Original Article

The Effects of Saturated and Unsaturated Fat Diets on the Histology of Adrenocortical Cells of Albino Rats - A Comparative Study

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ABSTRACT

Objectives: High fat diet stimulates Hypothalamic -pituitary- adrenal (HPA)axis activity and enhances the release of corticosteroids from adrenal gland which in turn contributes to metabolic syndrome by producing insulin resistance. Present study objectives are to compare the effects of two different types of fat that is saturated and polyunsaturated on the histology of adrenal gland with respect to its activity.

Study Design: Prospective experimental.

Place and Duration of Study: Department of Anatomy BMSI, JPMC August – October: 2008

Materials and Methods: A total of 30 adult male albino rats weighing 200-240 gm, aged 190 days were divided into three groups. group 'A' received standard laboratory diet and group 'B' received high saturated fats in the form of unsalted butter as 20% added fat of the total calories. Group 'C' received high unsaturated fats in diet i.e. 20% fat added in the form of corn oil of the total calories. Each group was subdivided according to their time of sacrifice i.e. 4 and 8 weeks respectively. Animals were sacrificed at the end of their respective periods by ether anesthesia. Adrenal glands were dissected out, weighed and processed for paraffin embedding and sectioned at 4µm thickness to be stained with H&E, for cell count and cell diameter in the 3 cortical zones.

Result: Observations on adrenal gland weight /100gm of body weight of butter and corn oil treated groups revealed moderately significant decrease in weight in corn oil treated group at 4 weeks while there was insignificant difference at 8weeks when compared to butter treated group. There was insignificant difference in cell count in the three zones between butter and corn oil treated groups. Cell diameter in zona fasciculata was significantly increased in butter treated groups at 8weeks when compared to corn oil treated group.

Conclusion: Insignificant differences were found in most of the observation between the 2 fat groups. Insignificant difference in adrenal gland weight /100gm body weight between butter and corn oil treated groups at 8 weeks was because of less marked increase in body weight in corn oil treated group. Zona fasciculata showed increase cell size in butter treated group reflecting increased stress hormone release.

Key Words: Adrenal gland, zona fasciculata, high fat diet, HPA axis.

INTRODUCTION

High fat diet has been reported to adversely affect the health of human and animal species[1].High dietary fat stimulates HPA axis activity[2,3,4], inducing both hyperplasia and hypertrophy of adrenal cortex [5,6] . Resultant glucocorticoid excess has been linked to obesity, hypertension, hyperlipidemia and glucose intolerance[7].

Disorders characterized by insulin resistance are associated with specific fatty acid pattern of serum lipids with increased proportion of palmitic and palmitoiec acid (saturated fatty acids) and reduced levels of linoleic acid which is a polyunsaturated fatty acid[8]. Myristic and palmitic acids (saturated fatty acids) are also more potent in increasing serum cholesterol. Butter is one of the sources of saturated fatty acids[9].

Diets high in polyunsaturated fatty acids (PUFAs) are negative regulator of lipogenesis that exert their effect primarily at the level of transcription[10]. Corn oil which contains PUFAs, has hypocholestrolemic action[11].

Adrenal gland is an essential stress response organ that is part of HPA axis[12,13]. Diet high in saturated fat increases circulatory corticosteroid levels from adrenal gland in addition to changes in glucose homeostasis[14].Cultured adrenocortical cells were experimentally stimulated by unsaturated fatty acids i.e. oleic acid and to lesser extent linoleic acid to produce increased levels of glucocorticoids[15]. Oxidized derivatives of linoleic acid also have the potential to stimulate corticosteroid production[16] .

A lot had been said about the comparison between saturated and unsaturated fats and the beneficial effect of unsaturated fats with respect to their effect on obesity and cardiovascular diseases. Adrenal gland which is the ultimate organ of stress axis has an established role in obesity and metabolic syndrome. How saturated and unsaturated fats affects the histology

of adrenal gland is of interest as it seems to play a decisive role and the impact of each type of fat on the development of metabolic syndrome through adrenal gland is the area for more research.

Most studies focused on the effects of variable amount of high fat diet with one or different types of fats on adrenal activity. But mostly they were assessed by the biochemical parameters. In present study a comparison of two types of fat in terms of their effect on the histological parameters was done.

