Role of Ventromedial hypothalamus in high fat diet induced obesity in male rats: association with lipid profile, thyroid profile and insulin resistance

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KEY WORDS

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ABSTRACT

Background: Ventromedial hypothalamus (VMH) plays a major role in food intake, obesity and energy homeostasis. There is a report of gender difference in energy balance with increased vulnerability of males to cardiac disease. **Purpose:** Body metabolism is greatly influenced by the diet we eat and some of the blood parameters like plasma glucose, insulin, lipid profile and thyroid profile depict a picture of energy homeostasis of the body. **Objective:** The present study was conducted to assess the effect of VMH in high fat diet (HFD) induced obesity and its link with insulin, glucose, thyroid and lipid profile of male Wistar rats. **Methods:** The male rats (n = 12) were given HFD for a period of 10 weeks to induce obesity. After obtaining a basal recording of food intake, body weight, glucose, insulin, thyroid and lipid profile, animals were divided into control and experimental group (n = 6 male in each). Experimental rats underwent electrolytic ablation of VMH whereas control rats underwent sham lesion. A post-lesion recording was taken at the end of four weeks. **Results:** The rats had a greater food intake and more body weight gain after HFD schedule in both the groups. After VMH lesion, food intake increased further, only in experimental group. Plasma glucose, Insulin, HOMA - IR, total cholesterol (TC) and triglycerides (TG) were significantly increased compared to the pre-lesion values in experimental group ($P<0.001$). **Conclusions:** Ten week of HFD resulted in obesity. VMH appears to prevent the development of insulin resistance and hypercholesterolemia which influences the energy homeostasis in male rats after high fat diet.

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Introduction

Obesity is defined as a derangement in energy homeostasis leading to an excessive accumulation of adipose tissue, a significant risk to health. The increasing prevalence of overweight and obesity, coupled with their associations with disability and disease, has led to their identification as a potentially preventable cause of premature morbidity and death.[1] Body weight is determined by an interaction between genetic, environmental and psychosocial factors.[2] These influences ultimately act by changing the energy balance equation, that is, the long-term balance between energy intake and expenditure. Excess intake of food is one of the risk factors for obesity. A study based on gene-expression profiling suggested that diet induced obese rats in general represent an appropriate obesity model.[3] Therefore, in present study, high fat diet (HFD) was used to create the obese model in rats.

VMH is designated as the principal satiety center governing feeding behavior.[4] Established pathways involving orexigenic neuropeptide Y (NPY) and agouti-related polypeptide (AgRP) (NPY/AgRP); and the anorexigenicpro-opiomelanocortin (POMC) and cocaine and amphetamine-related transcript (CART) (POMC/CART) neurons project from the arcuate nucleus (AR) to other important hypothalamic nuclei, including the paraventricular (PVN), dorsomedial (DMN), VMH and LH nuclei.[5] In addition, there are many projections to and from the brainstem, cortical areas and reward pathways, which modulate food intake. Neuronal pathways between these nuclei are organized into a complex network in which orexigenic and anorexigenic circuits influence food intake and energy expenditure.[5]

The obese males develop atherosclerotic disease and myocardial lesions while obese females and lean rats do not.[6] Body metabolism is greatly influenced by the diet we eat and some of the blood parameters like plasma glucose, insulin, lipid profile and thyroid profile depict a picture of energy homeostasis of the body. In the present study, we assessed the effect of VMH in HFD - induced obesity of male rats and its association with the biochemical parameters.

Methods

The study was conducted after obtaining approval of the JIPMER Scientific Advisory Committee and JIPMER Institutional Animal Ethics Committee. Twelve institute-bred healthy adult male albino rats of Wistar strain weighing between 150–250 g were obtained for the study. The rats were housed in individual plastic cages with wire lids. Twelve hour light–dark cycle was maintained. They were fed standard rat chow and allowed to habituate in their cage for 10 days.

The rats were fed on HFD for 10 weeks,[7] prepared freshly each day.

