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# Effect of Ventromedial Hypothalamus on Food Intake, Body Weight, Insulin Resistance, Lipid Profile and Thyroid Profile in High-Fat Diet Obese Female Wistar Rats

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## Abstract

**Background:** Diet is one of the risk factors for obesity and VMH plays a substantial role in food intake and obesity. Food intake and body weight differ preferentially with gender. Insulin resistance, thyroid, and lipid profile are intimately linked to body metabolism. Thus, this study was conducted to assess the role of VMH on metabolism

**Materials and Method:** The study was conducted in the Dept. of Physiology, JIPMER after ethics committee approval. The animals were fed on HFD (total of 12 female albino Wistar rats) for a period of 10 weeks. After obtaining a basal recording of food intake, body weight, glucose, insulin, thyroid, and lipid profile for a period of 1 week, animals were divided into control and experimental subgroups (each 6 female). Experimental rats underwent electrolytic ablation of VMH whereas control rats underwent sham lesion. Then a post-lesion recording was taken for four weeks and compared between groups with appropriate statistics.

**Results:** VMH lesion increased food intake, body weight, blood glucose, insulin, and insulin resistance in the experimental group. VMH lesion didn't have much influence on lipid profile and thyroid profile.

**Conclusion:** VMH has a significant role in feeding behavior, adiposity genesis, insulin-glucose homeostasis, and lipid metabolism.

***Keywords:*** *High-fat diet, ventromedial hypothalamus, obese rat.*

## Introduction

Globally obesity is a major health issue[(1)]. As per the reports of WHO, around 2.8 million deaths occur per year due to obesity[(2)]. The rate of obesity prevalence is doubled among adults and the rate was tripled among children[(3)]. Several factors have been shown to regulate the body weight or cause adiposity which includes environmental, genetic, social and nutrition[(4)]. The behavioral component of food intake and adiposity is proposed to be influenced by hypothalamic (Ventromedial hypothalamus, lateral hypothalamus, and arcuate nuclei) and limbic system. Both adiposity and calorie intake were integrated by many nuclei of the hypothalamus[(5, 6)]. VMH is considered to be the major satiety center which holds responsible for feeding behavior[(4)]. Among the brain regions, ventromedial hypothalamus (VMH) is linked to food intake regulation and body weight in animal models[(7)]. Li S. Zhang Hy et al have documented that diet-induced animal model to reflect a general apt obesity model[(8)] and high-fat diet is one of the accepted models for inducing obesity in rats. The diet intake alters the body metabolism. It has been shown that blood variables such as glucose, insulin, thyroid hormones, and lipid profile components represent energy homeostasis[(9, 10)]. Though lesion of VMH is found to cause obesity[(11)], its effect in already

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obese rats is not yet established and further, the effect of experimentally produced VMH lesion on parameters such as insulin, thyroid profile, lipid profile, and glucose concentrations were not studied till date. Hence, the present study was conceived.

## Materials and Method

**Study design and setting:** This is an Experimental, intervention-based animal study done in the Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry. We commenced the study after obtaining approval from both institute scientific advisory committee and ethics committee for animal studies. The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines were diligently followed in the study.

**Procedure:** We procured 12 female albino Wistar rats each weighing about 150-250g from the institute's animal house. The rats were randomly assigned to experimental (n=6) and control group (n=6). We accommodated the rats in plastic cages with a layer or husk closed with wire lids. Freshwater ad libitum was made available to all the cages. We ensured 12 hours light-dark cycle in the room where rats were housed. They were fed on standard rat chow and allowed to habituate for 10 days prior to the baseline data collection. After a habituation period of 10 days, rats were fed on a high fat diet (HFD group). HFD was prepared freshly each day in the laboratory by mixing the components in a given proportion (Table 1). Diet and water were provided ad-libitum to both the groups for a period of 10 weeks to produce the diet-induced obese model of rats.

HFD was given for a period of 10 weeks. Then the rats were fed on standard rodent chow for 10 days so as to get adapted to this diet. After 10 days of habituation, 40 g of standard rodent chow and 100 ml of fresh tap water ad libitum was provided every day. Daily food intake and body weight was measured every one week to determine the mean 24-hour basal recordings.

