Indian J Physiol Pharmacol 2012; 56(2) : 107–116

ROLE OF VENTROMEDIAL HYPOTHALAMUS ON ENERGY HOMEOSTASIS IN ALBINO RATS: EFFECT OF GENDER

SEBANTI DEV[1], PRAVATI PAL[1*], G. K. PAL[1], P. H. ANANTHANARAYANAN[2], V. LALITHA[1], ARCHANA GAUR[1] AND C. ADITHAN[3]

Departments of [1]Physiology, [2]Biochemistry and [3]Pharmacology, Jawaharlal Institute of Post-graduate Medical Education and Research (JIPMER), Puducherry – 605 006

(Received on April 9, 2011)

Abstract : Various brain areas like the ventromedial hypothalamus (VMH) are known to influence food intake and body weight. Though obesity is more common in females, the reports on gender difference in the neural regulation of energy homeostasis are not adequate. Therefore, the present study was conducted to assess the gender difference in the effect of VMH lesion on food intake (FI), body weight (BW), serum lipid profile, thyroid profile, glucose and insulin levels and glucose-insulin ratio (GIR) in Wistar albino rats. Twenty-four Wistar albino rats were divided equally into control and experimental groups with 6 male and 6 female rats in each. In the experimental group, bilateral electrolytic lesion of VMH was performed by stereotaxy and post-lesion parameters were recorded. In the control group, VMH sham lesion was made. Male-female difference in each parameter was determined. Following VMH lesion, FI was increased (females, $P<0.01$) and BW (males, $P<0.05$) and GIR decreased in males ($P<0.001$), which was significantly correlated with BW. T3 was more significantly correlated with FI and BW in females ($P<0.000$ and $P<0.001$). Following VMH lesion, male rats exhibited significant weight gain in the absence of proportionate hyperphagia indicating that weight-gain was mainly metabolic in nature. Also, the male rats developed more susceptibility to insulin resistance. The female rats developed resistance to weight-gain inspite of hyperphagia, which could be due to the higher T3 level.

Key words : ventromedial hypothalamus, food intake, body weight, lipid profile, thyroid profile, glucose-insulin ratio, Wistar rats

INTRODUCTION

Current WHO estimate suggests that over 1 billion people are overweight and over 300 million people are obese (1). Weight gain is due to an imbalance between energy expenditure and dietary intake (2). Regulation of body weight and adiposity is influenced by various factors such as nutritional, hereditary, social and environmental and so

*Corresponding Author : Dr. Pravati Pal, MD, Additional Professor, Department of Physiology, JIPMER, Puducherry – 605 006, India; E-mail: gopalpravati@sify.com, drppal@rediffmail.com; Fax : 0-91-413-2272067, Phone: 0-91-93606 82406

on (3). There are individuals who deposit fat even though they do not eat much and there are individuals who do not deposit fat even though they eat so much. This difference in adiposity could be due to the individual variation in regulation of body weight by central nervous system (4). However, our knowledge and understanding of neural regulation of adiposity is far from complete.

Hypothalamic areas like lateral hypothalamus (LH), ventromedial hypothalamus (VMH), arcuate nucleus (AR) and extrahypothalamic areas like nucleus septal medialis (NSM) and ventral medulla (VM) are known to influence food intake and body weight (4, 5). Nuclei within the hypothalamus integrate peripheral signals such as adiposity and calorie intake to regulate important pathways within the central nervous system controlling food intake and energy expenditure. Firmly established pathways involving orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP) (NPY/AgRP); and the anorexigenic pro-opiomelanocortin (POMC) and cocaine and amphetamine-related transcript (CART), (POMC/CART) neurons project from the AR to other important hypothalamic nuclei, including the paraventricular (PVN), dorsomedial (DMN), VMH and LH nuclei (2). In addition there are many projections to and from the brainstem, cortical areas and reward pathways, which modulate food intake. Central circuits in the brain rely on peripheral signals indicating satiety levels and energy stores, as well as higher cortical factors such as emotional and reward pathways. The hypothalamus is subdivided into interconnecting nuclei, including the arcuate nucleus (AR), paraventricular nucleus (PVN), ventromedial nucleus (VMH), dorsomedial nucleus (DMN) and lateral hypothalamic area (LH). Neuronal pathways between these nuclei are organised into a complex network in which orexigenic and anorexigenic circuits influence food intake and energy expenditure (2).

