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Possible Role of Neuromedin S (NMS) in Male Reproduction: Effect of NMS on Adipokines Secretion in Male Rhesus Monkeys (*Macaca mulatta*)

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ABSTRACT

Adipokines are known as important adiposity signals and play certain roles in various biological processes. Now their involvement in the regulation of hypothalamus pituitary gonadal (HPG) axis has also been established. In the present study, we investigated the role of peripheral administration of neuromedin S (NMS) on adipokines (adiponectin, leptin and resistin) secretion in 48-h fasting and normal fed adult male Rhesus monkeys. After NMS administration plasma adipokines levels were determined in fed and fasting monkeys. Four intact adult male monkeys were used in this study. 50 nmol of NMS was injected intravenously. Blood samples were collected individually 60 min before and 120 min after NMS administration at 15 min intervals. The plasma adipokines concentrations were determined by using specific Enzyme Immunoassay (EIA) kits. 48 h 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 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. In conclusion our study suggested that NMS has a 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. Further studies are required to confirm this relationship.

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Authors' Contributions
SA conducted all the experiments and wrote this manuscript. SJ supervised the work.

Key words
Neuromedin S, Adiponectin, Leptin, Resistin, Male reproduction, Rhesus monkeys

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 (Fischer-Posovszky *et al*., 2007). Leptin concentrations are positively related while adiponectin concentrations are negatively related to 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 play very important role in variety of physiological aspects, such as energy 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 (Kadowaki and Yamauchi, 2005; Fischer-Posovszky *et al*., 2007; Ahima and Lazar, 2008; 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).

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 (luteinizing hormone) levels (Henry *et al*., 1999, 2001). In rat hCG

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(human chorionic gonadotropin) 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. Resistin role in reproduction is not clearly understood. In rat testes 48 h 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 testosterone concentrations in both basal and hCG induced conditions (Nogueiras *et al*., 2004). These evidences show that adipokines might be playing an important role in the regulation of HPG axis.

Neuromedin S (NMS) an anorexigenic neuropeptide expressing in suprachiasmatic nucleus (SCN) of hypothalamus is involved in the regulation of HPA (Hypothalamus pituitary adrenal) axis (Jászberényi *et al*., 2007) and HPG axis (Vigo *et al*., 2007). NMS significantly induced testosterone concentrations in monkeys (Jahan *et al*., 2011). In this study it was hypothesized that NMS might be regulating HPG axis by affecting the secretion of adipokines from adipocytes.

MATERIALS AND METHODS

Animals and venous catheterization

Four adult male Rhesus 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.

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. All the sampling was performed after full recovery of animals from sedation. All experiments were approved by the Departmental Committee for Care and Use of animals.

Pharmacological reagents

Pharmacological reagents used in the study are: 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 administration. The time of NMS (50 nmol) administration was considered as 0 min. 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

Adipokines 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

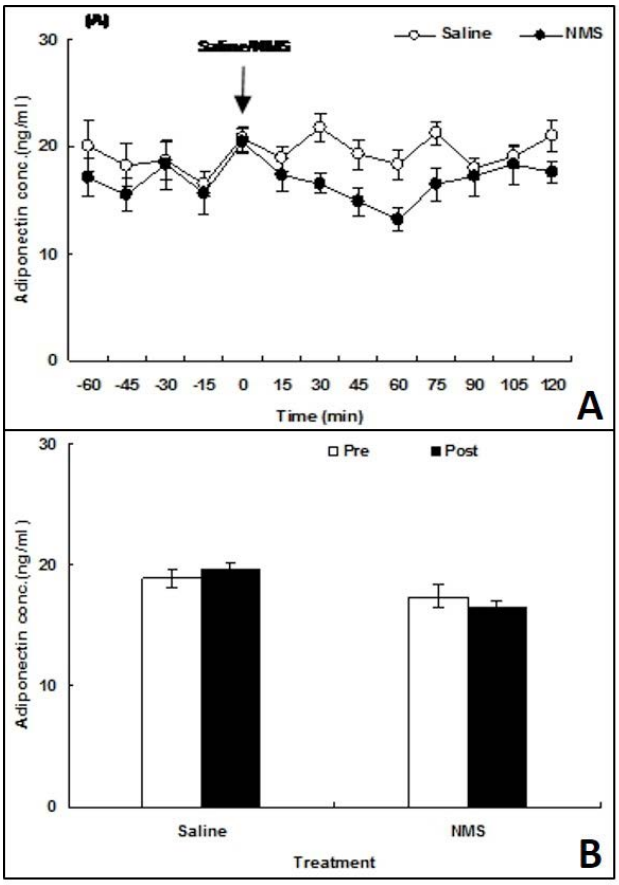

Fig. 1. **A,** Mean (±SEM) changes in plasma adiponectin levels (ng/ml) before and after saline/NMS administration (at 0 min) in normal fed adult male monkeys. P>0.05 vs 0 min sample (ANOVA followed by post hoc Dunnett's test). **B,** Comparison of mean (±SEM) plasma adiponectin levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in normal fed adult male monkeys. P>0.05 vs pre-treatment (Student's t test).