MATERIALS AND METHODS

Thirty male adult albino rats aged around 190 days weighing 200-240 gm were taken and kept on standard laboratory diet for one week observational period (12 hrs dark and light cycle) before study.

The animals were divided into group, A, B, C according to the diet they received. Group 'A' received normal diet, group 'B' received high fat diet (20 gram unsalted dairy butter/100 grams of normal diet, Lurpak, Denmark) and group 'C' received high unsaturated fat diet (20 ml of corn oil /100 gm of normal diet, Coroli-Cebag, Abu Dhabi). Animals were then subdivided into subgroups A1, A2, B1, B2, C1and C2 according to their time of sacrifice i.e. 4 and 8 weeks. Each subgroup comprised of 5 animal

Animals were individually housed after weighing in plastic cages, and were kept on 12:12 hrs light-dark cycle. Given food and water ad libitum. At the end of respective study period animals were weighed and anaesthetized with ether. Adrenal glands were excised after dissection and weighed and fixed in 10% buffered neutral formalin for 24 hours. After fixation they were processed in ascending grades of alcohol (70-100), cleared with xylene and infiltrated in paraffin. 4µm thick sections were cut with rotatory microtome and stained with H&E for morphometric study.

The cell count in the three cortical zones was done under 8 x ocular and 100 x oil immersion objective with counting reticule in randomly selected 3 fields from each cortical zone of each animal. Cell diameter was measured by randomly selecting ten cells per field in each zone with ocular micrometer scale under 8 x ocular and 100 x oil immersion objective.

The statistical analysis was done by student's't' test and P-value less than 0.05 was considered as significant. Calculations were done by utilizing computer software SPSS version 13.

RESULTS

Observations of adrenal gland weight per 100 gm of body weight of different groups revealed that group 'B' animals showed highly significant (P<0.001) increase at 4 and 8 weeks when compared to control group. Group 'C'animals, at 4 and 8 weeks also showed highly significant (P<0.001) increase in adrenal gland weight per 100 gm of body weight, when compared to control.

Comparison of the two fat groups revealed that Corn oil treated animals showed moderately significant (P<0.01) decrease in weight at 4 weeks while insignificant (P>0.05) difference at 8 weeks, when compared with butter treated group as shown in (Graph No. 1).

Graph No.1: Adrenal Gland wt/100gm body wt in different groups of albino rats.

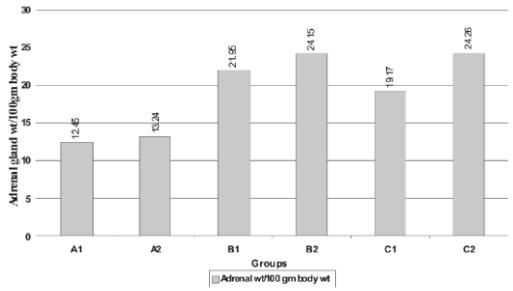

Table No.1: Mean number of cells (per unit area) in three cortical zones in different groups of albino rats

Groups	Sub-groups	Zona Granulosa	Zona Fasciculata	Zona Reticularis
A	A1	45±0.44	22±1.00	46±2.30
	A2	44±1.30	23.8±0.40	47±1.87
B	B1	39±0.63	17±0.70	47±0.70
	B2	32±1.58	17±0.83	48±0.70
C	C1	40±0.70	18±0.31	46±2.91
	C2	35.2±0.66	18±1.04	47±0.54

*Mean±SEM

Table No. 2: *Mean diameter of cells (µm) in three cortical zones in different groups of albino rats

Groups	Sub-groups	Zona Granulosa	Zona Fasciculata	Zona Reticularis
A	A1	13.60±0.20	11.50±0.19	7.80±0.26
	A2	13.62±0.50	12.50±0.18	7.90±0.09
B	B1	14.70±0.21	13.10±0.25	7.82±0.13
	B2	15.00±0.35	16.60±0.26	8.00±0.28
C	C1	14.00±0.28	12.30±0.22	7.81±0.25
	C2	14.50±0.17	15.50±0.19	8.20±0.21

*Mean±SEM

Cell count per unit area (Table-1) in different groups revealed that in group 'B' animals moderately significant (P<0.01) decrease in cell count in zona glomerulosa was found at 4 and 8 weeks when compared to control. Cell count in zona fasciculata showed moderately significant (P<0.01) decrease at 4 and 8 weeks, and zona reticularis showed insignificant (P>0.05) difference in count.