Sympathetic activity is decreased in rats with ventromedial hypothalamic obesity (6). Leptin produced in white adipose tissue activates sympathetic nerve activity via the VMH (7). Hyperleptinemia is associated with increased body fat, body mass index, waist-hip ratio and insulin resistance (8). Free tri-iodothyronine is positively associated with insulin secretion and hyper-thyrotropinemia is relatively common in obese children (9). A difference exists in the regulation of feeding behaviour between males and females (10). Also, plasma leptin level differs in male and female subjects (11). Obesity is associated with various disorders like hypertension, diabetes, hyperlipidemia and hypothyroidism. However, the exact mechanisms that cause obesity are not known. Whether VMH directly influence the feeding behaviour or they alter the sympathetic activity and thereby alter energy intake and expenditure, is not clear.

Therefore, in the present study, we propose to assess the effect of gender on VMH regulation of food intake and adiposity in rats correlating with their lipid profile, thyroid profile and insulin resistance.

MATERIALS AND METHODS

Animals

After obtaining approval of the research council and animal ethics committee of

JIPMER, a total of 24 (12 males and 12 females) institute-bred healthy adult albino rats of Wistar strain weighing between 150-275 g were obtained for the study. Animals were randomly divided into two groups: Control group (sham-lesion group) and Experimental group (VMH-lesioned animals). The sample size in each group was 12 (6 male and 6 female rats). The rats were housed in individual plastic cages with wire lids. 12 hour light-dark cycle was maintained. Standard rodent chow and fresh tap water was available *ad libitum*. Rats were allowed to habituate in individual cages for 10 days before basal measurements were taken.

Basal food intake and body weight recordings

After 10 days of habituation, standard rodent chow and fresh tap water *ad libitum* was provided every day. Daily food intake and body weight was measured for one week to determine the mean 24-hour basal recordings.

Procedures

Anesthesia

Because the depth of anesthesia required for different procedures was different, the anesthetic agent used was different for different procedures. As light anesthesia was required for blood collection, ether was used as anesthetic agent. For lesion making, Inj. Ketamine (0.25 ml/250 gm body weight) was injected intraperitoneally. For sacrificing the animal, double the dose of ketamine was used.

Blood collection

Jugular venous puncture : From control rats approximately 1.5- 2 ml of blood was collected by this method for obtaining basal biochemical values after 7 days of basal recordings of BW and FI.

Cardiac puncture : Approximately 5 ml of blood was collected with the help of a syringe and needle by puncturing the left ventricle during sacrifice before fixation of brain.

Electrolytic nuclear lesion

For making lesion, the stereotaxic procedure was performed as described earlier (12). Bilateral electrolytic lesions of VMH were made by introducing electrodes into the nuclei on both sides and allowing the anodal current of 0.5 mA to pass through the electrode. In animals undergoing sham lesions, all the above-mentioned steps were followed except that no current was passed.

Parameters

The parameters recorded were :

Physical parameters

i) Body weight (g)

ii) Food intake (g)

Biochemical parameters (Fasting)

i) Serum Glucose (mg/dl)

ii) Serum Insulin (ng/ml)

iii) Glucose-insulin ratio (insulin resistance was calculated by obtaining the glucose-insulin ratio) (13).

iv) Serum Total Cholesterol (mg/dl)

v) Serum Triglycerides (mg/dl)

vi) Serum TSH (µlU/ml)

vii) Serum Total T3 (ng/dl)

viii) Serum Total T4 (ng/dl)

All these biochemical parameters were estimated following the standard procedures as practiced in the clinical laboratory of department of Biochemistry of JIPMER, Pondicherry.

Recording of parameters

Body weight (BW) : It was measured everyday with an electronic weighing machine for the entire period of the study.

Food intake (FI) : Food intake was measured daily. After blood collection and lesion/sham lesion procedure the animals were allowed to recover from the stress of the intervention for a period of seven days during which food intake and body weight was not measured.

