobbs C** 1998 Hypothalamic proopiomelanocortin mRNA is reduced by fasting in ob/ob and db/db mice but is stimulated by leptin. *Diabetes.* 47: 294-297.

- **Moenter SM, DeFazio AR, Pitts GR and Nunemaker CS** 2003 Mechanisms underlying episodic gonadotropin-releasing hormone secretion. *Front Neuroendocrinol.* 24: 79-93.

- **Moller N and Jorgensen JO** 2009 Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. *Endocr Rev*. 30: 152-177.

- **Mondal MS, Date Y, Murakami N, Toshinai K, Shimbara T, Kangawa K and Nakazato M** 2003 Neuromedin U acts in the central nervous system to inhibit gastric acid secretion via CRH system. *Am J Physiol Gasterointest Liver Physiol*. 284: 963-969.

- **Monnikes H, Heymann-Monnikes I and Tache Y** 1992 CRF in the paraventricular nucleus of the hypothalamus induces dose-related behavioral profile in rats. *Brain Res.* 574: 70-76.

- **Moore RY, Speh JC and Leak RK** 2002 Suprachiasmatic nucleus organization. *Cell Tissue Res*. 309: 89-98.

- **Mori K, Miyazato M, Ida T, Murakami N, Serino R, Ueta Y, Kojima M and Kangawa K** 2005 Identification of neuromedin S and its possible role in the mammalian circadian oscillator system. *EMBO J*. 24: 325-335.

- **Mori Y, Nishihara M, Tanaka T, Shimizu T, Yamaguchi M, Takeuchi Y and Hoshino K** 1991 Chronic recording of electrophysiological manifestation of the hypothalamic gonadotropin-releasing hormone pulse generator activity in the goat. *Neuroendocrinology.* 53: 392-395.

- **Morin LP** 1975 Effects of various feeding regimens and photoperiod or pinealectomy on avulation in the hamster. *Biol Reprod.* 13: 99-103.

- **Morrison CD, Daniel JA, Holmberg BJ, Dijane J, Raver N, Gertler A and Keisler DH** 2001 Central infusion of leptin into well fed and undernourished ewe lambs; effects on feed intake abs serum concentrations of growth hormone and luteinizing hormone. *J Endocrinol*. 168: 317-324.

- **Mounzih K, Lu R and Chehab FF** 1997 Leptin treatment rescues the sterility of genetically obese ob/ob males. *Endocrinology*. 138: 1190-1193.

- **Nakahara K, Hanada R, Murakami N, Teranishi H, Ohgusu H, Fukushima N, Moriyama M, Ida T, Kangawa K and Kojima M** 2004 The gut–brain peptide neuromedin U is involved in the mammalian circadian oscillator system. *Biochem Biophys Res Commun*. 318: 156-161.

- **Nakazato M, Hanada R, Murakami N, Date Y, Mondal MS, Kojima M, Yoshimatsu H, Kangawa K and Matsukura S** 2000 Central effects of neuromedin U in the regulation of energy homeostasis. *Biochem Biophys Res Commun*. 277: 191-194.

- **Niall HD, Hogan ML, Sauer R, Rosenblum IY and Greenwood FC** 1971 Sequences of pituitary and placental lactogenic and growth hormones: evolution from a primordial peptide by gene reduplication. *Proc Natl Acad Sci*. 68: 866-870.

- **Nogueiras R, Barreiro ML, Caminos JE, Gaytán F, Suominen JS, Navarro VM, Casanueva FF, Aguilar E, Toppari J, Diéguez C and Tena-Sempere M** 2004 Novel expression of resistin in rat testis: functional role and regulation by nutritional status and hormonal factors. *J Cell Sci*. 117: 3247-3257.

- **Norman RL** 1993 Effects of corticotropin-releasing hormone on luteinizing hormone, testosterone, and cortisol secretion in intact male rhesus macaques. *Biol Reprod*. 49: 148-153.

- **Norrelund H** 2005 The metabolic role of growth hormone in humans with particular reference to fasting. *Growth Horm IGF Res*. 15: 95-122.