Group 'C' animals, when compared with control group showed moderately significant (P<0.01) decrease in cell count in zona glomerulosa at 4 and 8 weeks. Zona fasciculata showed significant (P<0.05) decrease in cell

count at both 4 and 8 weeks. Zona reticularis showed insignificant (P>0.05) difference.

When group 'B' and group 'C' were statistically compared insignificant (P>0.05) difference in cell count was found at both 4 and 8 weeks in the three cortical zones.

Cell diameter observed in different groups revealed that group 'B' animals when compared to control showed moderately significant (P<0.01) and significant (P<0.05) increase in cell diameter in zona glomerulosa at 4 and 8 weeks respectively. Moderately significant (P<0.01) and highly significant (P<0.001) increased diameter was seen in zona fasciculata at 4 and 8 weeks respectively. Zona reticularis showed insignificant (P>0.05) change.

Group 'C'animals when compared to control group showed insignificantly (P<0.05) increased cell diameter in zona glomerulosa at 4 and 8 weeks .Significant (P<0.05) and highly significant (P<0.001) increase in diameter in zona fasciculata was found at 4 and 8 weeks respectively. Zona reticularis showed insignificant (P>0.05) change.

Statistical comparison of group 'B' and group 'C' revealed insignificantly (P>0.05) increased diameter of cells in Group 'B' at both 4 and 8 weeks in zona glomerulosa when compared to group 'C'. In zona fasciculata insignificantly (P>0.05) increased and significantly (P<0.05) increased diameter was observed at 4 and 8 weeks respectively in group 'B' animals. Insignificant differences in diameter were observed in zona reticularis between the 2 groups.

DISCUSSION

Virtually every metabolic disorder characterized by elevated plasma free fatty acid levels is also associated with hypercorticoidism [15]. The pattern of effect of high fat diet is similar to that after chronic stress [2]. Long term stimulation of adrenal cortex by adrenocorticotropic hormone (ACTH) not only increases secretory activity but also causes hypertrophy and hyperplasia of adrenocortical cells [12] .ACTH increases synthesis of low density lipoprotein (LDL) and high density lipoprotein (HDL) receptors on adrenocortical cells. LDL and HDL deliver cholesterol to the adrenal gland. [5] Both saturated fat[14] and polyunsaturated fat[16] affects the activity of adrenal gland. Present study observations focus on the histological differences in their effect on adrenal cortex.

In present study adrenal gland weight per 100 gm of body weight increased in both fat treated groups. Butter treated group (Group 'B') revealed more weight increase at 4 weeks compared to corn oil treated group (Group 'C') while at 8 weeks both fat groups had equal weight because the body weight increment was less in corn oil treated group. Neilly et al (2009) used saturated fat and observed increased adrenal weight, which is in conformity with the present study observations, Kronenberg et al (2008) explained increase in adrenal gland weight after ACTH stimulation due to chronic stress as a result of hypertrophy and hyperplasia. Gotohda et al (2005) observed increased adrenal gland weight in response to toluene inhalation stress , the body weight decreased due to the stress while the organ weight increased due to hypertrophy.

Cell count in zona glomerulosa of both butter and corn oil treated groups showed decrease in cell number per unit area as the size of the cell increased at 4 and 8 weeks period. Difference in cell count in between the two fat groups was insignificant. In zona fasciculata the decrease in cell count /unit area was more evident in case of butter treated groups at 4 and 8 weeks compared to control and less in case of corn oil treated group when compared to control ,but statistical comparison of the two fat groups showed insignificant difference.

Tannenbaum et al (1997) and La Fleur et al (1995) used unsaturated and saturated fat respectively in their study and observed and assessed the activity of gland by biochemical parameters, in response to restraint stress and found exaggerated response by increased hormonal levels. Legendre and Harris (2006) used a mixture of corn oil and coconut oil equals to 40% of total calories and observed the activity of gland in response to mild stress and found similar results. Lai et al (2006) observed adrenocortical hyperplasia in response to chronic variable stress, the findings correlates well with present study observations as the decrease in count per unit area is relative due to increase in size.

