

A Biochemical and Morphological Comparison of the Role of L-Arginine on the High Saturated and Unsaturated Fat Diet Induced Changes on Adrenocortical Cells of Albino Rats

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ABSTRACT

Objective: Fatty diet produces both biochemical and histological changes in the adrenal cortex of albino rats. Present study objectives were to see if L-Arginine ameliorates the affects produced by both saturated and unsaturated fat equally.

Study Design: A prospective experimental study

Place and Duration of Study: This study was conducted at the Department of Anatomy, BMSI, JPMC, Karachi from August to October 2008.

Materials and Methods: A total of 50 Albino rats weighing 200-240gms, aged 190days were divided into 5 groups. Group A received standard laboratory diet. Group B received 20% saturated added fat as unsalted butter in diet. Group C received 20% unsaturated added fat as corn oil in diet. Group D received saturated fat with L-Arginine 300mg/kg body weight/day orally. Group E received unsaturated fat along with L-Arginine 300mg/kg body weight/day orally. After 8 weeks study period, animals were weighed and sacrificed and blood was drawn for hormonal assays. Adrenal glands were removed and fixed in buffered neutral formalin. They were then sectioned with cryostat in 10µm sections and stained with Oil red O to visualize fat in cells.

Results: Highly significant decrease observed in both ACTH and Corticosterone levels in Group D and E when compared to B and C respectively, but insignificant difference was found between D&E. Oil red O stained sections showed less densely packed fat globules in group D and E compared to B and C respectively. The results when compared between D and E were not significant.

Conclusion: L-Arginine lowers down the level of stress hormones in body and amount of fat in cortical cells in both groups receiving saturated and unsaturated fat diet with L-Arginine but their comparison didn't show significant difference statistically.

Key Word: Saturated fat, Unsaturated fat, Adrenal gland, L-Arginine.

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INTRODUCTION

Quality and quantity of diet influence organismal homeostasis adversely in different ways; one of the most important among them is activation of stress system with chronic elevation of stress mediators¹. Several studies investigated role of nutrients in the regulation of HPA(Hypothalamo-Pituitary - Adrenal) axis and stress responsiveness, studies in genetically obese (Zucker) rats have shown activation of HPA axis by them and hyper reactivity to stressful experimental conditions due to their consumption². Other studies shows high fat feeding alters both basal and stress induced increased HPA activity in rats^{3,4}.

It has been shown that high fat diet augment ACTH (Adrenocorticotrophic hormone) and Corticosterone hormone, therefore in terms of HPA axis stimulation, increased fat consumption functions as chronic stressor⁵. In animals lipid infusion induces increased levels of both hormones⁶. Type of fatty acid and therefore type of fat have different effects on HPA axis activation⁷.

Modern life style is characterized by diet high in fat, predominantly saturated, contributing to high prevalence of obesity and metabolic syndrome⁸.

Both psychological and fatty diet induced stress manifest their deleterious effects by stimulating the HPA axis chronically leading to abnormal secretion of stress hormones⁹. Agents which can prevent the excessive release of these stress hormones could prevent their hazardous contribution to disease phenomenon¹⁰. Among the agents used to decrease psychological stress L-Arginine is one of the semi-essential Amino acid which by means of Nitric oxide production down regulate excessive production of steroids from adrenal cortex¹¹. Present study was done to observe and compare the ameliorating effect of L

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Arginine on the morphological and functional changes produced by two different types of fatty diet.

MATERIALS AND METHODS

In this study 50 male adult Albino rats aged 190 days weighing 200-240gm were selected and kept on normal diet for a week under observation with 12 hours dark and light cycle, before commencement of study period. Animals were divided into groups A, B, C, D and E according to the diet they received, each comprising of 10 animals. Group A received normal laboratory diet. Group B received high saturated fat, 20gm unsalted dairy butter in 100gm of diet. Group C received high unsaturated fat, 20 ml corn oil in 100 gm of diet. Group D received high saturated fat along with 300mg L-Arginine /kg body weight/day orally (General Nutrition Corporation, Pittsburg, USA). Group E received high unsaturated fat along with 300mg L-Arginine /kg body weighed. Animals were housed in cages under, standard laboratory conditions of 12 hours day and night. After completion of study period animals were dissected after ether anesthesia. Blood sample were taken at the time of dissection through intra-cardiac puncture and shifted to lavender tubes containing EDTA and centrifuged for 15 minutes at 3500 Hz to get plasma, which was stored at -20°C for analysis. Plasma ACTH levels were determined by Elisa kit (Biomerica) and Corticosterone levels also by Elisa method (Neogen Corporation). Adrenals were removed and fixed in 10% buffered neutral formalin and sectioned by cryostat to $10\mu\text{m}$ sized sections which were stained with Oil Red O to demonstrate lipids. Statistical analysis was done by students 't' test and P value less than 0.05 was considered as significant. Calculations were done by utilizing computer software SPSS version 16.