- **Norrelund H, Nair KS, Jorgensen JO, Christiansen JS and Moller N** 2001 The proteinretaining effects of growth hormone during fasting involve inhibition of muscleprotein breakdown. *Diabetes*. 50: 96-104.

- **Nunez L and Frawley LS** 1998 Alpha-MSH potentiates the responsiveness of mammotropes by increasing Ca entry. *Am J Physiol*. 274: 971-977.

- **Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L** 2002 Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat Neurosci*. 5: 566-572.

- **Ohashi S, Kaji H, Abe H and Chihara K** 1995 Effect of fasting and growth hormone (GH) administration on GH receptor (GHR) messenger ribonucleic acid (mRNA) and GH-binding protein (GHBP) mRNA levels in male rats. *Life Sci*. 57: 1655-1666.

- **Ohki-Hamazaki H** 2000 Neuromedin B. *Prog Neurobiol*. 62: 297-312.

- **Ojeda SR, Lomniczi A and Sandau US** 2008 Glial-gonadotrophin releasing

hormone (GnRH) neuron interactions in the median eminence and the control of GnRH secretion. *J Neuroendocrinol.* 20: 732-742.

- **Olive DL, Schenken RS, Sabella V, Moreno A and Riehl RM** 1989 Gonadotropin-releasing hormone antagonists attenuate estrogen/progesterone-induced hyperprolactinemia in monkeys. *Fertil Steril.* 51: 1040-1045.

- **Ozaki Y, Onaka T, Nakazato M, Saito J, Kanemoto K, Matsumoto, T and Ueta Y** 2002 Centrally administered neuromedin U activates neurosecretion and induction of c-fos messenger ribonucleic acid in the paraventricular and supraoptic nuclei of rat. *Endocrinology.* 143: 4320–4329.

- **Page ST, Herbst KL, Amory JK, Coviello AD, Anawalt BD, Matsumoto AM and Bremner WJ** 2005 Testosterone Administration Suppresses Adiponectin Levels in Men. *J Androl*. 26**:** 85-92.

- **Parfitt DB, Church KR and Cameron JL** 1991 Restoration of pulsatile LH secretion after fasting in rhesus monkeys (*Macaca mulatta*): dependence on size of the refed meal. *Endocrinology.* 129: 749-756.

- **Pataky Z, Bobbioni-Harsch E and Golay A** 2010 Obesity: a complex growing challenge. *Exp Clin Endocrinol Diabetes.* 118: 427-433.

- **Perrigo G and Bronson FH** 1983 Foraging effort, food intake, fat deposition and puberty in female mice. *Biol Reprod.* 29: 455-463.

- **Petersen C and Soder O** 2006 The Sertoli cell—a hormonal target and 'super' nurse for germ cells that determines testicular size. *Horm Res.* 66: 153-161.

- **Piggins HD and Cutler DJ 2003** The roles of vasoactive intestinal polypeptide in the mammalian circadian clock. *J endocrinol.* 177: 7-15.

- **Pirke KM, Schweiger U, Laessle R, Dickhaut B, Schweiger M and Waechtler M** 1986 Dieting influences the menstrual cycle: vegetarian versus nonvegetarian diet. *Fertil Steril.* 46: 1083-1088.

- **Plant TM** 2008 Hypothalamic control of the pituitary-gonadal axis in higher

primates: key advances over the last two decades. *J Neuroendocrinol.* 20: 719-726.

- **Plant TM and Barker-Gibb ML** 2004 Neurobiological mechanisms of puberty in higher primates. *Hum Reprod Update.* 10: 67-77.

- **Plant TM and Shahab M** 2002 Neuroendocrine mechanisms that delay and initiate puberty in higher primates. *Physiol Behav.* 77: 717-722.

- **Pond WG, Mersmann HJ and Yen JT** 1988 Maternal tissue repartitioning in pregnant primiparous swine in response to restriction of calories or feed. *Proc Soc Exp Biol Med.* 188: 128-135.