Biochemical parameters

Collected blood was allowed to clot and then centrifuged to separate the serum. 0.5 ml of serum was used for analysis of fasting serum glucose and serum lipids (serum total cholesterol and serum triglycerides). The remaining serum samples were stored at –80°C in labelled containers for subsequent analyses of the following parameters :

Serum Insulin (Rat/mouse Insulin ELISA Kit, Millipore™, USA)

Serum TSH (Rat TSH ELISA Kit, Cusabio™, USA)

Serum Total T3 (Human TT3 RIA Kit, Immunotech ™, Czech)

Serum Total T4 (Human TT4 RIA Kit, Immunotech ™, Czech)

Insulin resistance was calculated by obtaining the glucose-insulin ratio (13).

Sacrifice

Animals were sacrificed following the standard procedure as described earlier (12).

Statistical analysis of data

For data analysis all values were expressed as mean±SD. Differences between means were compared by Student's *t* test. The differences among means were evaluated by one-way ANOVA (analysis of variance) using Graphpad InStat (Version 3, USA) software. Post hoc test was performed by Tukey Krammer multiple comparison test. The difference was considered statistically significant if probability of chance was less than 0.05 ($P<0.05$). For determining the correlation between the parameters Pearson's correlation test was used.

RESULTS

Control group

After undergoing sham lesions (Table I), the control male rats (n=6) did not show any significant difference in food intake (FI) and body weight (BW) from the pre- sham values. Similarly, biochemical parameters like serum glucose (SG), serum insulin (SI), serum total cholesterol (TC), serum triglycerides (TG), serum TSH, serum T3 (ST3) and serum T4 (ST4) also did not show any significant change from the pre-sham levels. But the GI ratio was significantly higher after sham procedure ($P<0.0156$).

Sham lesion did not significantly affect the FI, BW and biochemical parameters of the female control rats (n=6). But in female rats also the Gl ratio was significantly higher in the post sham period ($P<0.0285$) (Table II).

TABLE I: Food intake (FI), body weight (BW) and serum biochemical parameters in control male (n=6) rats before (pre sham) and after (post sham) lesion.

Parameters	*Pre-sham*	*Post-sham*	*P values*
FI (g)	15.59±1.32	16.56±1.90	0.3286
BW (g)	247.50±10.64	250.333±10.84	0.6579
Glucose (mg/dl)	72.33±10.91	74.16±7.30	0.7395
Insulin (ng/ml)	0.548±0.024	0.498±0.186	0.5285
G/I Ratio	131.98±11.90	148.91±7.84	0.0156
TC (mg/dl)	54.83±6.85	55.66±6.77	0.1231
TG (mg/dl)	55.83±5.60	55.33±13.79	0.9443
TSH (µlU/ml)	9.75±5.98	11.19±5.31	0.6683
T_3 (ng/dl)	19.16±4.15	25.19±9.77	0.1742
T_4 (µg/dl)	4.90±0.36	4.66±1.36	0.6416

Data expressed as mean±SD; The P<0.05 was considered significant. Analysis of data was done by Student's paired t test. Sham means the lesion making needle electrode was introduced in to the brain but current was not passed.

TABLE II: Food intake (FI), body weight (BW) and serum biochemical parameters in control female (n=6) rats before (pre-sham) and after (post-sham) lesion.

Parameters	*Pre-sham*	*Post-sham*	*P values*
FI (g)	12.21±1.56	13.32±1.87	0.2903
BW (g)	163.50±6.22	164.66±4.50	0.2387
Glucose (mg/dl)	66.83±7.08	73.83±7.16	0.1292
Insulin (ng/ml)	0.876±0.353	0.835±0.282	0.8286
G/I Ratio	76.28±8.24	88.42±8.20	0.0285
TC (mg/dl)	51.83±3.97	52.16±3.97	0.8893
TG (mg/dl)	107.01±14.39	114.50±16.73	0.4253
TSH (µlU/ml)	7.88±3.98	8.63±4.22	0.7580
T_3 (ng/dl)	33.80±4.25	36.36±8.08	0.3110
T_4 (µg/dl)	5.63±1.54	4.45±0.88	0.0798

Data expressed as mean±SD; The P<0.05 considered significant. Analysis of data was done by Student's paired t test. Sham means the lesion making needle electrode was introduced in to the brain but current was not passed.

