Journal of
Obesity & Weight Loss Therapy

Singh et al., J Obes Weight Loss Ther 2014, 4:4
DOI: 10.4172/2165-7904.1000234

Research Article Open Access

Altered Monoamine Metabolism in High Fat Diet Induced Neuropsychiatric Changes in Rats

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Received: September 08, 2014; **Accepted:** October 20, 2014; **Published:** October 25, 2014

Abstract

Objective: To investigate the role of central and peripheral monoamine metabolism on neuropsychological status in high fat diet induced obese rats.

Method: Animals were fed on high fat diet for 12 weeks. Periodically blood was collected to estimate serum Metanephrine (MN) levels. After 11 weeks, behavioural paradigms of depression, locomotor activity and cognition were performed. Central (brain) and peripheral i.e. Visceral White Adipose Tissue (vWAT) and Brown Adipose Tissue (BAT) MN levels, Monoamine Oxidase A and B (MAO-A, MAO-B) enzyme activity and Acetylcholinesterase (AChE) activity was estimated.

Results: High fat diet significantly increased the body weight which was negatively correlated with the serum metanephrine concentration. However, metanephrine concentration in brain was significantly decreased whereas it significantly increased in vWAT with no change in BAT. In obese animals, immobility time in forced swim test and transfer latency in elevated plus maze was significantly increased while locomotor activity was significantly decreased. Central MAO-A and MAO-B activities were increased while enzyme activities showed a significant reduction in vWAT with no change in BAT. Brain AChE activity was also increased significantly in obese rats.

Conclusion: Metabolism of biogenic monoamines plays a critical role in altered neuropsychiatric behaviours associated with diet induced obesity.

Keywords: Obesity; Neuropsychiatric illnesses; High fat diet; Metanephrine; Monoamine oxidase

Abbreviations:

MN: Metanephrine; vWAT: Visceral White Adipose Tissue; BAT: Brown Adipose Tissue; MAO: Monoamine Oxidase; AChE: Acetylcholinesterase

Introduction

Epidemiological data has demonstrated a strong correlation between weight gain and increase in neuropsychiatric illnesses and *vice versa* [1]. Monoamines (epinephrine, nor-epinephrine, dopamine and serotonin) are central to both obesity and neuropsychiatric disorders like depression, cognitive impairment and anxiety [2,3]. It has been suggested that monoamine levels and their metabolism in CNS plays a significant role in depression, locomotion and anxiety as evident by multiple classes of drugs (serotonin reuptake inhibitors, nor epinephrine/serotonin reuptake/metabolism inhibitors, atypical antidepressants) that have been designed based on this. Furthermore, the activity of biogenic amines has been reported in various adipose tissues [4]. Epinephrine activity in primary cell culture of adipocytes suggested their significant role in lipolysis and increased triglycerides release [5]. The activity of Monoamine Oxidase (MAO) enzymes has also been reported in different adipose tissue [6], which can be used as indirect marker of activity of these energy dissipating monoamines in different adipose tissues. Increased MAO activity in brain leads to depletion of monoamines that has been shown to result in major depressive disorder [7]. Metanephrine (MN), an intermediate metabolite of epinephrine generated by action of Catechol-o-methyl Transferease (COMT) is further attacked by MAO to form Vanillylmandelic Acid (VMA). It has a significant role as a marker of monoamine metabolism and in turn of energy expenditure and dissipation in adipose tissue [4].

Till date, there are very limited studies to assess the effect of monoamine metabolism on obesity associated neuropsychiatric illnesses. The present study is an attempt to provide evidence for the possible role of monoamine metabolism in central and peripheral tissues of High Fat Diet (HFD) fed obese rats.

Materials and Methods

Animals

Male Wistar rats (180-220 g, 3-4 month aged) bred in Central Animal House (CAH) facility of Punjab University, Chandigarh were used. Animals (3-4/cage) were housed under standard laboratory conditions (22 ± 2°C, relative humidity=55%) with free access to

water. Animals were divided into two groups: control animals (n=6) received Normal Rodent Diet (NRD) (Aashirwad industries, Mohali, India) and HFD group received diet containing 58% energy from fat (lard) using standard composition [8] described (Table 1). The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Punjab University, Chandigarh. Experiments were performed in accordance with Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) and Indian National Academy of Science (INSA) guidelines.

Ingredients	Diet (g/kg)
Powdered NRD	365
Lard	310
Casein	250
Cholesterol	10
Vitamin & mineral mix	60
DL-Methionine	03
Yeast powder	01
Sodium chloride	01

Table 1: Composition of HFD.