- **Pond WG, Mersmann HJ, Yen JT and Daris WJ** 1989 Reproduction and organ weight responses in female rats fed a restricted balanced diet or a restricted nonprotein calorie diet with or without supplemental glutamine or cysteine. *Growth Dev Aging.* 53: 17-24.

- **Porte D Jr and Woods SC** 1981 Regulation of food intake and body weight in insulin. *Diabetologia*. 20: 274-280.

- **Posner BI, Kelly PA, Shiu RPC, Paud R and Friesen HG** 1974 Studies of insulin, growth hormone and prolactin binding: Tissue distribution, species variation and characterization. *Endocrinology.* 95: 521-531.

- **Prins GS and Lee C** 1982 Comparison of prostatic response to pitiuitary grafts in castrate rats treated with testosterone or dihydrotestosterone. *J Androl.* 3: 305-312.

- **Purvis K, Clausen OPF, Olsen A, Hang F and Hansson V** 1979 Prolactin and Leydig cell responsiveness to LH/hCG. *Arch Androl.* 3: 219-230.

- **Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, Scherer PE and Ahima RS** 2004 Adiponectin acts in the brain to decrease body weight. *Nat Med.* 10: 524-529.

- **Quan H, Funabashi T and Kimura F** 2004 Intracerebroventricular injection of corticotrophin-releasing hormone receptor antagonist blocks the

suppression of pulsatile luteinizing hormone secretion induced by neuromedin U in ovariectomized rats after 48 hours fasting. *Neurosci Lett*. 369: 33-38.

- **Quan H, Funabashi T, Furuta M and Kimura F** 2003 Effects of neuromedin U on the pulsatile LH secretion in ovariectomized rats in association with feeding conditions. *Biochem Biophys Res Commun.* 311: 721-727.

- **Raddatz R, Wilson AE, Artymyshyn R, Bonini JA, Borowsky B, Boteju LW, Zhou S, Kouranova EV, Nagorny R, Guevarra MS, Dai M, Lerman GS, Vaysse PJ, Branchek TA, Gerald C, Forray C and Adham N** 2000 Identification and characterization of two neuromedin U receptors differentially expressed in peripheral tissues and the central nervous system. *J Biol Chem*. 275: 32452–32459.

- **Rajala MW, Qi Y, Patel HR, Takahashi N, Banerjee R, Pajvani UB, Sinha MK, Gingerich RL, Scherer PE and Ahima RS** 2004 Regulation of resistin expression and circulating levels in obesity, diabetes, and fasting. *Diabetes.* 53: 1671-1679.

- **Rasmussen KLR and Suomi SJ** 1989 Heart rate and endocrine responses to stress in adolescent male rhesus monkeys on Cayo Santiago. *P R Health Sci J.* 8: 65-71.

- **Reppert SM and Weaver DR** 2001 Molecular analysis of mammalian circadian rhythms. *Annu Rev Physiol*. 63: 647-676.

- **Reppert SM and Weaver DR** 2002 Coordination of circadian timing in mammals. *Nature*. 418: 935−994.

- **Roa J, García-Galiano D, Castellano JM, Gaytan F, Pinilla L and Tena-Sempere M** 2009 Metabolic control of puberty onset: New players, new mechanisms. *Mol Cell Endocrinol.* 324: 87-94.

- **Rodriguez-Pacheco F, Martinez-Fuentes AJ, Tovar S, Pinilla L, Tena-Sempere M, Dieguez C, Castaño JP and Malagon MM** 2007 Regulation of pituitary cell function by adiponectin. *Endocrinology.* 148: 401-410.

- **Rose RM, HoladayJW and Bernstein IS** 1971 Plasma testosterone, dominance rank and aggressive behaviour in male rhesus monkeys. *Nature.* 231: 366-368.

- **Roser JF** 2001 Endocrine and paracrine control of sperm production in stallions. *Anim Reprod Sci*. 68: 139-151.

- **Roux M, Martinat N, Richoux JP and Grignon G** 1985 Histoimmunological identification of a prolactin-like substance in rodent testis. *Cell Tissue Res.* 240: 663-667.