Composition of HFD[7]

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

HFD and water were provided *ad-libitum*. At the end of 10 weeks when all the rats turned obese, the diet was changed to standard rodent chow and water *ad libitum.* Following this, daily food intake and body weight was measured with the help of analytical weighing balance for one week and the average was taken to determine the 24 hour basal recordings.

The rats were then divided into two groups, experimental and control with six rats in each group. After one week of acclimatization to the lab environment, the experimental group underwent electrolytic ablation of VMH bilaterally and in control group, sham lesions were made, that is needle was passed into the VMH nucleus without passing any electric current. The location of the VMH was identified using co-ordinates from the stereotaxic atlas for rat brain by König and Klippel, 1963. The procedure was performed in anaesthetized (ketamine 40 mg/kg b.w. i.p.) animals. Daily food intake and body weight was measured after lesion for four weeks and the average was taken.

Biochemical Parameters

About 2ml of blood was collected from tail vein before the lesion and at the end of four weeks blood was obtained by cardiac puncture after the lesion for the biochemical parameters recording. The animals were sacrificed thereafter.

Fasting plasma glucose (glucose oxidase peroxidase method), fasting plasma insulin (rat/ mouse Insulin ELISA kit, Millipore™, USA), lipid profile viz, Total Cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low density lipoprotein (VLDL) (chemiluminescenceit, Siemens, USA), plasma TSH (Human TSH chemiluminescence Kit, Siemens, USA), total triiodothyronine (Human TT_3 RIA kit, Immunotech™, Czech) and totalthyroxine (Human TT_4 RIA Kit, Immunotech™, Czech) were measured. HOMA - IR, an index of insulin resistance assessed by the homeostasis model assessment was calculated as follows: HOMA- IR = Fasting insulin (μU/ml) x Fasting plasma glucose (mmol/l)/22.5.[8]

Statistical Analysis

For data analysis, all values were expressed as mean ± SD. Differences between means were compared by Student's *t* test (paired and unpaired) using Graph pad In Stat (Version 3, USA) software. $P<0.05$ was considered statistically significant.

Results

In control and experimental rats, food intake and body weight before the start of experiment was comparable (Table 1). After giving HFD diet, the food intake and body weight increased in both the groups.

Before undergoing lesion, both control and experimental group did not differ significantly in food intake and body weight.

Table 1: Comparison of food intake (FI) and body weight (BW) of male rats (n = 6) before giving high fat diet (HFD).

Parameter	Control	Experimental	P value
FI (g/day)	11.56 ± 1.64	11.96 ± 1.80	0.6959
BW (g)	217.70 ± 20.456	212.20 ± 15.80	0.6136

Data expressed as mean ± SD, HFD group is the group given high fat diet. The * represents comparison with control males, The analysis of data was done by unpaired 't' test. *p<0.05; **p<0.01; ***p<0.001.

Table 2: Comparison of food intake (FI), body weight (BW) & biochemical parameters of control (rats selected for sham lesion) and experimental (rats selected for VMH lesion) in male rats before lesion.

Parameter	Control (n = 6)	Experimental (n = 6)	P value
FI (g/day)	14.14 ± 2.035	15.936 ± 2.801	0.2326
BW(g)	285.66 ± 19.098	292.83 ± 18.358	0.5223
Plasma Glucose (mg/dl)	116.66 ± 16.621	108.83 ± 8.208	0.3252
Insulin (ng/ml)	3.069 ± 0.854	2.096 ± 0.757	0.0633
HOMA IR	17.200 ± 3.626	13.519 ± 2.125	0.0575
TC (mg/dl)	80.0 ± 4.099	78.33 ± 8.71	0.6799
TG (mg/dl)	75.33 ± 10.184	77.16 ± 13.74	0.7986
HDL (mg/dl)	21.33 ± 3.077	22.83 ± 4.355	0.5065
LDL (mg/dl)	36.6 ± 6.495	38.7 ± 7.473	0.6147
VLDL (mg/dl)	23.06 ± 5.837	29.43 ± 6.349	0.1005
TSH ((μIU/ml)	0.861 ± 0.143	1.13 ± 0.357	0.1174
T3 (ng/dl)	0.95 ± 0.265	1.102 ± 0.560	0.5612
Thyroxine (μg/dl)	5.27 ± 4.747	3.842 ± 1.830	0.5074

Data expressed as mean ± SD, HFD group is the group given high fat diet. The *represents comparison with control males, The analysis of data was done by unpaired 't' test. *p<0.05; **p<0.01; ***p<0.001.