All the high-fat diet food was packed properly in an airtight container to prevent oxidation.

**Table 1: The composition of a high fat diet**

| The composition of HFD (12) | |
|---|---|
| **Item** | **g/Kg of diet** |
| Casein | 164 |
| Corn starch | 303.1 |
| Dextrose | 115 |
| Sucrose | 89.9 |
| Butter oil | 190 |
| Cellulose | 58.6 |
| Soyabean oil | 10 |
| Mineral mix | 41 |
| Vitamin mix | 11.7 |
| L-Cysteine | 2.1 |
| Choline bitartate | 2.9 |

***Cont...*** **Table 1: The composition of a high fat diet...**

**Blood collection:** 1.5-2 ml of rat blood samples were collected after 7 days of baseline recording from jugular vein for biochemical analysis under mild anesthesia (Ether). Quantification of thyroid hormone profile and lipid profile was carried out using the isolated serum. Estimation of the lipid profile and thyroid hormones were analyzed as per the manufacturer guidelines. Blood glucose was measured using glucose oxidase and peroxidase method. Insulin concentration was measured using the ELISA procedure. Insulin resistance was calculated using the standard formulae.

5 ml of rat blood sample was collected under anesthesia (double dose of ketamine than the ketamine dose used during lesion making) by cardiac puncture before sacrificing. We administer two-fold increased amount of ketamine intraperitoneally before sacrificing the animal [13].

## Lesion making

Lesion of the VMH was made according to the coordinates provided from stereotaxic atlas for rat brain by Konig and Klippel, 1963. The position of the nucleus is given with reference to a three-dimensional system of coordinates determined by external landmarks on the skull. The point of intersection of three mutually perpendicular zero planes (horizontal, vertical and frontal) was used as zero.

| Nucleus | Anterior Coordinates | Lateral Coordinates | Vertical Coordinates |
|---|---|---|---|
| VMH | 0.45 mm | ±0.05 mm | 0.82 mm |

Following ketamine (5 mg/100 g Body Weight) administration intraperitoneally, the head was secured on the manual stereotaxy machine binaurally. We shaved the scalp and a midline scalp incision was made using a sterile scalpel. We applied pressure to control

the bleeding. Using artery forceps, we held the edges of skin and the skull was exposed following the removal of galea aponeurotica. VMH coordinates were set and marked on the skull with the marker dye. We drilled the skull bone using a 20 G trephine, bilaterally. Topical adrenaline (1:10, 000) and the pressure was applied to control the bleeding whenever required. We passed electric current for 10 seconds via 26-gauge lesion making needle bilaterally. The skin incision was sutured and we administered 0.5 ml paracetamol injection intramuscularly. The prophylactic antibiotic was applied on the surface of the skull. We also monitored the respiratory distress and bleeding for 10-15 minutes and then they were accommodated in a clean cage with fresh husk.

All the above-mentioned procedure was carried out to induce sham lesion except passing current. After the sham lesion, the rats were accommodated to their cages with standard rodent chow and water for a fortnight and we monitored them for bleeding and distress till their recovery. We recorded the post-lesion variables ensuring the complete recovery of the rats from the lesion inducing procedure.

**Parameters recorded:** At baseline, we monitored food intake behavior, weight and we measured circulating levels of glucose (glucose oxidase-peroxidase method), insulin (rat/mouse Insulin ELISA kit, MilliporeTM, USA),, lipid profile status and thyroid hormone profile (Human TSH chemiluminescence Kit, Siemens, USA) (Human TT3 RIA kit, ImmunotechTM, Czech) (Human TT4 RIA Kit, ImmunotechTM, Czech). Insulin resistance was calculated using HOMA–IR (HOMA-IR = Fasting insulin (μU/ml) x Fasting plasma glucose (mmol/l)/22.5) [14]. All the parameters were monitored again following post-lesion.

**Body weight (BW):** We used the electronic weighing machine and we measured once a week throughout the study

**Food intake (FI):** We monitored food intake on a daily basis. Following the lesion, we allowed the rat to recover from stress and we did not monitor food intake or their body weight for a period of fourteen days.