- **Rucinski M, Ziolkowska A, Neri G, Trejter M, Zemleduch T, Tyczewska M, Nussdorfer GG and Malendowicz LK** 2007 Expression of neuromedins S and U and their receptors in the hypothalamus and endocrine glands of the rat. *Int J Mol Med*. 20: 255-259.

- **Saeb-Parsy K, Lombardelli S, Khan FZ, McDowall K, Au-Yong IT and Dyball RE** 2000 Neural connections of hypothalamic neuroendocrine nuclei in the rat. *J Neuroendocrinol.* 12: 635-648.

- **Sakamoto T, Mori K, Miyazato M, Kangawa K, Sameshima H, Nakahara K and Murakami N** 2008 Involvement of neuromedin S in the oxytocin release response to suckling stimulus. *Biochem Biophys Res Commun.* 375: 49-53.

- **Sakharova AA, Horowitz JF, Surya S, Goldenberg N, Harber MP, Symons K and Barkan A** 2008 Role of growth hormone in regulating lipolysis, proteolysis, and hepatic glucose production during fasting. *J Clin Endocrinol Metab.* 93: 2755-2759.

- **Samson WK, Taylor MM and Baker JR** 2003 Prolactin-releasing peptides. *Regul Pept*. 114: 1-5.

- **Saper CB, Lu J, Chou TC and Gooley J** 2005 The hypothalamic integrator for circadian rhythms. *Trends Neurosci*. 28:152-157.

- **Sapolsky RM and Krey LC** 1988 Stress-induced suppression of luteinizing

hormone concentrations in wild baboons: Role of opiates. *J Clin Endocrinol Metab*. 66: 722-726.

- **Sawchenko PE, Swanson LW and Vale WW** 1984a Co-expression of corticotropin-releasing factor and vasopressin immunoreactivity in parvocellular neurosecretory neurons of the adrenalectomized rat. *Proc Natl Acad Sci*. 81:1883-1887.

- **Sawchenko PE, Swanson LW and Vale WW** 1984b Corticotropin-releasing factor: co-expression within distinct subsets of oxytocin-, vasopressin-, and neurotensin-immunoreactive neurons in the hypothalamus of the male rat. *J Neuroscience.* 4: 1118-1129.

- **Schenck PE, Koos-Slob A, Uilenbroek JTJ and van der Werff ten Bosch JJ** 1980 Effect of neonatal undernutrition on serum follicle slimulating hormone levels and ovarian development in the female rat. *Br J Nutr.* 44: 179-182.

- **Schibler U and Sassone-Corsi P** 2002 A web of circadian pacemakers. *Cell.* 7: 919-922.

- **Schneider JE** 2004 Energy balance and reproduction. *Physiol Behav.* 81: 289-317.

- **Schreihofer DA, Amico JA and Cameron JL** 1993a Reversal of fasting-induced suppression of luteinizing hormone (LH) secretion in male rhesus monkeys by intragastric nutrient infusion: evidence for rapid stimulation of LH by nutritional signals. *Endocrinology.* 132: 1890-1897.

- **Schreihofer DA, Parfitt DB and Cameron JL** 1993b Suppression of LH secretion during short-term fasting in male rhesus monkeys: the role of metabolic versus stress signals. *Endocrinology* 132: 1881-1889.

- **Schwartz M, Seeley R, Woods S, Weigle D, Campfield L, Burn P and Baskin D** 1997 Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. *Diabetes.* 46: 2119-2123.

- **Selmanoff M and Gregerson KA** 1986 Suckling-induced prolactin release is suppressed by naloxone and stimulated by beta-endorphin. *Neuroendocrinology.* 42: 255-259.

- **Shahab M, Zaman W, Bashir K and Arslan M** 1997 Fasting-induced suppression of hypothalamic-pituitary-gonadal axis in the adult rhesus monkey: evidence for involvement of excitatory amino acid neurotransmitters. *Life Sci.* 61: 1293-1300.

