

Effects of L-Arginine on Fatty Diet Induced Changes in Adrenal Cortex: A Morphometric Study

1. Iram Quddus 2. Ghulam Mujtaba Kolachi 3. Aisha Qamar 4. Rais Ahmad

1. Asstt. Prof. of Anatomy, DIMC, DUHC, Karachi 2. Assoc. Prof. of Anatomy, DMC, DUHC, Karachi 3. Asstt. Prof. of Anatomy, FJDC, Karachi 4. Assoc. Prof. of Anatomy, SMC, DUHC, Karachi

ABSTRACT

Aim: To study the effects of L-Arginine on high fat diet induced changes in adrenal cortex.

Study Design: A prospective experimental study.

Place and Duration of Study: This Study was conducted at the Department of Anatomy, Basic Medical Sciences Institute, Jinnah Post Graduate Medical Centre Karachi from August 2008 to October 2008.

Materials and Methods: Thirty male adult albino rats were taken for the study and were divided into three groups according to the dietary regimen. Group A received control diet. Group B received high fat diet with 20% added fat in the form of butter. Group C received high fat diet along with L-Arginine 300mg/kg / day orally. After the end of the study period that is 8 weeks, animals were weighed and sacrificed. The adrenal glands were removed, fixed in buffered neutral formalin and after processing embedded in paraffin to form blocks. 4 μ m thick sections were cut and stained with H&E and Mallory's trichrome stains for morphometric study.

Results: Highly significant increase in weight ($P<0.001$) was observed in animals of Group B and moderately significant ($P<0.01$) decrease was observed in Group C animals when compared to control and group B animals respectively. Cortical enlargement was found in zona glomerulosa and fasciculata and decreased thickness was observed in zona reticularis in Group B animals, when compared to control, the results were highly significant ($P<0.001$). In Group C decreased cortical thickness was noted in zona glomerulosa and fasciculata but in zona reticularis increase in thickness was noted when compared to Group B, results were highly significant ($P<0.001$). Mallory's trichrome stained sections of Group B animals showed dilated blood vessels in the three cortical zones, more numerous in zona fasciculata when compared to control animals. In Group C no marked change was observed in all three cortical zones when compared to control.

Conclusion: L-Arginine restricts the excessive weight gain caused by high fat diet. It also ameliorates the hypertrophic and vasodilatory effects on adrenal cortex caused by high fat diet.

Key Words: High Fat Diet, Hypertrophic, L-Arginine.

INTRODUCTION

Obesity is characterized by excessive body fat accumulation¹. Now a day it is considered a global epidemic and major public health problem. Its prevalence and tremendous cost of treatment necessitates the search for new alternative nutritional means². Obesity decreases life expectancy and is associated with medical complications such as insulin resistance, type 2 Diabetes mellitus, Dyslipidemia, Hypertension and Atherosclerosis³.

High fat diet, produces metabolic disorders in rats⁴, and increases the susceptibility to the hyperglycemia. Liver showed increased expression of lipogenic genes and inflammatory markers. Elevated levels of corticosterone were also demonstrated in response to high fat diet⁵. Obesity and metabolic syndrome are associated with a state of chronic low grade inflammation in white adipose tissue, characterized by cytokine production and macrophage infiltration⁶.

Key process involved in metabolic disturbances related to the obesity lies in the response of the hypothalamic-pituitary-adrenocortical (HPA) axis. The disturbances in the HPA axis functions play a permissive role in the metabolic disturbances associated with obesity⁷.

Chronic exposure to glucocorticoid hormones also result in symptoms similar to the metabolic syndrome⁸. Differentiating adipocyte produce high levels of 11 β -hydroxysteroid dehydrogenase type 1 (11 β HSD1) which converts inactive cortisone to active cortisol, thus increasing local glucocorticoid levels. Intracellular increase in glucocorticoids by the enzyme contributes to macrophage activation⁹. Chronic over nutrition might thus be a proinflammatory state with oxidative stress¹⁰. Hallmark of obesity and diabetes is a decrease in endothelial synthesis and bioavailability of nitric oxide. Physiological levels of nitric oxide play an important role in regulating the oxidation of energy substrate, insulin sensitivity and hemodynamics. Nitric oxide is synthesized from L-Arginine, underscoring a crucial role for this amino acid in maintaining health and treating a wide array of chronic diseases¹¹.