## Biochemical Parameters

Blood was collected in EDTA coated tubes and centrifuged to obtain serum. Approximately 0.5 ml of serum was immediately handed over to the technical personnel to the biochemistry laboratory of JIPMER, Puducherry for analysis of fasting glucose and lipid profile (total cholesterol, triglycerides, LDL, HDL, and VLDL). The remaining plasma samples were stored at-20$^{o}$C in labeled containers for analyses of other parameters

- Plasma Insulin (Rat/Mouse Insulin ELISA Kit, Millipore TM, USA)
- Plasma TSH (Human TSH chemiluminescent Kit, Siemens, USA)
- Total T3 (Human TT3 RIA Kit, Immunotech TM, Czech)
- Total T4 (Human TT4 RIA Kit, Immunotech TM, Czech)

**Statistical Analysis:** All the data underwent normality testing and based on their distribution they were expressed in mean±SD. Unpaired‘t’ test was done between the groups and paired t-test done before and after the intervention. All the data analysis was carried out in IBM SPSS statistics software (Version 20, New York, USA). The significance was set at p-value <.05.

## Results

**Table 2: Comparison of body weight and food intake of control (female rats selected for sham lesion) and experimental (female rats selected for VMH lesion) of High Fat Diet group before and after lesion**

| Parameters | Lesion | Control Group (n=6) | Experimental Group (n=6) | P value |
|---|---|---|---|---|
| Food intake (g/day) | Pre | 9.07 ± 2.975 | 9.53 ± 0.495 | .716 |
| | Post | 9.821 ± 0.707 | 12.766 ± 0.65 | <.001 |
| | Pre vs post | .560 | <.001 | |
| Body weight (kg) | Pre | 172.5 ± 11.862 | 178.33 ± 15.371 | .478 |
| | Post | 210.50 ± 10.621 | 256.60 ± 15.504 | <.001 |
| | Pre vs post | <.001 | <.001 | |

Data were expressed in mean±SD. The unpaired t-test was done between the groups and paired t-test was done to analyze the pre-intervention and post-intervention outcomes. A p value <.05 is considered to be significant

Table 2 shows no significant difference in food intake or body weight between control and experimental groups at baseline. Whereas, following lesion experimental group demonstrated significantly increased food intake behavior and marked rise in body weight than its baseline value and control group value. Control group post-interventional results have shown increased body weight than its baseline value following sham lesion. However, there are no significant changes in food intake behavior of control group following sham lesion.

**Table 3: Comparison of blood glucose, insulin and HOMA-IR of control (female rats selected for sham lesion) and experimental (female rats selected for VMH lesion) of High Fat Diet group before and after lesion**

| Parameter | Lesion | Control Group | Experimental Group | P value |
|---|---|---|---|---|
| | | Female Rats (n=6) | Female Rats (n=6) | |
| Blood Glucose (mg/dl) | Pre | 92.16 ± 14.303 | 90.66 ± 14.733 | . 861 |
| | Post | 105.83 ± 13.527 | 125.33 ± 11.165 | .021 |
| | Pre vs post | .119 | <.001 | |
| Insulin (ng/ml) | Pre | 1.313 ± 0.331 | 1.439 ± 0.357 | . 540 |
| | Post | 1.908 ± 0.592 | 3.036 ± 0.856 | .024 |
| | Pre vs post | .057 | .001 | |
| HOMA-IR | Pre | 7.165 ± 2.037 | 7.725 ± 2.154 | . 653 |
| | Post | 11.956 ± 3.446 | 22.530 ± 4.821 | .001 |
| | Pre vs post | .015 | <.001 | |

Data were expressed in mean±SD. The unpaired t-test was done between the groups and paired t-test was done to analyze the pre-intervention and post-intervention outcomes. A p value <.05 is considered to be significant. HOMA-IR: Homeostasis of Model Assessment of Insulin Resistance.

Table 3 shows no significant difference in blood glucose, insulin or HOMA-IR between control and experimental groups at baseline. Whereas, following lesion experimental group demonstrated significantly increased concentration of blood glucose, insulin, and HOMA-IR than its baseline value and control group value. Control group post-interventional results have shown a borderline increase in insulin concentration and a significant increase in insulin resistance than its baseline value following sham lesion. However, there are no significant changes in blood glucose concentrations of the control group following sham lesion.