Cell diameter was increased in butter treated groups both at 4 and 8 weeks period, in zona glomerulosa and zona fasciculata cells. In corn oil treated group increase in size of cell was only appreciable in zona fasciculata. Comparing the two fat groups there wasn't much difference in cell size in zona glomerulosa and reticularis ,only in the longer duration(8week) butter treated animals showed increased cell size in zona fasciculata compared to corn oil treated group .Gotohda et al (2005) observed increased cell size in response to toluene induced stress.Lai et al (2006) observed both hyperplasia and hypertrophy in response to chronic variable stress which involved zona fasciculata mainly, which in accordance with present study results.

CONCLUSION

Despite of all the debate which proves that polyunsaturated fats are superior or less harmful in excess, to saturated fats, present study observations indicated that no remarkable difference was found related to stressful effects on adrenal gland in between two types of fat.

REFERENCES

1. Sreekumar R, Unnikrishnan J, Fu A, Nygren J, Short KR, Schimke J, et al. Impact of high-fat diet and antioxidant supplement on mitochondrial functions and gene transcripts in rat muscle. Am J Physiol Endocrinol Metab 2002;282:1055-1061.
2. Tannenbaum BM, Brindley DN, Tannenbaum GS, Dallman MF, McArthur MD, Meaney MJ. High-fat feeding alters both basal and stress-induced hypothalamic-pituitary-adrenal activity in the rat. Am J Physiol Endocrinol Metab 1997;273:1168-1177.
3. La Fleur SE, Houshyar H, Roy M, Dallman MF. Choice of lard, but not total lard calories, damps adrenocorticotropin responses to restraint. Endocrinology 2005;146: 2193–2199.
4. Legendre A, Harris RB. Exaggerated response to mild stress in rats fed high-fat diet. Am J Physiol Regul Integr Comp Physiol 2006; 291: 1288- 1294.
5. Kronenberg HM, Melmed S, Polonsky KS, Larsen PR. Williams textbook of endocrinology. 11th ed. Philadelphia: Saunders Elsevier;2008.
6. Gotohda T, Tokunaga I and Kubo S. Toluene inhalation-induced adreno-cortical hypertrophy and endocrinological changes in rat. Life Sci 2005;76: 1929-37.
7. Wang M. The role of glucocorticoid action in the pathophysiology of the metabolic syndrome. Nutrition & metabolism 2005;2:1172–1178.
8. Vessby B. Dietary fat and insulin action in humans. Br J Nut 2000; 83: 91-96
9. Champe PC, Harvey RA, Ferrier DR. Lippincott's Illustrated Reviews: Biochemistry. 4th ed. Philadelphia: Lippincott Williams and Wilkins; 2008.
10. Cruz MR, Tovar AR, Gonzalez BP, Prado MD , Torres N. Synthesis of long-chain polyunsaturated fatty acids in lactating mammary gland: role of Δ5 and Δ6 desaturases, SREBP-1, PPARα, and PGC-1. J of Lipid Res 2006;47:553-560.
11. Mooradian AD, Haas MJ, Wong NC. The effect of select nutrients on serum high-density lipoprotein cholesterol and apolipoprrptein A-1 levels. Endocrine Reviews 2005;27: 2-16.
12. Guyton AC, Hall JE. Textbook of medical physiology. 11th ed. Philadelphia: Saunders Elsevier; 2006.
13. Lai YM, Figueiredo HF, Ostrander MM, Choi DC, Engeland WC, Herman JP. Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. Am J Physiol Endocrinol Metab 2006;291: 965-973.
14. Neilly AM, Williamson R, Balfour D, Sutherland C, Stewart C. Adrenal hypertrophy occurs in concert with insulin resistance following high fat feeding of two different strains of rat. Endocrine Abstracts 2009; 19: 133.
15. Widmaier EP, Margenthaler J, Sarel I. Regulation of pituitary- adrenocortical activity by free fatty acids in vivo and in vitro. Prostaglandins Leukot Essent Fatty Acids 1995; 52:179-83
16. Bruder ED, Ball DL, Goodfriend TL, Raff H. An oxidized metabolite of linoleic acid stimulates corticosterone production by rat adrenal cells. Am J Physiol Regul Integr Comp Physiol 2003;284: 1631-1635.

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