L-Arginine, a conditionally essential amino acid for adult mammal and is a precursor for the synthesis of biologically important molecules including nitric oxide, polyamines and agmatine etc². L-Arginine increases expression of genes that promote whole body oxidation of energy substrates¹². Nitric oxide (precursor of L-Arginine) regulates the metabolism of glucose, fatty acids and amino acids in mammals¹³. Physiological

levels of nitric oxide promote fat oxidation and decrease fat synthesis. L-Arginine supplementation reduces white adipose tissue mass by 20-40%, and decreases adipocyte size¹⁴ and enhances lipolysis in adipocyte and lowers triglyceride and leptin levels². Fat mass is also reduced in diabetic fatty rats¹³.

L-Arginine treatment showed reduction in markedly increased blood glucose levels in diabetic rats. The polyamines, the product of L-Arginine, play a role in insulin biosynthesis and beta cell replication¹⁵. Nitric oxide is the key mediator of immune response. L-Arginine supplementation attenuates the oxidative stress induced by burn injury¹⁶. On adrenocortical cells, it significantly decreases both basal and adrenocorticotrophic hormone (ACTH) induced corticosterone production in rats¹⁷.

MATERIALS AND METHODS

Thirty male adult albino rats aged around 190 days weighing 200-230 gm were taken and kept on normal diet for one week observational period (12 hours dark and light cycle) before study.

The animals were divided into three groups, A, B, C (10 animals in each group) according to the diet they received. Group 'A' received normal diet. Group 'B' received high saturated fat diet (20 grams unsalted dairy butter/100 grams of normal diet, Lurpak, Denmark). Group 'C' received high saturated fat diet same as group B, along with L-Arginine (300mg/kg body weight/day, General Nutrition Corporation, Pittsburg, USA).

Animals were individually housed in plastic cages, and were kept on 12:12 hour light-dark cycle. Given food and water ad libitum. They were weighed at the initiation of study period, fortnightly and just before sacrifice. At the end of study period i.e. 8 weeks animals were dissected out after ether anaesthesia. Adrenal glands were excised, weighed and fixed in 10% buffered neutral formalin for 24 hours. After fixation they were processed in increasing strengths of alcohol, and were cleared with xylene and were infiltrated in paraffin. 4µm thick sections were cut with rotatory microtome. Sections were stained with H&E for morphometric study and Mallory's trichrome stain for vascular pattern (dilatation of blood vessels).

Thickness of the three cortical zones was measured under 8 x ocular and 40 x objective with ocular micrometer in x and y axis from randomly selected 10 sections from each animal.

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

Highly significant ($P<0.001$) increase in weight was observed in animals of Group B when compared to

control. Moderately significant ($P<0.01$) decrease in weight was observed in Group C animals when compared to group B animals.

Micrometry of H&E stained sections revealed the cortical thickness of three zones in different groups. There was highly significant ($P<0.001$) increase in thickness of zona glomerulosa observed in Group B when compared to control. Group C showed highly significant ($P<0.001$) decrease in thickness when compared to Group B as shown in Table 1.

Table-1: *Mean width of the three adrenocortical zones (µm) in different groups of albino rats

Groups	Zona Glomerulosa	Zona Fasciculata	Zona Reticularis
A	57.45±0.125	267.0±0.166	274.0±0.033
B	67.02±0.129	434.0±0.047	210.0±0.093
C	58.60±0.159	296.4±0.128	250.0±0.072