**Table 4: Comparison of the lipid profile of control (female rats selected for sham lesion) and experimental (female rats selected for VMH lesion) of High Fat Diet group before and after lesion.**

| Parameter | Lesion | Control group | Experimental group | P value |
|---|---|---|---|---|
| | | Female rats (n=6) | Female rats (n=6) | |
| TC (mg/dl) | Pre | 51.16 ± 5.380 | 50.16 ± 7.477 | .795 |
| | Post | 75.33 ± 6.543 | 91.0 ± 8.235 | .004 |
| | Pre vs post | <.001 | <.001 | |
| TG (mg/dl) | Pre | 132.33 ± 12.41 | 121.0 ± 14.904 | .182 |
| | Post | 87.83 ± 6.063 | 136.66 ± 20.617 | <.001 |
| | Pre vs post | <.001 | .162 | |
| HDL (mg/dl) | Pre | 29.16 ± 3.616 | 31.83 ± 4.262 | .269 |
| | Post | 30.83 ± 3.251 | 31.0 ± 5.725 | .950 |
| | Pre vs post | .419 | .781 | |

***Cont...*** **Table 4: Comparison of the lipid profile of control (female rats selected for sham lesion).....**

| | | | | |
|---|---|---|---|---|
| LDL (mg/dl) | Pre | 19.86 ± 4.478 | 20.13 ± 6.773 | .936 |
| | Post | 18.36 ± 3.074 | 20.63 ± 6.116 | .435 |
| | Pre vs post | .514 | .895 | |
| VLDL (mg/dl) | Pre | 18.46 ± 6.28 | 18.2 ± 5.981 | .942 |
| | Post | 11.13 ± 3.135 | 12.20 ± 3.707 | .601 |
| | Pre vs post | .028 | .063 | |

Data were expressed in mean±SD. The unpaired t-test was done between the groups and paired t-test was done to analyze the pre-intervention and post-intervention outcomes. A p value <.05 is considered to be significant. TC: Total cholesterol; TG: Triglycerides, HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: very low-density lipoprotein.

Table 4 shows no significant difference in lipid profile parameters (Total cholesterol, HDL, LDL, and VLDL) between control and experimental groups at baseline. Whereas, following lesion experimental group demonstrated significantly increased concentration of total cholesterol than its baseline value and increased concentration of triglycerides than control group value. Control group post-interventional results have shown significantly increased total cholesterol concentration, triglycerides, and VLDL than its baseline value following sham lesion. However, there is no significant changes between the groups was observed at baseline. Pre and post intervention among control group rats revealed no significant changes in any other parameter except pre-post changes of total cholesterol and triglyceride

**Table 5: Comparison of thyroid profile of control (female rats selected for sham lesion) and experimental (female rats selected for VMH lesion) of High Fat Diet group before and after lesion.**

| Parameter | Lesion | Control group | Experimental group | P value |
|---|---|---|---|---|
| | | Female rats (n=6) | Female rats (n=6) | |
| TSH (μIU/ml) | Pre | 0.49 ± 0.185 | 0.341 ± 0.224 | .237 |
| | Post | 0.583 ± 0.118 | 0.47 ± 0.192 | .247 |
| | Pre vs post | .323 | .309 | |
| $T_3$ (ng/dl) | Pre | 1.107 ± 0.87 | 0.923 ± 0.144 | .620 |
| | Post | 1.085 ± 0.213 | 1.270 ± 1.003 | .667 |
| | Pre vs post | .953 | .421 | |
| $T_4$ (μg/dl) | Pre | 4.005 ± 3.230 | 3.014 ± 0.886 | .485 |
| | Post | 4.181 ± 3.713 | 4.316 ± 1.850 | .938 |
| | Pre vs post | .931 | .151 | |

Data were expressed in mean±SD. The unpaired t-test was done between the groups and paired t-test was done to analyze the pre-intervention and post-intervention outcomes. A p value <.05 is considered to be significant. TSH: Thyroid stimulating hormone.

