Abstract

Diets rich in high fats have shown to exert detrimental effects on brain chemistry and cognitive functions. The present study was designed to examine the high fat (HF) diet induced age related perturbations in cholinergic and antioxidant systems of brain and associated dysfunctions in cognitive behavior of rats. Wistar albino rats (2 and 6 months) were fed with control and HF diet separately for a period of 8 weeks. It is evident that no significant changes were observed in body weight except in 7th and 8th week after consumption of HF diet. However, significant increased levels in total cholesterol and triglycerides were recorded in both 4 and 8 months age groups of HF diet fed rats. Results also showed decreased synaptosomal acetylcholinesterase (AChE) and, mitochondrial superoxide dismutase (SOD) activities where as MDA levels increased in the cortex, hippocampus and cerebellum regions of both age groups following consumption of HF diet. These HF diet induced alterations in cholinergic and antioxidant systems were greater in cortex and more pronounced at 8 months age rats. Spatial learning, memory and exploratory behaviors also decreased in 4 and 8months age groups of HF diet fed rats. In conclusion, our results suggest that HF diet induces impairments in spatial learning and alterations in brain cholinergic and antioxidant systems in age dependent manner.

Keywords: high fat diet, cholinergic system, antioxidant system, behavior, brain.

Introduction

The consumption of fat-rich diets has increased significantly over the past decade and contributing to the world wide epidemic obesity (1). The high-fat (HF) diet consumption has been linked to development of chronic diseases including diabetes, hypertension, cardiovascular diseases and also cognitive function deficits (2-4). Epidemiological studies have established an association between high intake of fats and increased risk of developing cognitive impairment (5,6). Different experimental studies also showed that consumption of HF diet is associated with increased vulnerability of cholinergic system and deficits in cognitive functions (7,8).

Recent studies showed altered brain cholinergic system and deficits in memory and learning, exploratory behavior after consumption of HF diets (9,10). Experimental and epidemiological studies have also showed that obesity and metabolic dysfunctions are highly associated with increased oxidative stress (11,12). Rinder et al., (13) and Dhibi et al., (14) reported that HF diet increased the lipid peroxidation and alterations in the markers of apoptosis by impairment in the activities of antioxidant enzymes superoxide dismutase, (SOD) catalase (CAT) and glutathione peroxidase (GPx) in the tissues of rat. Most of the available research studies on the impact of HF diet concentrated on old age rats and less attention has been focused on age-related impairments in cognitive functions. Therefore,
this study was undertaken to determine the HF diet induced age related impairments in brain cholinergic and antioxidant systems and associated dysfunctions in spatial learning and exploratory behavior of rats.

Materials and Methods

For the present study, 2 months and 6 months old male Wistar albino rats were fed with a control and high fat diets separately for a period of 8 weeks.

Diet: The animals were fed in the laboratory with high fat and control diets supplied by National Institute of Nutrition (NIN), Hyderabad, India and water *ad libitum*. The High fat diet contained the following ingredients: Casein-2.736 Kg, L-cystine-0.024 Kg, Starch - 1.376 Kg, Sucrose - 1.376 Kg, Cellulose-0.40 Kg, Ground Nut oil-0.200 Kg, Tallow- 1.52 Kg, Mineral Mix (AIN 93 grade) - 0.28 Kg, Vitamin Mix (AIN 93 grade) - 0.08 Kg. The animals were housed in clear plastic cages with hardwood bedding in a room maintained at 28° ± 2° C and relative humidity 60 ± 10% with a 12 hour day/night cycle. Animal experiments were carried out in accordance with NIH and ICMR (India) guidelines. The approval to conduct the experiments was obtained from the Institutional animal ethical clearance committee, S. V. University. Both age groups of rats were weighed every week for the period of two months.

Serum total cholesterol and triglycerides: Total cholesterol and triglycerides were estimated by using commercially available kits (bio systems) with auto analyzer in serum of control and high fat diet fed rats at 4 and 8 months.