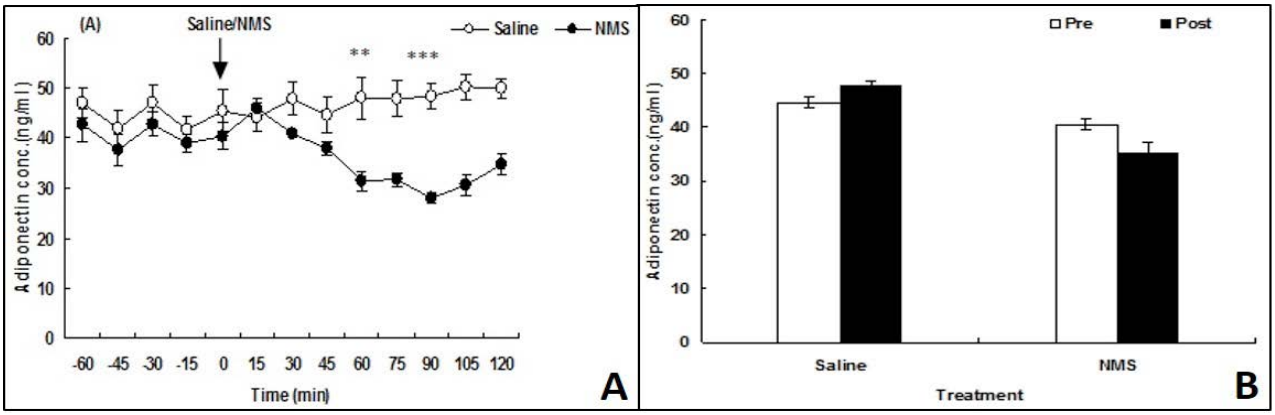

Fig. 2. **A,** Mean (±SEM) changes in plasma adiponectin levels (ng/ml) before and after saline/NMS administration (at 0 min) in 48 h fasting adult male monkeys. **P<0.01, ***P<0.001 vs 0 min sample (ANOVA followed by post hoc Dunnett's test). **B,** Comparison of mean (±SEM) plasma adiponectin levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in 48 h fasting adult male monkeys. P>0.05 vs pre-treatment (Student's t test).

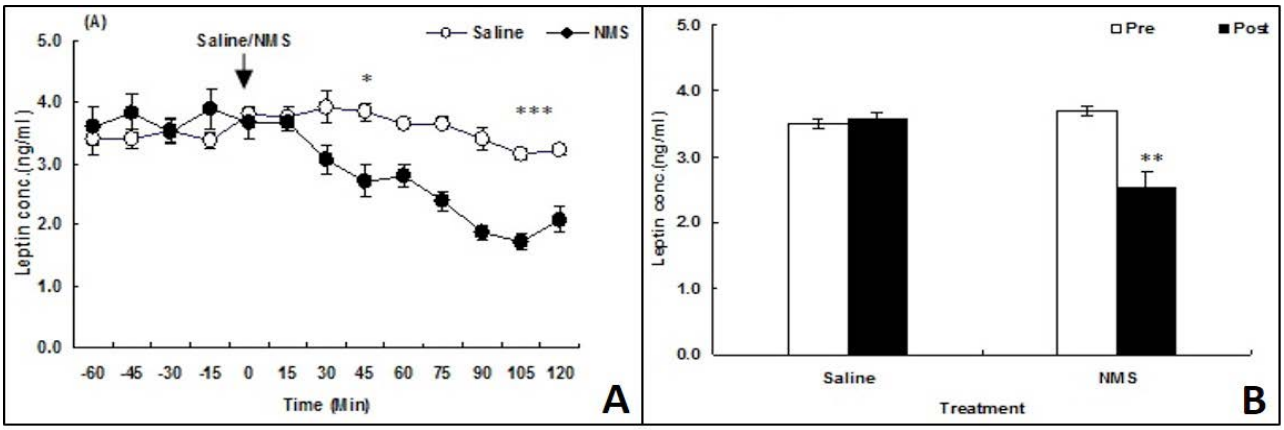

Fig. 3. **A,** Mean (±SEM) changes in plasma leptin levels (ng/ml) before and after saline/NMS administration (at 0 min) in normal fed adult male monkeys. *P<0.05, ***P<0.001 vs 0 min sample (ANOVA followed by post hoc Dunnett's test). **B,** Comparison of mean (±SEM) plasma leptin levels (ng/ml) in 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).