Similarly, no significant difference was observed in biochemical parameters (Table 2).

Effect of lesion

Comparison of experimental group with control group

There was a significant increase in food intake ($p<0.05$) and body weight ($p<0.001$) following lesion in experimental group (Table 3). Though there was increase in fasting plasma glucose and insulin, the increase was significant only for insulin ($p<0.05$) (Table 3). HOMA - IR increased significantly ($p<0.05$) (Table 3). There was a significant increase in total cholesterol and triglycerides ($p<0.001$) (Table 3).

Comparison of post lesion values with pre-lesion values in experimental group

Though there was increase in both food intake and body weight, body weight increase was significant ($p<0.001$) following lesion. Fasting plasma glucose, insulin, HOMA - IR, TC and TG levels were significantly increased ($p<0.001$) (Table 4). HDL and VLDL did not show any significant difference, but LDL was significantly decreased ($p<0.01$), (Table 4). Thyroid profile did not show any significant change (Table 4).

Discussion

VMH has long been considered as the major brain structure for appetite control in energy homeostasis. Lesion of VMH leads to hyperphagia and morbid obesity, which is called as 'hypothalamic obesity'.[9] But the role of VMH on food intake and body

Table 3: Comparison of food intake (FI), body weight (BW) & biochemical parameters of control (rats selected for sham lesion) and experimental (rats selected for VMH lesion) in male rats after lesion.

Parameter	Control (n = 6)	Experimental (n = 6)	P value
FI (g/day)	14.98 ± 1.565	18.928 ± 1.979*	0.0033
BW (g)	293.5 ± 14.927	362.66 ± 16.20***	<0.0001
Plasma Glucose (mg/dl)	145.52 ± 35.138	169.66 ± 22.932	0.1891
Insulin (ng/ml)	2.996 ± 0.892	4.48 ± 1.025*	0.0233
HOMA IR	25.815 ± 4.717	45.006 ± 15.651*	0.0165
TC (mg/dl)	106.0 ± 7.314	152.83 ± 11.210***	<0.0001
TG (mg/dl)	65.83 ± 5.212	104.83 ± 5.193***	<0.0001
HDL (mg/dl)	19.16 ± 4.401	20.66 ± 2.503	0.4847
LDL (mg/dl)	29.66 ± 8.350	25.2 ± 5.015	0.2882
VLDL (mg/dl)	27.16 ± 2.642	26.96 ± 4.039	0.9212
TSH ((μIU/ml)	1.76 ± 1.090	1.925 ± 1.193	0.8076
T3 (ng/dl)	0.753 ± 0.242	0.851 ± 0.647	0.7354
Thyroxine (μg/dl)	3.564 ± 1.719	4.036 ± 2.208	0.6882

Data expressed as mean ± SD, HFD group is the group given high fat diet. The * represents comparison with control males, The analysis of data was done by unpaired 't' test. *p<0.05; **p<0.01; ***p<0.001.

Table 4: Comparison of food intake (FI), body weight (BW) & biochemical parameters of control (rats selected for sham lesion) in both male rats before & after lesion.

Parameter	Pre-lesion (n = 6)	Post-lesion (n = 6)	P value
FI (g/day)	15.936 ± 2.801	18.928 ± 1.97	0.0583
BW (g)	292.83 ± 18.358	362.66 ± 16.20***	<0.0001
Plasma Glucose (mg/dl)	108.83 ± 8.208	169.66 ± 22.932***	0.0001
Insulin (ng/ml)	2.096 ± 0.757	4.48 ± 1.025***	0.0010
HOMA IR	13.519 ± 2.125	45.006 ± 15.651***	0.0006
TC (mg/dl)	78.33 ± 8.71	152.83 ± 11.210***	<0.0001
TG (mg/dl)	77.16 ± 13.74	104.83 ± 5.193***	0.0010
HDL (mg/dl)	22.83 ± 4.355	20.66 ± 2.503	0.3149
LDL (mg/dl)	38.7 ± 7.473	25.2 ± 5.015**	0.0043
VLDL (mg/dl)	29.43 ± 6.349	26.96 ± 4.039	0.4401
TSH ((μIU/ml)	1.13 ± 0.357	1.925 ± 1.193	0.1489
T3 (ng/dl)	1.102 ± 0.560	0.851 ± 0.647	0.4889
Thyroxine (μg/dl)	3.842 ± 1.830	4.036 ± 2.208	0.8717