- **Shankar KKP, Harrell A, Zhong Y, Marecki JC, Ronis MJ and Badger TM** 2010 Maternal overweight programs insulin and adiponectin signaling in the off spring. *Endocrinology*. 151: 2577-2589.

- **Shimazu T** 1987 Neuronal regulation of hepatic glucose metabolism in mammals. *Diabetes Metab Rev.* 3: 185-206.

- **Shousha S, Nakahara K, Sato M, Mori K, Miyazato M, Kangawa K and Murakami N** 2006 Effect of neuromedin S on feeding regulation in the Japanese quail. *Neurosci Lett*. 391: 87-90.

- **Sipols AJ, Baskin DG and Schwartz MW** 1995 Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes*. 44: 147-151.

- **Sirek AM, Horvath E, Ezrin C and Kovacs K** 1976 Effect of starvation on pituitary growth hormone cells and blood growth hormone and prolactin levels in the rat. *Nutr Metab* 20: 67-75.

- **Sirotkin AV** 2005 Control of reproductive processes by growth hormone: Extra- and intracellular mechanisms. *Vet J*. 170: 307-317.

- **Skutella T, Montkowski A, Stohr T, Probst JC, Landgraf R, Holsboer F and Jirikowski GF** 1994 Corticotropin-releasing hormone (CRF) antisense oligodeoxynucleotide treatment attenuates social defeat-induced anxiety in rats. *Cell Mol Neurobiol*. 14: 579-588.

- **Smith AI and Funder JW** 1988 Proopiomelanocortin processing in the

pituitary, central nervous system, and peripheral tissues. *Endocr* Rev. 9: 159-179.

- **Smith JT, Acohido BV, Clifton DK and Steiner RA** 2006a KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. *J Neuroendocrinol.* 18: 298–303.

- **Smith SR, Ckhetri MK, Johanson J, Radfar N and Migeon CJ** 1975 The pituitary-gonadal axis in men with protein-calorie malnutrition. *J Clin Endocrinol Metab.* 41: 60-69.

- **Spiteri-Grech J and Nieschlag E** 1992 The role of growth hormone and insulin-like growth factor I in the regulation of male reproductive function. *Horm Res.* 38: 22-27.

- **Spiteri-Grech J and Nieschlag E** 1993 Paracrine factors relevant to the regulation of spermatogenesis. *J Reprod Fertil.* 98: 1-14.

- **Stephan FK, Berkley KJ and Moss RL** 1981 Efferent connections of the rat suprachiasmatic nucleus. *Neuroscience*. 6: 2625-2641.

- **Steppan CM, Bailey ST, Bhat S, Brown E J, Banarjee RR, Wright CM, Patel HR, Ahima RS and Lazar MA** 2001a The hormone resistin links obesity to diabetes. *Nature.* 409: 307-312.

- **Steppan CM, Bailey ST, Bhat S, Brown EJ, Banarjee RR, Wright CM, Patel HR, Ahima RS and Lazar MA** 2001a The hormone resistin links obesity to diabetes. *Nature.* 409: 307-312.

- **Stockhorst U, de Fries D, Steingrueber HJ and Scherbaum WA** 2004 Insulin and the CNS: effects on food intake, memory, and endocrine parameters and the role of intranasal insulin administration in humans. *Physiol Behav.* 83: 47-54.

- **Suckale J and Solimena M** 2008 Pancreas islets in metabolic signaling--focus on the beta-cell. *Front Biosci.* 13: 7156-7171.

- **Swanson LW, Sawchenko PE, Rivier J and Vale WW** 1983 Organization of

ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology.* 36: 165-186.

- **Tal J, Price MT and Olney JW** 1983 Neuroactive amino acids influence gonadotrophin output by a suprapituitary mechanism in either rodents or primates. *Brain Res.* 273: 179-182.

- **Tanaka Y, Atsumi Y, Asahina T, Hosokawa K, Matsuoka K, Kinoshita J, Onuma T and Kawamori R** 1998 Usefulness of revised fasting plasma glucose criterion and characteristics of the insulin response to an oral glucose load in newly diagnosed Japanese diabetic subjects. *Diabetes Care.* 21: 1133-1137.