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. Adipokines 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 h fasting and normal fed conditions.

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

RESULTS

Plasma adiponectin secretion

Fasting caused overall increase in adiponectin levels. A single peripheral injection of NMS did not cause any significant change (P>0.05) change in adiponectin levels in normal fed monkeys (Fig.1A and 1B). In 48 h fasting monkeys individual decrease in Adiponectin levels was observed at 60 min (P<0.01) and 90 min (P<0.001) after

NMS injection compared to 0 min sample (Fig. 2A) but Comparison between pre- and post-treatment showed a non significant (P>0.05) decrease in adiponectin levels after NMS administration (Fig. 2B).

Plasma leptin secretion

An overall decrease in leptin concentrations were observed in 48 h fasting monkeys. In normal fed monkeys NMS injection caused a significant decrease (P<0.01) in leptin concentrations (Fig. 3B). Maximum decrease in leptin levels was observed at 45 min (P<0.05) and 105 min (P<0.001) after NMS injection compared to 0 min sample (Fig. 3A). NMS treatment in 48 h fasting monkeys caused a significant (P<0.01) decrease in leptin concentrations (Fig. 4B). The most significant decrease (P<0.001) in leptin concentrations was observed at 90 min and 105 min of NMS injection compared to 0 min sample (Fig. 4A).

Plasma resistin secretion

48 h fasting caused an overall decrease in plasma resistin levels in monkeys. A significant increase (P<0.05) was observed after the injection of NMS in normal fed monkeys (Fig. 5B). This increase in resistin levels was more prominent at 60 min of NMS injection compared to 0 min sample (Fig. 5A). NMS treatment in 48 h fasting monkeys also induced a significant (P<0.05) increase in resistin concentrations (Fig. 6B). The prominent increase in resistin concentrations was observed after 45 min and 75 min of NMS injection compared to 0 min sample (Fig. 6A).

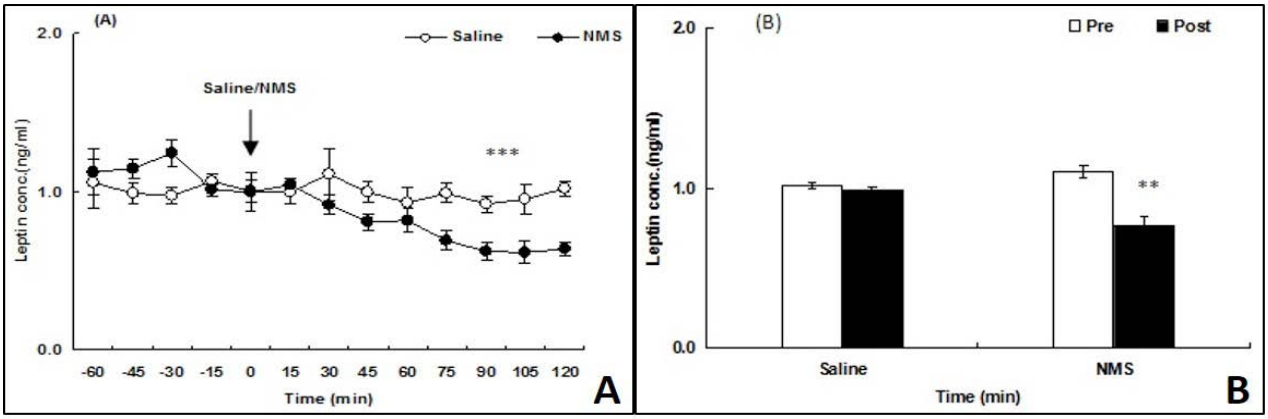

Fig. 4. **A,** Mean (±SEM) changes in plasma leptin levels (ng/ml) before and after saline/NMS administration (at 0 min) in 48 h fasting adult male monkeys. ***P<0.001 vs 0 min sample (ANOVA followed by post hoc Dunnett's test). **B,** Comparison of mean (±SEM) plasma leptin levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in 48 h fasting adult male monkeys. **P<0.01 vs pre-treatment (Student's t test).