Experimental protocol

Animals were acclimatized for one week to the experimental condition. Their initial body weights were measured and animals were fed with group specific NRD and HFD for 90 days. The major difference in diets is by energy from fat content (12% fat, 25% protein and 63% carbohydrate, as a % of total kcal) and the diets are not ketogenic in nature HFD (58% fat, 25% protein and 17% carbohydrate, as a % of total kcal). Body weights were measured every week and blood was collected retro-orbitally once per month for the estimation of serum MN levels during the experimental period. After 85 days, behavioural analysis was performed. Rats were sacrificed by cervical dislocation after performing behavioural assays. Whole brain, vWAT and BAT were dissected out and preserved at -80°C until further analysis.

Behavioural assessment

Evaluation of depressive behavior:

Forced swim test [9] was used to evaluate the depressive stature of the animals by measuring the immobility in water filled tank (60 cm*30 cm*45 cm). Animals were forced to swim in that glass tank on 85th day of the experiment for 15 minutes and on 86th day the immobility (stage where no or minimal movement observed) or the condition of learned helplessness precipitated was recorded using stopwatch for 5 minutes.

Evaluation of cognitive stature in elevated plus maze test:

Transfer latency, the time taken by animals to enter in closed arm was evaluated in elevated plus maze [10], was considered as measure of learned task or memory process. It was recorded by training the animals on 88th day of the experiment and the retrieval of memory process was performed on 89th day.

Locomotor activity:

Gross locomotor activity was measured on 90th day of the experiment for evaluation of general activity of the animals in photoactometer instrument (IMCORP, Ambala, India). Animals were placed individually in the instrument for a total period of 8 minutes, in which first 3 minutes were considered as acclimatization period during which no recording was made. Recording of total ambulatory and rearing movements were counted automatically by the 12 photocell (6 on each wall) placed on the opposite two walls of the arena (30 cm*23 cm*22 cm) [11].

Serum metanephrine analysis

Metanephrine (MN) levels were estimated in serum collected on various days (Day 0, 30, 60, 90) and in brain, vWAT and BAT tissues as per the manufacturer's instruction (Mybiosource Inc. San Diego, CA, USA; catalog # MBS887295).

Tissue monoamine oxidase enzyme activity estimation

Isolation of mitochondrial samples:

MAO-A and MAO-B activity were measured in brain, vWAT and BAT tissue using method described by McEwen and Charls [12]. Half of the brain section, through midsagittal plane were cut using scalpel blade. For other tissues approximately 200 mg samples were used. Briefly, tissue mitochondria were extracted using method of Schurr and Livne [13]. Tissues were homogenized (10% w/v) in homogenization buffer (0.25 M sucrose, 0.1 M tris, 0.02 M EDTA-pH 7.41). Homogenate was centrifuged at 800 g, 10 min, at 4°C. Pellet was discarded and the supernatant was then centrifuged at 12,000 g, 20 min. and the precipitate was washed twice with sufficient amount of same buffer and resuspended in 50 mM potassium phosphate buffer, pH 7.4. Final protein concentration was estimated using Bradford's reagent.

Estimation of MAO-A activity:

1 part of the homogenate was added to 1 part of serotonin and 1 part of buffer. The reaction tube was placed at 37°C for 20 minutes and the reaction was arrested by the addition of similar volume of 1 M HCl. The reaction product was extracted with 20 part of butyl acetate. The organic phase was separated and measured at 280 nm using a spectrophotometer. Blank samples were prepared by adding 1 M HCl (200 µl) prior to reaction and the reaction was carried out. The MAO-A is expressed in nmoles/ mg protein.

Estimation of MAO-B activity:

1 part of the homogenate was added to 1 part of serotonin and 1 part of buffer. The reaction tube was placed at 37°C for 20 minutes and the reaction was arrested by the addition of similar volume of 1 M HCl. The reaction product was extracted with 5 ml of Cyclohexane. The organic phase was separated and measured at 242 nm using a spectrophotometer. Blank samples were prepared by adding 1 M HCl (200 µl) prior to reaction and the reaction was carried out. The MAO-B activity is expressed in nmoles/ mg protein.

Estimation of AChE activity

In the second half of the brain activity of AChE, a degrading enzyme to acetylcholine was estimated by method of Ellman et al. [14].

AChE is a prominent marker in cognition loss because of its ability to breakdown the important neurotransmitter acetylcholine. Briefly, the samples were homogenized in 10 mM sodium phosphate buffer, pH 7.4 and then centrifuge at 10,000 g for 20 minute, 4°C. Supernatant was collected and used for AChE estimation. In 3.0 mL of sodium phosphate buffer (0.1 M, pH 8.0), 0.1 mL of Ellman's reagent was mixed and 0.1 mL of acetyl thiocholine iodide was added, afterwards 0.05 mL samples were added and change in absorbance over a period of 2 minute at 412 nm. Results were calculated using the molar extinction coefficient of chromophore ($1.36\times104\ M^{-1}\ cm^{-1}$). Results were expressed as µM substrate hydrolyzed/ min/mg protein.