Isolation of mitochondrial and synaptosomal fractions: Synaptosomes were isolated from brain homogenates using Ficoll-sucrose gradients (15). The cerebral cortex, cerebellum and hippocampus were isolated in cold conditions. The tissues were weighed and homogenized in 10 ml of ice-cold homogenizing buffer (320 mM sucrose, 10 mM imidazole, 1 mM EDTA) and the volume was brought up to 25 ml with homogenizing buffer. The homogenates were centrifuged at 750 x g for 10 min. The pellets were discarded. The supernatants were centrifuged at 17,000 x g for 20 min. The pellets were suspended in 10 ml of 0.32 M sucrose and were layered on a two-step discontinuous Ficoll–sucrose gradient consisting 13 and 7.5% Ficoll and centrifuged at 65,000 x g for 45 min. The milky layer was formed at the interface of 13 and 7.5% Ficoll. The pellet formed at the bottom of the centrifuge tube (mitochondrial fraction) was taken and suspended in homogenizing buffer. The milky layer fraction was diluted with nine volumes of 0.32 M sucrose and centrifuged again 17,000 x g for 30 min. The supernatant was discarded and the pellet (synaptosomal fraction) was suspended in 0.32 M sucrose.

Cholinergic system

*Estimation of Acetylcholine (ACh):* The acetylcholine content was estimated by the method of Metcalf (16) as given by Augustinson (17). The synaptosomal fractions of hippocampus and cerebellum were placed in boiling water for 5 minutes to terminate the AChE activity and also to release the bound ACh. To the synaptosomal fractions 1 ml of alkaline hydroxylamine hydrochloride followed by 1 ml of 50% hydrochloric acid were added. The contents were mixed thoroughly and centrifuged. To the supernatant 0.5 ml 0.37 M ferric chloride solution was added and the brown colour developed was read at 540 nm against a reagent blank in a spectrophotometer and expressed as acetylcholine (ACh) content (µ moles of ACh/gm wt.).

*Estimation of Acetylcholinesterase activity (AChE):* AChE specific activity was determined following the method of Ellman *et al.*, (18). The reaction mixture contained 3.0 ml of phosphate buffer (pH 8.0), 20 µl of 0.075M acetylthiocholine iodide (substrate) and 100 µl of 0.01 M DTNB (5, 5-Dithiobis-2-Nitrobenzoic acid). The reaction was initiated with the addition of 100 µl of synaptosomal fraction. The contents were incubated for 30 min at room temperature and the color absorbance was measured at 412 nm in spectrophotometer.

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Oxidative stress marker enzymes

Estimation of Superoxide dismutase (SOD) activity: Measurement of total SOD activity was performed according to Misra and Fridovich (19) based on the inhibition of autoxidation of epinephrine. The total reaction mixture contained 880 µl of carbonate buffer, 20 µl of epinephrine and 100 µl of enzyme source and absorbance was recorded at 480 nm against reagent blank. The enzyme activity was expressed as µ moles of superoxide anion reduced/ mg of protein.

Estimation of MDA levels: The lipid peroxides were determined by the TBA method of Hiroshi et al., (20). The tissues were homogenized in 1.15% KCl (20% W/V). To 1 ml of tissue homogenate, 2.5 ml of 20% trichloro acetic acid (TCA) was added and the contents were centrifuged at 3500g for 10 minutes. Residue was dissolved in 2.5 ml of 0.05 M sulphuric acid (H2SO4). To this 3 ml of thiobarbituric acid (TBA) was added and the samples were kept in a hot water bath for 30 minutes. The samples were cooled and malondialdehyde (MDA) was extracted with 4 ml of n-butanol and read at 530 nm in a spectrophotometer against the reagent blank. The results were expressed as micromoles of MDA formed/mg of tissue/hr.

Estimation of protein content: Protein content of the tissues was estimated by the method of Lowry et al., (21). To 0.1 ml of synaptosomal fraction, 1ml 10%TCA was added and the samples were centrifuged at 1000g for 15 minutes. Supernatant was discarded and the residues were dissolved in 1ml of 1N sodium hydroxide (NaOH). To this, 2 ml of alkaline copper reagent was added followed by 0.2 ml of folin-phenol reagent (1:1folin:H2O). The color was measured at 600 nm in spectrophotometer (Hitachi model U-2000) against a blank. The protein content in mitochondrial fraction was also measured with the same procedure. The protein content of the tissues was calculated using the standard graph. The values were expressed as mg protein/gm wet weight of the tissue.