*Mean±SEM

Comparison of weight gain in different experimental groups of albino rat

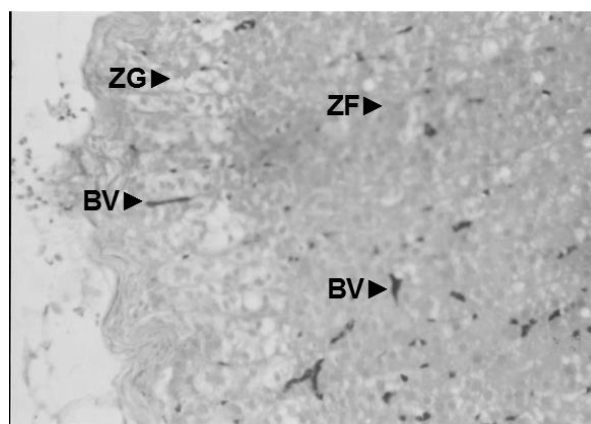
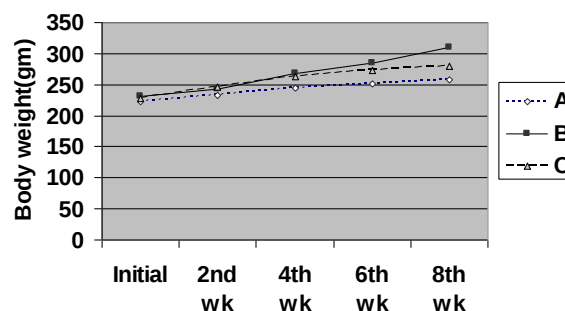


Figure No.-1 Mallory trichrome stained 4µm thick section of adrenal cortex from group A rat, showing normal blood vessels. Photomicrograph x 400.

Zona fasciculata (ZF) of Group B when observed showed highly significant ($P<0.001$) increase in thickness compared to control. Group C showed highly significant ($P<0.001$) decrease in thickness when compared to Group B as shown in Table 1.

Zona reticularis of Group B animals showed highly significant ($P < 0.001$) decrease in thickness when compared to control. Group C animals showed highly significant ($P < 0.001$) increase in thickness when compared to Group B as shown in Table 1.

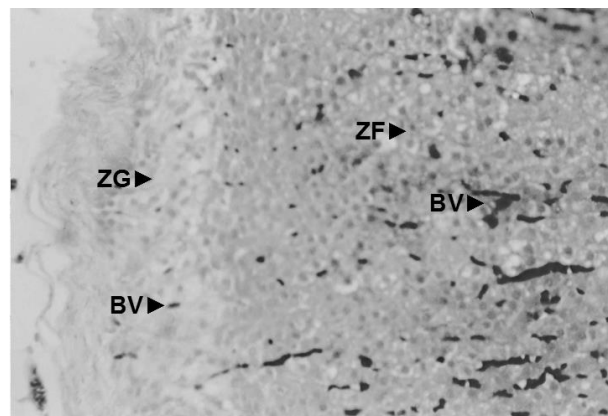


Figure No.-2 Mallory trichrome stained 4 μ m thick section of adrenal cortex from group B rat, showing dilated blood vessels. Photomicrograph x 400.

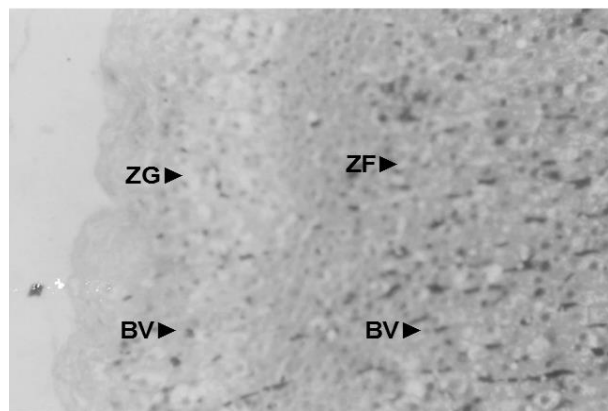


Figure No.-3 Mallory trichrome stained 4 μ m thick section of adrenal cortex from group C rat, showing near to normal blood vessels. Photomicrograph x 400.

Mallory's trichrome stained sections of Group B animals (fig 2) showed dilated blood vessels in the three cortical zones, more numerous in zona fasciculata when compared to control animals (fig 1). In group C animals near to normal vascular pattern was observed in the three cortical zones (fig 3).