On analyzing the gender differences of the control group of rats (Table III), it was seen that the female rats were significantly lower in BW (P<0.001) and their FI was much lesser than the male rats (P<0.05). In spite of that, there was no significant gender difference in SG and SI. However, the females had a lower Gl ratio than the males (P<0.001). Similarly, TSH & T4 did not show any appreciable gender difference. However, the gender difference in T3 did not exist following sham lesion. When the lipid profile was analyzed, it was observed that though TG levels in the females were significantly higher (P<0.001) the TC levels did not show any difference.

Experimental group

Effect of the lesion

After the lesion (Table IV), FI increased in both the sexes, but it was significant in females (P<0.01). Both males and females showed an increase in BW, but the increase was significant in males (P<0.05). The males showed a significant decrease (P<0.001) in Gl ratio whereas the females showed a significant increase (P<0.001) in Gl ratio. In the female rats, VMH lesion did not cause any significant change in any of the other parameters. In the male rats, there was significant increase in the SI levels (P<0.001) and a significant decrease in SG levels (P<0.01). Following VMH lesion, male rats also did not show any change in the lipid profile, however, there was a significant decrease in TSH levels (P<0.01) with no detectable change in ST3 and ST4.

Effect of gender

Even though the BW remained significantly lower (P<0.001) in female rats the difference in FI was not seen after VMH lesion. Post lesion, SG was significantly lower (P<0.01) with significantly higher SI levels (P<0.01) in male rats compared to females. Even though in control female

TABLE III : Food intake (FI), body weight (BW) and serum biochemical parameters in control male (n=6) and female (n=6) rats before (Pre sham) and after (post sham) lesion.

Parameters	*Pre-sham*		*Post-sham*		*P values*
	Male	*Female*	*Male*	*Female*	
FI (g)	15.59±1.32	12.21±1.56*	16.56±1.90$^{\#\#}$	13.32±1.87^{f}	0.0008
BW (g)	247.50±10.64	163.5±6.22***	250.33±10.84$^{\#\#\#}$	164.66±4.50***,^{fff}	<0.0001
Glucose (mg/dl)	72.33±10.91	66.83±7.08	74.16±7.302	73.83±7.16	0.4077
Insulin (ng/ml)	0.548±0.024	0.876±0.353	0.498±0.186	0.835±0.282	0.5480
G/I Ratio	131.98±11.40	76.28±8.52***	148.91±7.84*,$^{\#\#\#}$	88.42±8.20**,^{fff}	<0.0001
TC (mg/dl)	54.83±6.85	51.83±3.97	55.66±6.77	52.16±3.97	0.5608
TG (mg/dl)	55.83±5.60	107.01±14.39***	55.33±13.79$^{\#\#\#}$	114.50±16.73***,^{fff}	0.0001
TSH (µlU/ml)	9.75±4.98	7.88±3.98	11.19±5.31	8.63±4.22	0.6382
T_3 (ng/dl)	19.16±4.15	33.8±4.25**	25.19±9.77	36.36±8.083**	0.0013
T_4 (µg/dl)	4.90±0.36	5.63±1.54	4.66±1.36	4.45±0.88	0.3231

Data expressed in mean±SD. The * represents comparison with pre-sham males, $^{\#}$ represents comparison with pre-sham females; ^{f} represents comparison with post-sham male. The analysis of data was done by one-way ANOVA and post hoc by Tukey- Krammer test. *P<0.05; **P<0.01; ***P<0.001; $^{\#\#}$P<0.01; $^{\#\#\#}$P<0.001; ^{f}P<0.05; ^{fff}P<0.001.

TABLE IV : Comparison of food intake (FI), body weight (BW) and serum biochemical parameters in control and VMH lesion rats in both male (n=6) and female (n=6).