RESULTS

The results of hormonal assays showed that highly significant decrease in plasma ACTH levels (Graph-1) was observed in butter with L-Arginine and corn oil with L-Arginine receiving groups, when compared to only butter and corn oil receiving groups respectively. Plasma Corticosterone levels (Graph-2) in butter with L-Arginine and corn oil with L-Arginine receiving groups when compared with butter and corn oil alone showed moderately significant decrease. When ACTH and Corticosterone levels were compared in butter with L-Arginine and corn oil with L-Arginine receiving groups there was no statistically significant difference.

Microscopic study of sections stained with Oil Red O, of adrenal cortex of butter receiving animals showed more densely packed fat globules (Fig-1), while corn oil receiving group showed densely packed fat globules (Fig-2). Both butter with L-Arginine and corn oil with L-Arginine showed less densely packed fat globules (Fig 3&4) and there was not much difference among the two.



Figure No.1: Oil Red O & Haematoxylin stained, $10\mu\text{m}$ thick frozen section of rat adrenal cortex showing more densely packed fat globules (FA) in zona glomerulosa (ZG) and zona fasciculate (ZF) after 8 weeks treatment with Butter. Photomicrograph X400



Figure No.2: Oil Red O & Haematoxylin stained, $10\mu\text{m}$ thick frozen section of rat adrenal cortex showing densely packed fat globules (FA) in zona glomerulosa (ZG) and zona fasciculate (ZF) after 8 weeks treatment with Corn oil. Photomicrograph X400

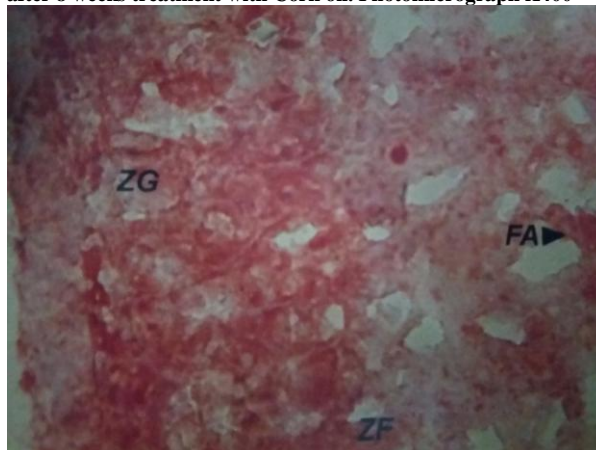
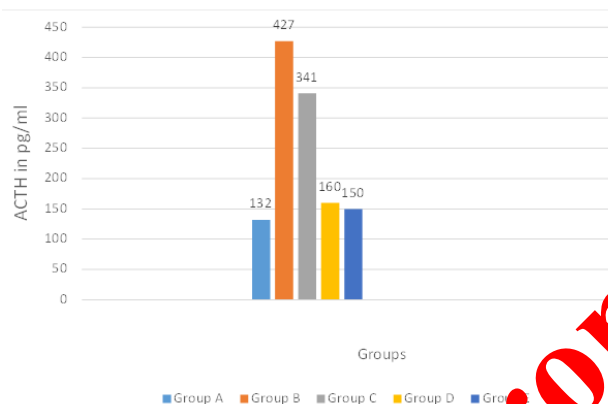


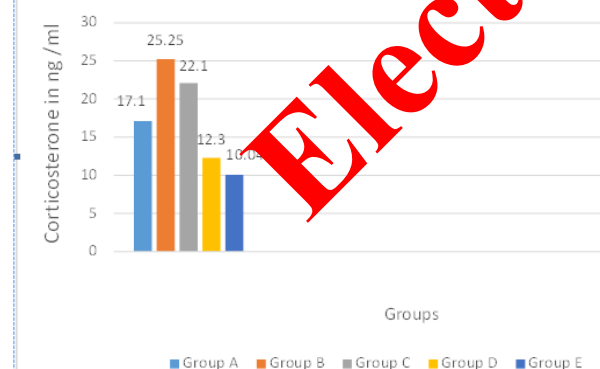
Figure No.3: Oil Red O & Haematoxylin stained, $10\mu\text{m}$ thick frozen section of rat adrenal cortex showing less densely packed fat globules (FA) in zona glomerulosa (ZG) and zona fasciculate (ZF) after 8 weeks treatment with Butter & L-Arginine. Photomicrograph X400