Data expressed as mean ± SD, HFD group is the group given high fat diet. The * represents comparison with pre- lesion, The analysis of data was done by paired 't' test. *p<0.05; **p<0.01; ***p<0.001.

weight in already obese individuals has not been fully delineated. In the present study, we observed an increase in body weight after lesion even in HFD-induced obese rats. Hence, VMH lesion can lead to further increase in body weight irrespective of the initial body weight in obese rats.

The role of VMH on satiety and body weight control has been reassessed and it has been proposed that VMH-lesion obesity is in large a metabolic obesity due to autonomic dysfunctions independent of hyperphagia.[10] We have not measured the autonomic parameters in this study, but we noted a significant

increase in food intake after lesion in study group. Hence, the mechanism of hyperphagia as a cause of increased body weight after VMH lesion cannot be ruled out.

Anorexigens like insulin and leptin act on arcuate nucleus and paraventricular nucleus to increase sympathetic activity and energy expenditure and decrease food intake.[11] However, the observation that the increase in body weight and food intake after VMH lesion alone, could be due to the fact that VMH acts as the final common pathway controlling the feeding behavior cannot be concluded as there are other hypothalamic areas regulating feeding.

Important parameters of energy homeostasis are levels of plasma glucose and insulin. There was a significant increase in insulin level following VMH lesion which is in corroboration with the previous studies.[9,12–16] In the present study, though there was an increase in plasma glucose concentration, it was not significant when compared to controls. However, the plasma glucose concentration was significantly increased compared to pre-lesion values. Many reports state that normal serum glucose level, in the presence of hyperinsulinemia suggests insulin resistance.[13,15] Hence, in the present study experimental rats were in the insulin resistant state after VMH lesion. This is further supported by the significant increase in HOMA – IR compared to the controls as well as pre-lesion values. Suga et al showed that despite a marked adiposity and hyperinsulinemia, insulin resistance was not increased in VMH-lesioned rats whereas fructose diet brought about substantial insulin resistance and hyperinsulinemia in both lean and obese rats.[17] This difference could be due to the type of diet given. Thus, it appears that following VMH lesion, HFD induced obese male rats are susceptible to insulin resistance.

In the present study, serum cholesterol in experimental rats was significantly increased following VMH lesion, which indicates that VMH has a cholesterol suppressing effects in high-fat induced obesity. Following lesion, triglyceride was significantly increased inexperimental rats (Table 4). Our findings are in conformity with the previous report that VMH lesion causes hypertriglyceridemia.[18] Another study suggests that lipoprotein synthesis markedly increases in the livers of VMH lesioned rats. [19] From the findings of the present study, it is proposed that VMH has an inhibitory effect on triglycerides and cholesterol in males and the effect is reduced after lesion.

Thus, our study suggests increased susceptibility of obese male rats to insulin resistance and dyslipidemia.

Thyroid hormone largely influences energy balance of the body. Our study did not show any significant difference in TSH, T_3 and thyroxinelevels in experimental rats following VMH lesion compared to that of control rats.

Conclusion

VMH is the important center for satiety and adiposity and could be the ultimate pathway for control of food intake and obesity. The mechanism of hyperphagia as a cause of increased body weight after VMH lesion could be the possible mechanism for obesity. VMH lesion can lead to further increase in body weight in HFD-induced obese rats. VMH appears to prevent the development of insulin resistance. The present study reveals the susceptibility of male rats on HFD to insulin resistance, adiposity and hyperlipidemia after VMH lesion.

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