Parameters	*Control*		*Experimental*		*P values*
	Post-sham male	*Post-sham female*	*Post-lesion male*	*Post-lesion female*	
FI (g)	16.56±1.90	13.32±1.87*	19.16±2.11$^{\#\#\#}$	17.58±1.25$^{\#\#}$	0.0002
BW (g)	250.33±10.84	164.66±4.50***	268.66±13.20*,$^{\#\#\#}$	173.33±7.84***,^{fff}	0.0001
Glucose (mg/dl)	74.16±7.30	73.83±7.16	55.83±8.75**,$^{\#\#}$	74.33±10.32^{ff}	0.0022
Insulin (ng/ml)	0.498±0.186	0.835±0.282	1.32±0.42***,$^{\#}$	0.630±0.144^{ff}	0.003
G/I Ratio	148.91±7.84	88.42±8.20***	42.29±6.86***,$^{\#\#\#}$	117.98±11.56***,$^{\#\#\#,fff}$	<0.0001
TC (mg/dl)	55.66±6.77	52.16±3.97	52.01±2.96	55.50±6.25	0.4629
TG (mg/dl)	55.33±13.79	114.50±16.73***	53.16±14.56$^{\#\#\#}$	1 18.66±12.48***,^{fff}	0.0001
TSH (µlU/ml)	11.19±5.31	8.63±4.22	2.99±0.86**	5.65±1.75	0.0038
T_3 (ng/dl)	25.19±9.77	36.36±8.08	28.86±8.66	43.82±7.07**,^{f}	0.0053
T_4 (µg/dl)	4.66±1.36	4.45±0.883	5.04±0.98	4.86±0.761	0.7735

Data expressed in mean±SD. The * represents comparison with post sham males, $^{\#}$ represents comparison with post sham females; ^{f} represents comparison with post lesion male. The analysis of data was done by one-way ANOVA and post hoc by Tukey-Krammer test. *P<0.05; **P<0.01; ***P<0.001; $^{\#}$P<0.05; $^{\#\#}$P<0.01; $^{\#\#\#}$P<0.001; ^{f}P<0.05; ^{ff}P<0.01; ^{fff}P<0.001.

animals there was a significantly lower Gl ratio, after lesion the females had a significantly higher Gl ratio (P<0.001). Lipid profile did not have any appreciable change due to the lesion between the genders. Even though the thyroid profile did not show any gender difference in the control animals, after VMH lesion, female rats had significantly higher ST3 levels (P<0.05) (Table IV).

Correlation results

In the male and female rats with VMH lesion (Table VI), both FI and BW were significantly correlated with TSH and ST3. In both the genders, BW also showed a strong correlation with Gl ratio.

TABLE V : Correlation of change in food intake and body weight with alteration in total cholesterol (TC), glucose-insulin ratio (GIR), triglyceride (TG), thyroid stimulating hormone (TSH), tri-iodothyronine (T3) and thyroxine (T4) in male and female experimental rats (n=6, in each group) following lesion of VMH.

Parameters	*Food intake*				*Body weight*			
	Male		*Female*		*Male*		*Female*	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
GIR	–0.18	0.132	0.20	0.110	–0.50	0.010	–0.40	0.030
TC	–0.16	0.164	0.20	0.098	–0.17	0.150	0.22	0.086
TG	–0.15	0.186	0.18	0.146	–0.12	0.320	0.18	0.140
TSH	–0.30	0.034	–0.38	0.024	–0.36	0.048	–0.42	0.032
T3	0.50	0.014	0.75	0.001	0.48	0.003	0.70	0.000
T4	0.16	0.160	0.21	0.090	0.18	0.130	0.24	0.084

P<0.05 was considered significant.

DISCUSSION

Lesion of VMH in experimental animals resulted in increase in food intake in both males and females compared to the values of rats of their own control group (Table IV). This confirms the inhibitory nature of VMH on food intake, for which VMH has been designated as satiety center (5). However, the increase in food intake in female was more significant (P<0.01) compared to the nonsignificant increase in males indicating that satiation effect induced by VMH in females is more than that of the males.

Though there was increase in body weight in both male and female rats compared to their own control rats following VMH lesion, the increase was significant in male rats (P<0.05) and not significant in female rats (Table IV). This indicated that body weight gain in male rats was more than the female rats. This is a very interesting finding that body weight gain in male was more than female inspite of food intake was more in females. This indicates the dissociation of mechanisms controlled by VMH regulating food intake and body weight. It is well known that lesion of VMH leads to massive obesity in four to six weeks, which is called as 'hypothalamic obesity' (14). In our study, the body weight gain was not massive, because we had recorded all the parameters in third week following lesion, when body weight gain was in the first phase (the rising phase) as we were interested to assess the acute effects of lesions in the present study.