Statistical analysis

Data were analysed with Graphpad 6.0 software (Graphpad software Inc., CA, USA). All the values were presented as mean ± S.E.M. A two way Analysis of Variance (ANOVA) with Bonferonni post-test were used for analysis of body weight data and serum MN concentration at different time intervals. Unpaired t-test was used for analysis of central and peripheral MN concentration, average weight gain, average MN change, behavioural tests, MAO-A and MAO–B and AChE activity. Pearson correlation was used for correlation statistics. A minimum criterion for significance was set to $P \leq 0.05$.

Results

Administration of HFD significantly increased the body weight of rats (Figure 1A) Behavioural analysis (Figure 1 B–D) showed significant increase in immobility time for HFD fed rats as compared to NRD fed rats in forced swim test (P<0.0001). Likewise transfer latency in elevated plus maze test was significantly increased (P<0.0001) in HFD fed rats. On the contrary, there was a significant decrease (P=0.0041) in total locomotor activity of HFD fed rats as compared to control (Figure 1B–D). There was significant correlation between weight gain and behavioural changes (Figure 1E–G).

Serum MN levels were decreased with weight gain (although non-significant) [F(3,52)=0.7674, P=0.5175]. MN levels in vWAT of HFD rats was significantly high (P=0.0302) as compared to control rats whereas there was no change MN concentration in BAT. On the contrary, brain MN levels were significantly decreased (P=0.0404) in HFD fed rats (Figure 2A). A significant increase (P<0.0001) in average weight gain and decrease in average change in MN concentration were observed in HFD fed rats (Figure 2B). In HFD fed animals, a significant negative correlation was found between weight gain of animals and change in their MN levels (Figure 2C) (r=-0.4098, P=0.0123). Central MAO-A and MAO-B activity was significantly enhanced in obese rats as compared to normal rats (P<0.0001) (Figure 2D). In contrast, peripheral MAO-A (in BAT and vWAT) was significantly reduced (P=0.008 and 0.0034 respectively). MAO-B activity in BAT was not changed significantly in HFD rats as compared to normal rats (P=0.0902), whereas, it was decreased significantly in vWAT of HFD fed rats as compared to control (Figure 2D). The enzymatic activity of AChE (Figure 2E) was found to be significantly increased in obese rats (P=0.0164) as compared to normal rats.

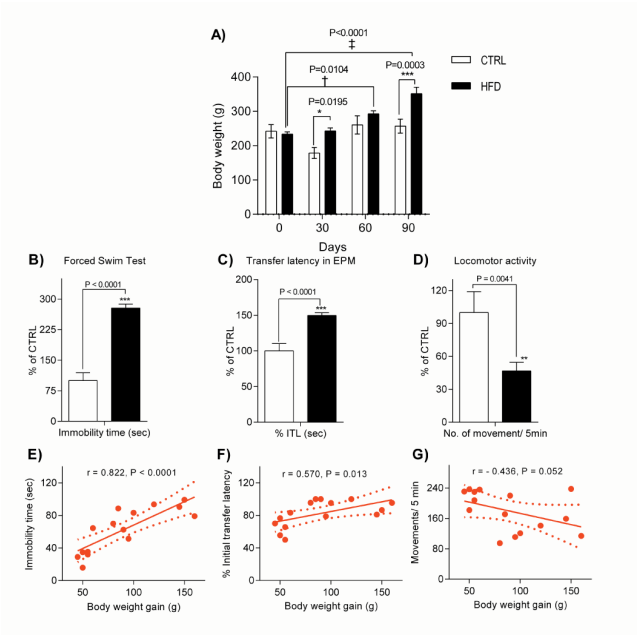

Figure 1: Effect of high fat diet on different behaviour paradigms in rats: [A] body weights for 90 days; [B] immobility time in forced swim test; [C] transfer latency in elevated plus maze test; [D] locomotor activity in photoactomreter; [E–G] correlation between body weight gain and different behaviour parameters (dotted line represent 95% confidence interval band). All the values (A–D) are expressed as mean ± S.E.M. (Two way ANOVA or student t-test was applied; *P<0.05 versus its respective control, **P<0.01 versus its respective control, ***P<0.001 versus its respective control, †P<0.01 versus day 0 control, ‡P<0.001 versus day 0 control). CTRL=Control (n=6), HFD=High Fat Diet (n=10), EPM=Elevated Plus Maze, ITL=Initial Transfer Latency.

Discussion

The present study suggests a positive correlation between body weight gain and development of neuropsychiatric illnesses like depression and cognitive deficits. Further, while evaluating (a) serum and tissue (brain and vWAT/BAT) metanephrine levels (b) MAO A & B enzyme activity in brain and adipose tissue, we have established a link between perturbations in monoamine metabolism and development of psychiatric disorders in high fat fed rats.