Behavioral Studies

Morris Water Maze: The water maze is a circular water tank measuring 1.85 m in diameter and 0.7 m deep constructed according to a basic design similar to that of Morris (22). Four points along the circumference of the water tank are designated arbitrarily North (N), South (S), East (E) and West (W), thus dividing the maze into four quadrants. The pool was filled to a depth of 30 cm with water made opaque with white, nontoxic water-based paint. A circular platform (diameter 12.5 cm) is placed 2-3 cm below the surface of the water. Rats were allowed to swim to the hidden platform and the escape latency (time to find the hidden platform) was noted. Animals were tested for a total of 2 daily trials for 5 days. Each trial was separated by 30 minutes. Rats were placed in water facing the wall of the pool and allowed 60 seconds to find the escape platform. If the animal reached the escape platform within 60 seconds, the escape latency was recorded and the mouse was allowed to rest on the platform for 10 seconds before removal. Each age group was separately tested. In tests of reference memory, the hidden platform remained in the same location throughout each phase of the experiment. During the acquisition phase, the platform was placed in the centre of the quadrant, 15 cm from the edge of the pool, and it was moved to the opposite quadrant for the reversal phase. In tests of working memory, each rat was required to find the hidden platform located in a new position. The location of the hidden platform was changed daily in a pseudo-randomized fashion such that different rats were tested in all quadrants on a given day, and all rats were trained in each quadrant at least twice during the experiment.

Exploratory behavior: Exploratory behavior was measured in a box with a hole board bottom (90 cm × 90 cm) containing three equally spaced holes (3 cm in diameter) in the floor. Each rat was placed in the center of the arena for 5 min during which time the number of head dips and head-dipping duration (in seconds) were
recorded. A head dip was scored if both eyes disappeared into the hole.

**Analysis of Data:** The data were subjected to one way analysis of variance (ANOVA) followed by student Newman-Keuls (SNK) post hoc test. The 0.05 level of probability was used as the criterion for significance.

**Results**

In the present study, analysis of body weight over the 8 experimental weeks, established no significant changes (P >0.05) in body weight except in 7th and 8th week of 4 and 8 months age groups of HF diet fed rats, compared to controls (Fig.1). Total cholesterol and triglycerides levels in serum were estimated using Bio-system kits. A significant increase was observed in both total cholesterol and triglyceride levels in both the age groups of HF diet fed rats compared to controls (Fig. 2 and 3). The specific activity of synaptosomal AChE and ACh levels were estimated in cortex, cerebellum and hippocampus regions at 4 and 8 months age groups of rats (Fig. 4 and 5). The hippocampus region exhibited higher levels of AChE activity and ACh levels than cortex and cerebellum (Fig. 4 and 5). The specific activity of AChE as well as ACh levels were decreased in HF diet fed rats (The decrease in AChE was 3.9% and 21.1% in cortex, 2.3% and 16% in cerebellum, and 9.4% and 29.1% in hippocampus regions respectively of 4 and 8 months age groups of rats where as the decrease in ACh levels were 25.3% and, 33% in cortex, 13.3% and 31% in cerebellum and 9.2% and 21.1% in hippocampus regions respectively of 4 and 8 months age groups of rats) compared to controls. (Fig. 4 and 5).

Further, we evaluated the mitochondrial SOD activity and tissue MDA levels in cortex, cerebellum and hippocampus regions (Fig. 6 and 7). The SOD enzyme activity and MDA levels were increased with age and the highest activity of SOD and MDA levels were observed in cortex than cerebellum and hippocampus. HF diet fed rats showed decrease in the activity of SOD (cortex: 9.7% in 4 months, 32% in 8 months; cerebellum: 5.4% in 4 months, 20.1% in 8 months) and MDA levels (cortex: 11.3% in 4 months, 20.1% in 8 months; cerebellum: 13.3% in 4 months, 21.1% in 8 months) compared to controls.
Fig. 3. Effect of HF diet on serum triglyceride levels in 4 and 8 months age groups of rats. Male rats were fed with control and high fat diets separately for a period of 8 weeks. Values are mean ± SD of eight observations. The values marked with “asterisk” are significantly different from corresponding controls as evaluated by the ANOVA followed by student Newman–Keuls (SNK) post hoc test (P < 0.05).

Fig. 4. Effect of HF diet on synaptosomal acetylcholinesterase (AChE) activity in cortex, cerebellum and hippocampus regions of 4 and 8 months age groups of rats. Male rats were fed with control and high fat diets separately for a period of 8 weeks. Values are mean ± SD of eight observations. The values marked with “asterisk” are significantly different from corresponding controls as evaluated by the ANOVA followed by student Newman–Keuls (SNK) post hoc test (P < 0.05).

Fig. 5. Effect of HF diet on synaptosomal acetylcholine (ACh) levels in cortex (CC), cerebellum (CB) and hippocampus (HP) regions of 4 and 8 months age groups of rats. Male rats were fed with control and high fat diets separately for a period of 8 weeks. Values are mean ± SD of eight observations. The values marked with “asterisk” are significantly different from corresponding controls as evaluated by the ANOVA followed by student Newman–Keuls (SNK) post hoc test (P < 0.05).