- **Tena-Sempere M** 2006 GPR54 and kisspeptin in reproduction. *Hum Reprod Update.* 12: 631-639.

- **Tena-Sempere M and Huhtaniemi I** 2003 Gonadotropins and gonadotropin receptors. In: Fauser BCJM (ed). Reproductive Medicine – Molecular, Cellular and Genetic Fundamentals, New York, NY: *Parthenon Publishing*. pp. 225-244.

- **Tena-Sempere M, Pinilla L, Gonzalez LC, Dieguez C, Casanueva FF and Aguilar E** 1999 Leptin inhibits testosterone secretion from adult rat testis in vitro. *J Endocrinol*. 161: 211–218.

- **Tena-Sempere M, Pinilla L, Gonzalez LC, Navarro J, Dieguez C, Casanueva FF and Aguilar E** 2000 In vitro pituitary and testicular effects of the leptin-related synthetic peptide leptin (116–130) amide involve actions both similar to and distinct from those of the native leptin molecule in the adult rat. *Eur J Endocrinol*. 142: 406-410.

- **Ter Horst GJ and Luiten PG** 1986 The projections of the dorsomedial hypothalamic nucleus in the rat. *Brain Res Bull*. 16: 231-248.

- **Terasawa E and Fernandez DL** 2001 Neurobiological Mechanisms of the Onset of Puberty in Primates. *Endocr Rev.* 22: 111-151.

- **Thind KK and Goldsmith PC** 1997 Expression of estrogen and progesterone receptors in glutamate and GABA neurons of the pubertal female monkey hypothalamus. *Neuroendocrinology.* 65: 314-324.

- **Thissen JP, Ketelslegers JM and Underwood LE** 1994 Nutritional regulation of the insulin-like growth factors. *Endocr Rev.* 15: 80-101.

- **Tsutsui K** 2010 Phylogenetic aspects of gonadotropin-inhibitory hormone and its homologs in vertebrates. *Ann N Y Acad Sci.* 1200: 75-84.

- **Uyama N, Geerts A and Reynaert H** 2004 Neural connections between the hypothalamus and the liver. *Anat Rec A Discov Mol Cell Evol Biol.* 280: 808-820.

- **Vale W, Spiess J, Rivier C and Rivier J** 1981 Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. *Science*. 213: 1394-1397.

- **Vigo E, Roa J, Lopez M, Castellano JM, Fernandez-Fernandez R, Navarro VM, Pineda R, Aguilar E, Dieguez C, Pinilla L and Tena-Sempere M** 2007 Neuromedin S as novel putative regulator of luteinizing hormone secretion. *Endocrinology*. 148: 813-823.

- **Volk A, Renn W, Overkamp D, Mehnert B, Maerker E, Jacob S, Balletshofer B, Häring HU and Rett K** 1999 Insulin action and secretion in healthy, glucose tolerant first degree relatives of patients with type 2 diabetes mellitus. Influence of body weight. *Exp Clin Endocrinol Diabetes*. 107: 140-147.

- **Vrang N, Larsen PJ and Mikkelsen JD** 1995 Direct projection from the suprachiasmatic nucleus to hypophysiotrophic corticotropin-releasing factor immunoreactive cells in the paraventricular nucleus of the hypothalamus demonstrated by means of Phaseolus vulgaris – leucoagglutinin tract tracing. *Brain Res.* 684: 61-69.

- **Wade GN and Jones JE** 2004 Neuroendocrinology of nutritional infertility. *Am J Physiol Regul Integr Comp Physiol.* 287: 1277-1296.

- **Wade GN, Schneider JE and Li HY** 1996 Control of fertility by metabolic cues. *Am J Physiol.* 270: 1-19.

- **Wahab F, Bano R, Jabeen S, Irfan S and Shahab M** 2010 Effect of peripheral kisspeptin administration on adiponectin, leptin, and resistin secretion under fed and fasting condition in the adult male rhesus monkey (Macaca mulatta). *Horm Metab Res.* 42: 570-574.