DISCUSSION

Obesity epidemic in developed countries caused financial burden on the economy of country¹. Developing countries like ours couldn't afford lot of budget spent on treatment of such preventable disorder. Obesity and metabolic syndrome are multi-factorial disorders. Type of diet, genetic factors and hormones all play important role yet adrenal gland the end organ of hypothalamic pituitary adrenal axis (HPA) could not be ignored while considering these disorders¹⁸. Any

agent which could affect the activity of adrenal gland may be helpful in the fight against obesity. L-Arginine, which has antioxidant properties, decreases fat mass^{13,14}. It influences the hormonal synthesis in adrenal cortex¹⁹.

Dietary interventional study in human indicates that high intake of fat contributes to the development of obesity²⁰, as the present study results showed that there was more weight gain in animals taking fatty diet. Woods et al (2003) found similar results when used butter oil in diet, with 10% increase in weight. Neilly et al (2009) in their study observed significant weight gain when given lard to animals, however they also observed increased adrenal weight and high corticosterone levels in these animals. Bjorntorp (2001) observed relationship of increased adrenal activity with increased visceral obesity and weight gain.

Decreased gain in weight in fat with L-Arginine receiving animals correlate well with other studies. Fu et al (2005) observed 16% lower weight in ten weeks study compared to control group, he gave L-Arginine in drinking water to obese diabetic Zucker rats. Jobgen et al (2006) observed reduced fat mass in diabetic fatty rats, while Jobgen and shi (2007) observed 20-40% reduced white adipose tissue mass in non diabetic rats with L-Arginine supplementation.

Adrenocortical hypertrophy more specifically zona glomerulosa and zona fasciculata was observed in fatty diet treated group, this finding indicates increased cortical activity. Gotohda et al (2005) observed cortical hypertrophy due to the stimulation of cortical cells as a result of toluene inhalation-induced stress. Hyperactivity of adrenocortical cells by biochemical analysis was observed by Widmaier et al (1995) who observed that high concentration of free fatty acid stimulates adrenocortical activity. Carsia et al (2008) compared the dietary effects of three different types of fats on adrenocortical activity by measuring hormonal contents and found that both corticosterone and aldosterone production increased in response to high saturated fat diet which is similar to the present study findings in which both zona glomerulosa and fasciculata showed hypertrophy in fat treated group.

Zona reticularis thickness was decreased in fat treated animals as a result of recruitment of zona reticularis cells into zona fasciculata (Kelly et al. 1998).

L-Arginine with fatty diet treated animals showed decrease thickness of zona glomerulosa and fasciculata probably because L-Arginine is a negative modulator of steroidogenesis in adrenocortical cells as mentioned by Repetto et al (2006). Cymeryng et al (2002) observed decreased activity of adrenocortical cells, both basal and ACTH stimulated, in response to L-Arginine treatment which is consistent with present study findings.

Mallory's trichrome stained sections revealed dilated blood vessels in fat treated animals. Obesity seems to

be a systemic low grade inflammatory state (Kyrou et al 2006), which together with ACTH release in response to high fat diet (Tannenbaum et al. 1997) leads to excessive vasodilatory effect observed in the vessels of adrenal cortex. Milovanovic et al (2003) observed the cortical vessels with Azan stain in response to ethanol administration, found dilated small blood vessels and prominent hyperemia.

L-Arginine, although a precursor of nitric oxide, which causes endothelial induced vasodilatation probably revert the inflammatory and ACTH induced excessive vasodilatation. Chattopadhyay et al (2009) observed the effect of L-Arginine on liver which decreased the congestion produced by ischemic reperfusion injury.

CONCLUSION

The study results confer to already existing evidence of adrenal cortex in its contribution to development of obesity and related problems. L-arginine although not the ultimate solution but, its observed role in reducing the stimulatory effects of fatty diet on adrenal cortex and preventable role in excessive weight gain supports its rightful role in the management of obesity and related issues. Further studies required for quantitative contribution of this organ keeping other causative factors in view.

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Address for Corresponding Author:**Dr. Iram Quddus**

Assistant Professor

Anatomy Department

Dow International Medical College,

DUHS ,Karachi.

Cell No: 0333-3865001