Important parameters of energy homeostasis are levels of serum glucose and insulin. In the present study, serum glucose concentration was significantly decreased in male rats (P<0.01), but not in female rats compared to their control counterparts following VMH lesion (Table IV). There was no male-female difference in serum glucose levels in the control rats, but post-lesion the difference was highly significant due to acute hypoglycemia in males (P<0.01). The decrease in serum glucose in males could primarily be due to increased plasma insulin in these rats. Hyperinsulinemia is a common feature of lesion of VMH (6, 14, 15, 16). Though many studies report a state of euglycemia in the presence of sustained hyperinsulinemia and suggest insulin resistance even in the presence of normal serum glucose following VMH lesion (6, 15);

hypoglycemia was observed in the present study. The difference could be due to the timing of observation, as many investigators have assessed the parameters after one month of lesion by which pancreatic adaptation occurs to VMH-induced obesity (16). Therefore, we had assessed the acute effects of lesion in the present study. It has been reported that in the acute phase after VMH lesion, rats are hyperinsulinemic and hypersensitive to insulin; and in the later phase when obesity is well established, VMH lesion rats become insulin resistant (15).

The alteration in energy homeostasis in VMH-lesion is considered mainly due to the alteration in autonomic (vagosympathetic) output (6). It has been observed that VMH lesion results in reduced sympathetic and increased parasympathetic activity (6, 17, 18). It is known that sympathetic activation causes body weight loss and parasympathetic activation causes body weight gain. Therefore, sympathovagal balance is the major contributor to energy homeostasis of the body (19).

Moreover, the glucose-insulin ratio (GIR), an index of insulin resistance was significantly decreased in males ($P<0.001$) and increased in females ($P<0.001$) following VMH lesion. The decrease in GIR indicates insulin resistance and increase in GIR is associated with increased insulin sensitivity (13). Therefore, from the present study it appears that insulin resistance is induced by VMH lesion in male rats, whereas female rats are protected from this. This was further supported by the findings of the present study that the body weight but not the food intake was significantly correlated with the Gl Ratio in control rats (Table V). Moreover, in experimental rats, body weight gain (but not the increase in food intake) following VMH lesion was significantly correlated with Gl Ratio, in which the correlation was more pronounced for male rats compared to female rats (Table VI). All these findings indicate that male rats following VMH lesion are more susceptible to develop insulin resistance.

There was no difference in alteration in total cholesterol and triglycerides in experimental rats following VMH lesion compared to controls rats that had undergone sham lesion (Table III), which indicates that VMH has no direct influence on lipid profile in rats. Moreover, there was significant correlation of food intake and body weight with lipid profile parameters in rats before and after lesion.

In the present study, there was significant decrease in TSH level in rats following VMH lesion, in which decrease was more pronounced in male rats ($P<0.01$) compared to female rats (not significant) (Table IV). Also, T3 level was increased (though not significant) following VMH lesion. These findings indicate that VMH lesion induces some degree of hyperthyroidism (low TSH and high T3), which may contribute to alteration in energy homeostasis. The influence of VMH on thyroid hormone secretion is executed via hypothalamo-pituitary-thyroid axis of neurohumoral control. Though the increase in T3 in male and female rats was not significant compared to their control rats, there was a significantly high T3 in female rats compared to male rats ($P<0.05$). As thyroid hormones facilitate metabolism and decreased adiposity, a higher level of T3 in female rats

may be among the contributory factors for less body weights gain following VMH lesion in these animals. Increase in T3 level was significantly correlated with increase in body weight in experimental rats (Table V), indicating the direct association of thyroid hormones with food intake and body weight. The degree of correlation of both TSH and T3 was more in females compared to males, indicating thyroid control of food intake and body weight is more prominent in females.

From the present study, it is confirmed that neurophysiological control of energy homeostasis in rat model is mainly dictated by hypothalamic influences originating primarily from ventromedial hypothalamus. It appears that VMH controls energy balance by its influence through sympathovagal output, hypothalamopituitary-thyroid axis and hypothalamopituitary-pancreas axis.