Fig. 6. Effect of HF diet on mitochondrial superoxide dismutase activity (SOD) in cortex (CC), cerebellum (CB) and hippocampus (HP) regions of 4 and 8 months age groups of rats. Male rats were fed with control and high fat diets separately for a period of 8 weeks. Values are mean ± SD of eight observations. The values marked with “asterisk” are significantly different from corresponding controls as evaluated by the ANOVA followed by student Newman–Keuls (SNK) post hoc test (P < 0.05).
**Fig. 7.** Effect of HF diet on MDA levels in cortex (CC), cerebellum (CB) and hippocampus (HP) regions of 4 and 8 months age groups of rats. Male rats were fed with control and high fat diets separately for a period of 8 weeks. Values are mean ± SD of eight observations. The values marked with “asterisk” are significantly different from corresponding controls as evaluated by the ANOVA followed by student Newman-Keuls (SNK) post hoc test (P < 0.05).

**Fig. 8.** Effect of HF diet on average escape latency (time in sec) to find the hidden platform during acquisition, reversal phase tests and working memory test at 4 months age group rats. Values are mean ± SD of eight observations. The values marked with “asterisk” are significantly different from corresponding controls as evaluated by the ANOVA followed by student Newman-Keuls (SNK) post hoc test (P < 0.05).

**Fig. 9.** Effect of HF diet on average escape latency (time in sec) to find the hidden platform during acquisition, reversal phase tests and working memory test at 8 months age group rats. Values are mean ± SD of eight observations. The values marked with “asterisk” are significantly different from corresponding controls as evaluated by the ANOVA followed by student Newman-Keuls (SNK) post hoc test (P < 0.05).

**Fig. 10.** Effect of HF diet on exploratory behavior at 4 and 8 months age groups of rats. Values are mean ± SD of eight observations. The values marked with “asterisk” are significantly different from corresponding controls as evaluated by the ANOVA followed by student Newman-Keuls (SNK) post hoc test (P < 0.05).

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months; hippocampus: 6.8% in 4 months, 28.5% in 8 months) and increase in tissue MDA levels (cortex: 8.6% in 4 months, 30.9% in 8 months; cerebellum: 6.4% in 4 months, 20.9% in 8 months; hippocampus: 12.2% in 4 months, 28.9% in 8 months) in both age groups of rats, compared to controls (Fig. 6 and 7). However, the alterations in specific enzyme activities, neurotransmitter and MDA levels were more pronounced in cortex (P <0.001) than hippocampus and cerebellum regions and greater at 8 months (P <0.001) age groups of rats (Fig. 4 to 7) after consumption of HF diet.

Spatial learning and memory were tested in Morris water maze and results from the reference (acquisition and reversal phases) and working memory tests are summarized in Fig. 8 and 9. HF diet fed rats showed increase in the escape latency to find the hidden platform in acquisition, reversal phases and working memory of both age groups of rats, compared to control (Fig.8 and 9). The exploratory behavior recorded in three whole board showed shorter head dip duration and lesser head dip counts in HF diet fed rats of both age groups of rats (Fig.10). However, the alterations observed in spatial learning, memory and exploratory behavior in older adults were significant (P <0.001) (Fig. 8 to 10) and marginal (P >0.05) in young adult rats.

Discussion

The major findings of the present study are that, consumption of HF diet can lead to impairments in spatial learning, memory and brain cholinergic and antioxidant systems in different age groups of rats. HF diet induced an overt obesity characterized by body weight gain and fat deposition in different tissues (1,23). Rats fed with the HF diet had consistently greater body weight than control and the majority of the weight gain occurred from the fourth week in both age groups of rats after consumption of HF diet. Furthermore, the increase in body weight was greater in older adults (8 months) compared to young adults after consumption of HF diet. Chen et al.,(24) reported similar body weight gain after six weeks in HF diet fed rats.