Table 5 shows no significant difference in thyroid hormone profile (TSH, T3, T4) at baseline and after intervention in both the groups

## Discussion

Previous studies have shown that VMH lesions resulted in hyperphagia and obesity in a number of species including humans [6]. However, studies on the effect of VMH lesion in obese rats is sparse. High-fat diet is an accepted model to induce obesity in rats [15]. In the present study, we considered 12 albino Wistar rats (6 control group and 6 experimental groups) to study the impact of VMH lesion on food intake, glucose-insulin dynamics, lipid, and thyroid profile after creating obesity in the rats. Groups were gender and weight matched as these factors are known to influence our study variables[13].

We observed that both food intake and body weight increased significantly in the experimental group after

lesion. However, the body weight increased significantly even in the control group without any change in their feeding behavior. Hence, the increase in body weight continues even with normal feeding behavior with standard chows. This increase might be due to the effect of the stress of sham lesion on rats. Further, the increase in body weight is higher in the experimental group as compared to the control group. Hence, we can hypothesize that increase in body weight in the experimental group might be due to VMH lesion causing hypothalamic obesity [16]. One of the reasons for the increase in body weight might be due to an increase in food intake as observed in our study in the experimental group. The increase in food intake behavior occurred in already obese rats. This gives us the insight that VMH is still functioning in obese rats and controlling the feeding behavior. As regards to the link between VMH lesion and autonomic activity, available evidence suggest that lesion of VMH is associated with enhancing parasympathetic activity and reduced sympathetic activity; increased parasympathetic activity results in weight gain [17-19]. This could be one of the mechanisms for the increase in body weight.

In the control group, there was an increase in insulin resistance and insulin levels with no change in blood glucose levels. This was expected as high-fat diet induces obesity along with insulin resistance and this leads to a vicious cycle leading to diabetes [20, 21]. In our study, we hypothesize that the increase in insulin levels was able to keep the blood glucose values within the normal range in the control group. However, in the experimental group, there was significantly increased blood glucose, insulin, insulin resistance. The increase in insulin levels and resistance were higher in the experimental group as compared to the control group. Hence, these observations could be attributed to a lesion in VMH. This shows that VMH lesion not only plays a role in feeding behavior but has a role in metabolic homeostasis too. VMH lesion is able to increase insulin resistance even in obese rats and lead them into diabetes. This might be due to an increase in food intake and also by altered autonomic balance caused by VMH lesion as discussed earlier.

Our observation on lipid profile is mixed. In the control group, there was an increase in total cholesterol, a decrease in triglycerides and VLDL, while HDL and LDL remained unchanged. In the experimental group, both Total cholesterol and triglycerides increased, while HDL and LDL remain unchanged and only VLDL showed a decrease. We hypothesize that most of the changes in lipid profile might be due to high-fat diet-induced obesity. Similar to our findings Ishibashi S et al [22] and Marion M. Marsh et al [23] also found increased cholesterol concentration and triglycerides in the plasma following high-fat diet among mice respectively. There was not much significant difference between the control group and experimental group in lipid profile except for triglycerides. From our study, we are not able to determine the effect of VMH lesion, which would have required stabilization of the metabolic profile in the obese model we have created.

This suggests that VMH could be the major hypothalamic nuclei responsible for metabolic regulation and dietary pattern also partly contributes to metabolic regulation in animal models.

In our study, there was no change in thyroid profile in both the control and experimental group. Although previous studies have suggested that role of thyroid hormone on VMH in regulating the food intake and energy expenditure component [24, 25], findings from our study suggest no influence of VMH on thyroid hormone profile occurs in rats within a short duration. Contrary to our findings, Shan-Shan Shao et al., have observed increased thyroid hormone (T3, T4, and TSH) and morphological changes in thyroid gland following high-fat diet on a long term basis (24 weeks) among rats [26].

## Conclusion

VMH lesion is able to increase food intake, increase body weight and increase insulin resistance and blood glucose values even in already obese female Wistar rats.

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**Conflict of Interest:** None

**Ethical Approval:** The study has been approved by the institute animal ethics committee

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