Conclusion

To conclude VMH is the important center for satiety and adiposity in rat models and has differential influence on control of food intake and body weight in male and female rats. Lesion of VMH predisposes the male rats to insulin resistance, but not the female rats. Hypothalamo-pituitary-thyroid axis is involved in VMH control of energy homeostasis, which appears to be stronger in females than in males.

REFERENCES

1. World Health Organisation. Genomics and World Health, Report of the Advisory Committee on Health Research - Summary. 2002.
2. Simpson KA, Martin NM, Bloom SR. Hypothalamic regulation of food intake and clinicaltherapeutic applications. *Arq Bras Endocrinol Metab* 2009; 53: 120–128.
3. Epstein FH. Epidemiology of obesity. In: Bjorntorp P, Brodoff BN Obesity, ed Philadelphia: PAJB Lippincot, 1992; 330–342.
4. Peters A, Pellerin L, Dallman MF, et al. Causes of obesity: looking beyond Hypothalamus. *Prog Neurobiol* 2007; 81: 61–88.
5. King BM. The rise, fall and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. *Physiol Behav* 2006; 87: 221–244.
6. Valensi P, Doare L, Perret G, Germarc R, Paries J and Mesangeau D. Cardiovascular vagosympathetic activity in rats with ventromeduial hypothalamic obesity. *Obes Res* 2003; 11: 54–56.
7. Tanida M, Nagai K and Kaneko H. Activation of the renal sympathyetic nerve by leptin microinjection into the ventromedial hypothalamus in rats. *In vivo* 2003; 17: 213–217.
8. Kennedy A, Gettys TW, Watson P, et al. The metabolic significance of leptin in humans: gender- based differences in relationship to adiposity, insulin sensitivity, and energy expenditure. *J Clin Endocrinol Metab* 1997; 82: 1293–1300.
9. Ortega E, Koska J, Pannacciulli N, Bunt JC and Krakoff J. Free triiodothyronine plasma concentrations are positively associated with insulin secretion in euthyroid individuals. *Eur J Endocrinol* 2008; 158: 217–221.
10. Bella DD, Zuckera I. Sex differences in body weight and eating; organization and activation by gonadal hormones in the rat. *Physiol Behav* 1971; 7: 27–34
11. Licinio J, Negrao AB, Mantzoros C, et al. Sex differences in circulating human leptin pulse amplitude: clinical implications. *J Clin Endocrinol Metab* 1998; 83: 4140–4147.
12. Pal GK, Pravati Pal, Nivedita Nanda and Suman Saurabh. Study of interaction of estrogen and dopamine injected into ventromedial hypothalamus on control of obesity in ovariectomized albino rats. *Annals Neurosci* 2007; 14: 8–12.
13. Vuguin P, Saenger P, Dimartino-Nardi J. Fasting glucose insulin ratio: a useful measure of insulin resistance in girls with premature adrenarche.

J Clin Endocrinol Metab 2001; 86: 4618–4621.

14. Bray GA. Hypothalamic and genetic obesity: an appraisal of the autonomic hypothesis and the endocrine hypothesis. *Int J Obes* 1984; 8: 119–137.
15. Pénicaud L, Kinebanyan MF, Ferré P, Morin J, Kandé J, Smadja C, Marfaing-Jallat P, Picon L. Development of VMH obesity: *in vivo* insulin secretion and tissue insulin sensitivity. *Am J Physiol* 1987; 257: 255–260.
16. Smith FJ, Campfield LA. Pancreatic adaptation in VMH obesity: *in vivo* compensatory response to altered neural input. *Am J Physiol* 1986; 251: 70–76.
17. Balkan B, Strubbe JH, Bruggink JE, Steffens AB. Altered sympathetic control of nutrient mobilization during physical exercise after lesions in the VMH. *Am J Physiol* 1991; 260: 368–372.
18. Sakaguchi T, Bray GA, Eddlestone G. Sympathetic activity following paraventricular or ventromedial hypothalamic lesions in rats. *Brain Res Bull.* 1988; 20: 461–465.
19. Straznicky NE, Lambert GW, Lambert EA. Neuroadrenergic dysfunctions in obesity: an over view of the effects of weight loss. *Curr Opin Lipidol* 2010; 21: 21–30.