Diets rich in HF induce alterations in lipid profiles which consequently increase the serum total cholesterol and triglyceride levels in rats (25). Our results also showed significant increase in serum total cholesterol and triglyceride levels following consumption of HF diet in both age groups of rats. Roberst et al., (26) reported that HF diet caused an increase in serum triglycerides and total cholesterol levels of rats. Different experimental studies also showed increased levels of lipid profiles in serum and tissues of rats fed with HF diets (27,28). Interestingly, the present study results showed HF diet induced alterations in serum lipid profiles were greater in older rats than young adults, suggesting age dependent accommodation to the higher caloric density of the HF diet. The link between dyslipidemia and risk of cognitive impairment or dementia is unclear (29). Indeed, rats fed with higher amount of dietary fat showed widespread cognitive deficits on various tasks of learning and memory (10,30). The results of present study showed HF diet fed rats took longer time to find hidden platform and caused impairments in acquisition, reversal phases and working memory in Morris water maze test at 4 and 8 months age groups of rats. Molteni et al.,(31) reported that HF diet consumption increases the deficits in spatial learning and memory of rats. Impairments in learning and memory have also been observed in young, adult and aged rats after consumption of HF diet (10,32). Our findings also showed decreased exploratory behavior in both age groups of rats following HF diet consumption. However, HF diet induced impairments in spatial learning, memory and exploratory behavior were greater in older age group rats compared to young adult rats suggesting age dependent effects of HF diet. However, the mechanisms of effects of dietary fat on cognitive functions are still not completely understood.

Cognitive dysfunctions are characterized by a substantial loss of cholinergic system in different brain regions of rat (6,33). Morganstern et al.,(10) reported that changes in cognitive and exploratory behaviors induced by consumption
of HF diet may stem from disturbances in cholinergic system in specific brain regions. Another study also reported that HF diet consumption disturb the brain cholinergic system in rats (34). However, most of the studies were concentrated on whole brain evaluation and missed the presence of brain region specific elevations in response to HF diet consumption (5,7,35). In this connection, present study evaluated the brain region specific alterations in cholinergic system (ACh and AChE) in two different age groups of rats. Our present findings demonstrated that synaptosomal ACh levels and AChE enzyme activity decreased following consumption of HF diet in cortex, hippocampus and cerebellum regions at 4 and 8 months age groups of rats. Our present findings demonstrated that synaptosomal ACh levels and AChE enzyme activity decreased following consumption of HF diet in cortex, hippocampus and cerebellum regions at 4 and 8 months age groups of rats. Kaizer et al. (36) also reported that AChE activity and ACh levels were significantly decreased in cortex, hippocampus and hypothalamus regions following consumption of HF diet. Additionally in vitro studies have also suggested that fat intake decreases the AChE activity and ACh levels (37-39). It is also proved in another study, which showed that 8 weeks of HF diet consumption depletes the brain ACh levels by modifying the AChE enzyme activity (9,10). However, the decreased activity of AChE enzyme and levels of ACh in different brain regions indicate that HF diet influences the cholinergic system in brain region specific manner and contributing to the observed changes in spatial learning, memory and exploratory behaviors of rats.

The mechanisms by which HF diet induce alterations in cholinergic system are not clearly known (40). HF diets increase oxidation of fatty acids through the peroxisomal oxidation pathway, that is associated with increased generation of free radicals and reduce the antioxidant enzyme activities (11,41). The present study confirms that HF diet consumption caused decrease in mitochondrial SOD enzyme activity where as MDA levels increased in cortex, cerebellum and hippocampus regions at both age groups of rats. Several recent studies also suggested that HF diet fed rats showed decrease in the antioxidant enzyme activities and increase in the MDA levels in brain of rats (13,42,43). HF diet caused oxidative damage in brain by enhancing peroxidation of membrane lipids due to the generation of ROS and decreases the activity levels of antioxidant enzymes leading to oxidative stress (12,43). Our results clearly showed that an increased MDA level has been accompanied by reduction in the activity of SOD enzyme in different brain regions of rat. The decreased antioxidant enzymes activities in HF diet fed rats are indicative of the oxidative stress and a response to the cholinergic system dysfunction.

We have chosen to evaluate brain regions instead of whole brain because different regions may respond differently to oxidative stress and vulnerability of the cholinergic system (35,40). In the present study, the alterations were more pronounced in cortex compared to hippocampus and cerebellum regions. Previous studies also suggested that cortex region is more susceptible to HF diet consumption (6,35). However, HF diet induced impairments in cognitive functions and brain chemistry appears to be age specific (10,44,45). In our study, HF diet induced alterations in cognitive functions and brain cholinergic and antioxidant systems were marginal in young adults compared to older adults, perhaps longer HF diet consumption is required to alter cognitive functions in young adult rats. In conclusion, the present data indicate that HF diet influences spatial learning, memory and exploratory behavior and associated cholinergic and antioxidant systems in a brain region specific manner and suggest that age is a major factor in determining the detrimental effects of high fat diet.

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References