HFD promoted time-dependent increase in body weight over the period of 3 months. During first month, there was no significant change in HFD rats whereas in normal rats showed a decrease in body weight. This can be attributed to external factors such as novel environment, change in feed and handling of animals. HFD fed rats showed depression (significant increase in immobility time in forced swim test), learning abnormality (increase in transfer latency in plus maze test) and decreased locomotion (decrease in total number of rearing and ambulatory movements in photoactometer). Body weight gain was positively correlated with depression like symptoms and learning abnormality whereas it was negatively correlated with locomotor activity. It is in accordance with already existing literature where stress reactions and depression have been linked to visceral fat

accumulation and abdominal obesity [15]. It underscores the importance of understanding mental health in obese patients and also suggests obesity is associated with an increased risk of developing psychiatric disorders.

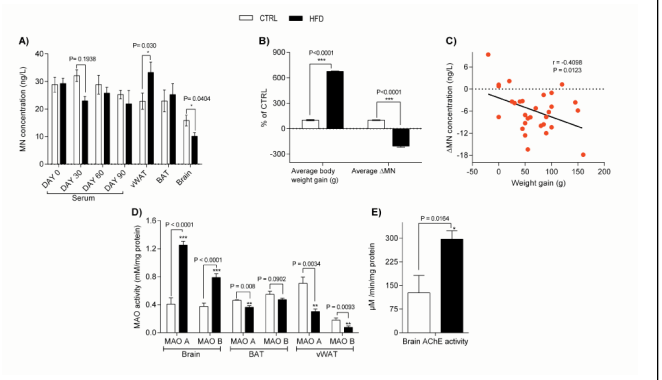

Figure 2: Effect of high fat diet on different molecular markers and enzyme activities in rats: [A] Metanephrine concentration (ng/L) in serum, visceral white adipose tissue, brown adipose tissue, and in brain; [B] average weight gain and average change in serum metanephrine concentration was represented as function of their respective control values; [C] correlation between change in serum metanephrine concentration to weight gain; [D] MAO activity in brain, BAT and vWAT; [E] AChE in brain of rat. All the values are expressed as mean ± S.E.M. Student t-test was applied to check the statistical significance. *P<0.05 versus control, **P<0.01 versus control, ***P<0.001 versus control. CTRL=Control (n=6), HFD=High Fat Diet (n=10), vWAT=Visceral White Adipose Tissue, BAT=Brown Adipose Tissue, MN=Metanephrine, Δ=Change, MAO A and MAO B=Monoamine Oxidase A and B, AChE=Acetylcholinesterase.

The mean weight gain was negatively correlated with serum metanephrine levels. This is quite evident as a decreased level of metanephrine signifies decreased levels of adrenergic activity, decreased energy dissipation, thermogenesis and anti stress response [16]. It has been well studied in rodents and recently it has been shown in humans too that levels of metanephrine are positively correlated with brown adipose tissue activity [4,17]. We can argue here that consumption of high fat diet results in decrease in energy expenditure and brown adipose tissue activity. Both these are positively correlated with increase in central adiposity.

Considering the role of monoamine metabolism in these psychiatric behaviours, we assayed for MAO-A and MAO-B activity in brain as well as peripheral tissue like adipose tissue. Both MAO-A and B were significantly increased in brain suggesting a decrease in the levels of epinephrine, serotonin and dopamine in diet induced obesity in rats [7,18]. This can explain the change in behaviour pattern of high fat diet fed animals (i.e. reduction in epinephrine and serotonin lead to depression like behaviour and decrease in dopamine may result in decreased locomotion). Similarly, Acetylcholinesterase activity (AChE) was also significantly increased in high fat diet fed animals, hence lowering acetylcholine required for memory and learning. We can here rationally argue that HFD-induced obesity might induce activity of monoamines metabolizing enzymes. Surprisingly, the activity of MAO-A and B enzymes were significantly decreased in peripheral tissues like visceral white adipose tissue. This can be attributed to decrease in substrate concentration (i.e. signal from brain to periphery) or a decrease in the enzyme production/activity to preserve the levels of monoamines, which could be due to compensatory mechanism of increased activity in brain or serum. Simultaneous increase in the tissue metanephrine levels are also there. In BAT, there was no change in metanephrine levels as well as MAO-B enzyme activity with a significant decrease in MAO-A enzyme activity.

Conclusion

In conclusion, weight gain is associated with altered psychiatric behaviors. Our study points out differential involvement of central and peripheral monoamine metabolism in weight gain. Further studies should be carried out to understand the link and possible role of synthetic or natural enzyme modifying agents as preventive or therapeutic options in obesity-induced psychiatric changes.

Acknowledgement

Authors would like to thank Department of Biotechnology, Government of India for research grants given to National Agri-Food Biotechnology Institute (NABI).

Conflict of Interest

Authors declare that there are no conflicts of interest.

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