Figure No.4: Oil Red O & Haematoxylin stained, 10 µm thick frozen section of rat adrenal cortex showing less densely packed distribution of fat globules (FA) in zona glomerulosa (ZG) and zona fasciculata (ZF) after 8 weeks treatment with Corn oil & L-Arginine. Photomicrograph X400



Graph No.1: Mean Plasma ACTH Hormone in different groups of Albino rats



Graph No.2: Mean plasma Corticosterone in different groups of Albino rats

DISCUSSION

In modern society we are facing stress in two ways psychological stress and consumption of fatty diet which appeared to have synergistic affect^{1,12}. High fat diet is associated with high HPA axis responsiveness which leads to obesity and metabolic syndrome¹³. In present study fat was given in two forms Butter

(saturated) and corn oil (unsaturated), 20% of total calories. Both ACTH and Corticosteroid levels were found to be high in animals receiving saturated and unsaturated fat. Tannenbaum et al³, used corn oil and Wood et al¹⁴ used butter in the same amount alone, while Legender and Harris² used a mixture of corn and coconut oil and observe the hormonal levels after mild stress. Widmaier⁶ and his colleagues observed that infusions of fatty acid in rats increased stress hormone level indiscriminately but when fatty acids were introduced to cultured adrenal cortical cells there was no stimulatory affect when saturated fatty acids were used. Hisanao et al⁷ on the other hand found in their study that olive oil compared to corn oil and safflower oil when fed to rats and exposed to repeated stress result in higher levels of corticosterone.

L-Arginine seems to decrease the levels of both hormones when used with the two types of fat, there was no statistically significant difference in levels of both hormones in the two fat groups. Michalska et al¹⁵ observed the effect of L-arginine in decreasing the level of stress hormones induced by nicotine and found a significant decrease in levels, while Smriga et al¹⁶ used L-Arginine to reduce corticosterone levels and anxiety. Gastel et al¹⁷ used L-Arginine to decrease stress hormone levels in turbot after repeated handling.

Oil red O stained sections of saturated and unsaturated fat groups showed increased amount of fat globules in the three zones particularly zona fasciculata and zona glomerulosa (Fig-1&2). Comparison of the stained section showed that fat globules are more in saturated fat diet group. D-Souza¹⁸ and his fellows in their study induced metabolic syndrome by giving high fat and carbohydrate diet similar to junk food consumed nowadays having lard in it, their finding showed fat accumulation in adrenal cortex similar to us.

Oil red O stained sections of Saturated and unsaturated fat with L-Arginine showed decreased amount of fat globules in the cortical zones when compared with only fat consuming group (Fig -3&4). Comparison of protection provided by L-Arginine among the 2 type of fat is not markedly different. L-Arginine possibly prevent fat accumulation and decreased steroidogenesis by decreasing amount of lipid in blood¹⁹ which could decrease influx in adrenal gland and by acting through NO (nitric oxide) production decrease steroidogenesis as observed by Repetto et al²⁰.

Diaz et al in their study used a mixture of saturated fatty acids and unsaturated fatty acid and observed the protection provided by vitamin C. Vitamin C could inhibit fatty diet induced excessive steroidogenesis from adrenal gland but that is because of down regulation of steroidogenic acute regulatory protein and hydroxysteroid 11 beta dehydrogenase 2 genes as observed by Diaz et al⁶. They didn't compare the types of fat.

CONCLUSION

Despite of well documented superiority of unsaturated fats in terms of health benefits present study results does not show marked difference regarding fat accumulation in adrenal cortex and increased steroidogenesis. Use of L- Arginine along the two type of fatty diets does not show much difference in its affects which is promising in case of saturated fat.

Author's Contribution:

Concept & Design of Study: Iram Qudous
 Drafting: Iram Qudous
 Data Analysis: Imtiaz Manzoor
 Revisiting Critically: Aisha Qamar
 Final Approval of version: Iram Qudous

Conflict of Interest: The study has no conflict of interest to declare by any author.

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