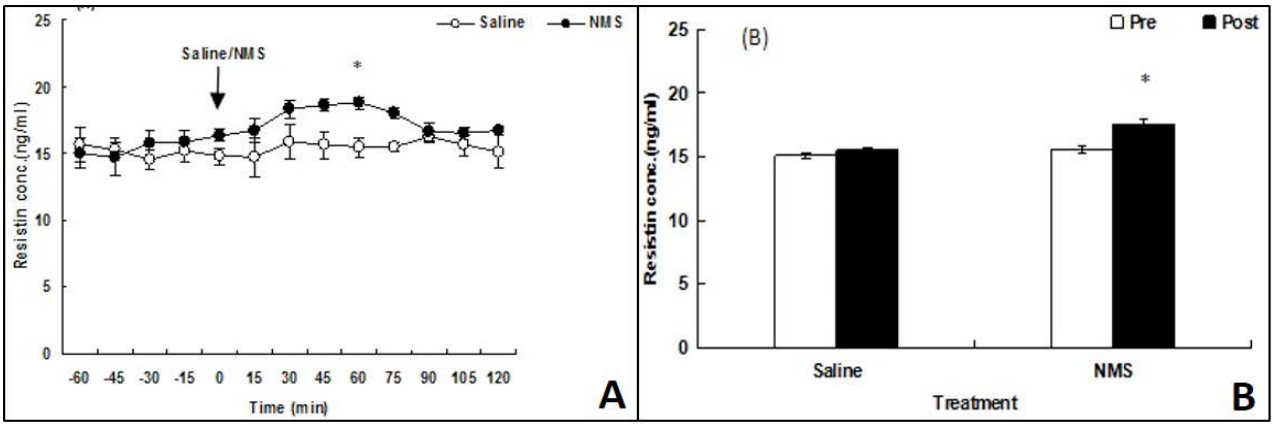

Fig. 5. **A,** Mean (±SEM) changes in plasma resistin levels (ng/ml) before and after saline/NMS administration (at 0 min) in normal fed adult male monkeys. *P<0.05 vs 0 min sample (ANOVA followed by post hoc Dunnett's test). **B,** Comparison of mean (±SEM) plasma resistin levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in normal fed adult male monkeys. *P<0.05 vs pre-treatment (Student's t test).

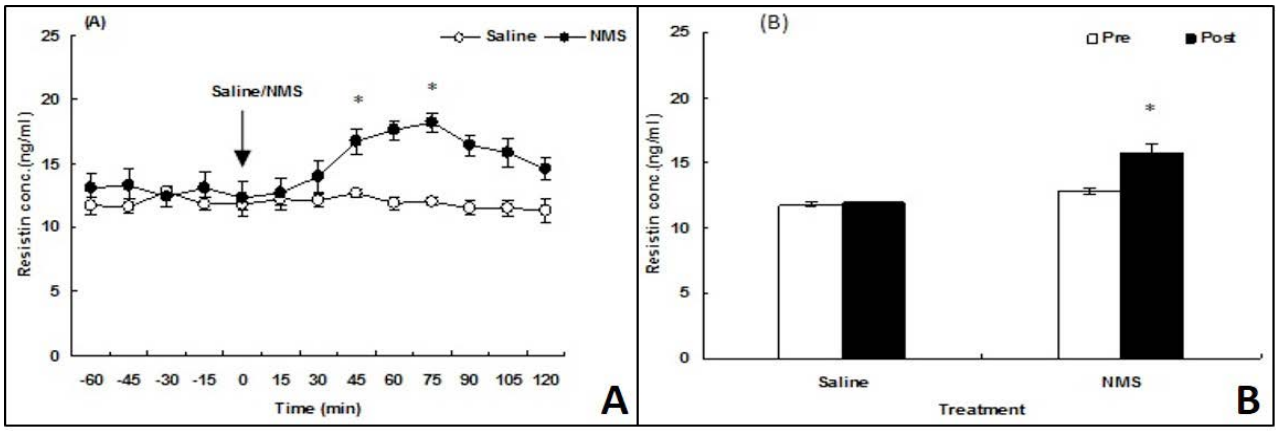

Fig. 6. **A,** Mean (±SEM) changes in plasma resistin levels (ng/ml) before and after saline/NMS administration (at 0 min) in 48 h fasting adult male monkeys. *p<0.05 vs 0 min sample (ANOVA followed by post hoc Dunnett's test). **B,** Comparison of mean (±SEM) plasma resistin levels (ng/ml) in 60 min pre- and 120 min post saline/NMS in 48 h fasting adult male monkeys. *P<0.05 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) secretions in 48 h 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 first 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 h 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 testosterone 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 testosterone 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 h 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 h 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 h 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 h 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 testosterone 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 testosterone secretion and regulation of HPG axis.

CONCLUSION

NMS plays very interesting role in regulation of adipokines. Role of NMS on adiponectin is not clearly understood. However, NMS plays a significant role in regulation of leptin and resistin suggesting their possible role in NMS regulation of HPG axis. 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 adipokines secretion on the basis of this single study is very difficult to prove. Therefore, further studies are required to explore the pathways, involved in this regulation.

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Conflict of interest statement

We declare that we have no conflict of interest.

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