- **Wahab F, Aziz F, Irfan S, Zaman WU and Shahab M** 2008 Short-term fasting attenuates the response of the HPG axis to kisspeptin challenge in the adult male rhesus monkey (*Macaca mulatta*). *Life Sci*. 83: 633-637.

- **Walkden-Brown SW, Restall BJ, Norton BW, Scaramuzzi RJ and Martin GB** 1994 Effect of nutrition on seasonal patterns of LH, FSH and testosterone concentration, testicular mass, sebaceous gland volume and odour in Australian cashmere goats. *J Reprod Fertil.* 102: 351-360.

- **Watts AG, Swanson LW and Sanchez-Watts G** 1987 Efferent projections of the suprachiasmatic nucleus: I. Studies using anterograde transport of Phaseolus vulgaris leucoagglutinin in the rat. *J Comp Neurol*. 258: 204-229.

- **Weiner RI and Martinez de la Escalera G** 1993 Pulsatile release of gonadotrophin releasing hormone (GnRH) is an intrinsic property of GT1 GnRH neuronal cell lines. *Hum Reprod.* 8: 13-17.

- **Welt C, Sidis Y, Keutmann H and Schneyer A** 2002 Activins, inhibins, and follistatins: from endocrinology to signalling. A paradigm for the new millennium. *Exp Biol Med*. 227: 724-752.

- **Werther GA, Hogg A, Oldfield BJ, McKinley MJ, Figdor R and Mendelsohn FAO** 1989 Localization and characterization of insulin-like growth factor I receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry. *J Endocrinol*. 1: 369-377.

- **Whisnant CS and Harrell RJ** 2002 Effect of short-term feed restriction and refeeding on serum concentrations of leptin, luteinizing hormone and insulin in ovariectomized gilts. *Domest Anim Endocrinol.* 22: 73-80.

- **Wiersma A, Baauw AD, Bohus B and Koolhaas JM** 1995 Behavioral activation produced by CRH but not alpha-helical CRH (CRH-receptor antagonist) when microinfused into the central nucleus of the amygdala under stress-free conditions. *Psychoneuroendocrinology.* 20: 423-432.

- **Williams GL, Amstalden M, Garcia MR, Stanko RL, Niezielski SE, Morrison CD and Keisler DH** 2002 Leptin and its role in the central regulation of reproduction in cattle. *Domest Anim Endocrinol*. 23: 339–349.

- **Woodside BC** 1991 Effects of food restriction on the length of lactational diestrus in rats. *Horm Behav.* 25: 70-83.

- **Wren AM, Small CJ, Abbott CR, Jethwa PH, Kennedy AR, Murphy KG, Stanley SA, Zollner AN, Ghatei MA and Bloom SR** 2002 Hypothalamic Actions of Neuromedin U. *Endocrinology.* 143: 4227-4234.

- **Xie QW** 1991 Experimental studies on changes of neuroendocrine functions during starvation and refeeding. *Neuroendocrinology.* 53: 52-59.

- **Xu A, Chan KW, Hoo RL, Wang Y, Tan KC, Zhang J, Chen B, Lam MC, Tse C, Cooper GJ and Lam KS** 2005 Testosterone selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from adipocytes. *J Biol Chem*. 280: 18073-18080.

- **Xu J, Kirigiti MA, Grove KL and Smith MS** 2009 Regulation of Food Intake and GnRH/LH during Lactation: Role of Insulin and Leptin. *Endocrinology.* 150: 4231-4240.

- **Yen SCC, Hoff JD, Lasley BL, Casper RJ and Sheehan K** 1980 Induction of prolactin release by LRF and LRF agonist. *Life Sci.* 26: 1963-1967.

- **Zachmann M** 1992 Interrelations between growth hormone and sex hormones – physiology and therapeutic consequences. *Horm Res.* 38: 1-8.

- **Zipf WB, Payne AH and Kelch RP** 1978 Prolactin, growth hormone, and luteinizing hormone in the maintenance of testicular luteinizing receptors. *Endocrinology.* 103: 595-600.