

COMPARISON OF EFFECT OF HIGH FAT DIET INDUCED OBESITY AND SUBSEQUENT ATORVASTATIN ADMINISTRATION ON DIFFERENT ANTHROPOMETRIC MEASURES IN SPRAGUE DAWLEY RATS

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ABSTRACT

Objective: To compare the effect of high fat diet induced obesity and atorvastatin administration on different anthropometric parameters in Sprague Dawley rats.

Study Design: Randomized control trial (RCT).

Place and duration of study: The study was conducted at Department of Physiology, Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad and was completed in 12 months.

Material and Methods: Ninety healthy Sprague Dawley rats were taken and divided into three equal groups with 30 rats in each group. Group I rats (normal control) were given normal diet for 3 weeks. Group II rats (obese control) were given high fat diet for 3 weeks. Group III rats (obese treated) were orally given 10mg/kg/day of atorvastatin through gavage method for 3 weeks after obesity induction. At the start of the study, initial body weight and naso-anal length of all the rats in each group were recorded and then weight change was regularly recorded thrice weekly. Naso-anal length was again recorded at the end of the experiment.

Results: There was a significant increase in body weight in high fat diet induced obese and obese atorvastatin treated rats ($p < 0.05$). No significant change in either body mass index or Lee index was observed in any of the groups.

Conclusion: Atorvastatin administration to high fat diet induced obese rats had no significant effect on any of the anthropometric measure of obesity.

Keywords: Atorvastatin, BMI, LEE Index, Obesity, Rats.

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INTRODUCTION

Obesity has become an epidemic worldwide and is a risk factor for development of various diseases which include diabetes mellitus, hypertension, dyslipidemia and cardiovascular diseases¹. Sedentary life styles with decreased physical activity and intake of energy rich foods has resulted in tripled rate of obesity in developing countries². Body mass index is used as a standard tool by World Health Organization to classify obesity. According to this criterion, individuals having BMI ≥ 30 kg/m² are obese, 25 to 29.9 kg/m² BMI are overweight and 18.5 to

24.9 kg/m² BMI are normal weight. This criterion helps in identifying subjects who are at a higher risk of developing diseases later in life. A direct relationship was found between BMI and morbidity and mortality in various epidemiological studies³.

Rats are commonly used in experimental trials to observe various consequences of obesity because their behavior of gaining weight on taking high calorie diet resembles that of humans⁴. Using high fat diet is a preferred method to induce obesity in rats because this is the usual way by which humans tend to gain weight and become obese. When rats are given high fat diet for a suitable time period, they develop more adipose tissue, have a significant increase in abdominal weight and develop obesity related problems like insulin resistance⁵.

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Statins inhibit the enzyme 3 Hydroxy methylglutaryl coenzyme A (HMG-CoA) reductase and effectively decrease blood cholesterol level. Currently they are routinely used for prevention of adverse atherosclerotic cardiovascular events. Results of various clinical trials show that statins effectively decrease acute myocardial infarction and ischemic stroke incidence in dyslipidemic individuals⁶⁻⁸. Statin use in normocholesterolemic subjects has also shown beneficial effects. These effects that are other than lipid lowering include anti-inflammatory effects, immunomodulation, cardio protective effects, endothelial protection and anti-oxidant effects and are known as pleiotropic effects⁹⁻¹¹. These effects are currently the area of interest in research.

Information is lacking on anthropometric parameters measured in laboratory rats with no single definite criteria of obesity. Obesity is usually considered as a significant weight gain as compared to control animals¹². The aim of the

MATERIALS AND METHODS

This was a randomized control trial. A total of 90 healthy male and female Sprague-Dawley rats with an average weight of 220 ± 30 grams were selected and purchased from National Institute of Health, Islamabad. Rats were selected using systematic random sampling. For this purpose, one rat was selected and every alternative rat was discarded till a total of 90 rats were selected out of 180. Seven rats developed disease during the study period and were excluded. Initially all rats were acclimatized and fed standard chow diet for five days before the start of experiment. Room temperature was adjusted to 23 ± 5 °C and a daily photo period of 12 hours light and 12 hours dark was ensured. Rats were divided into three equal groups randomly. Rats in group I were fed normal diet and water ad libitum for three weeks duration (normal control). Rats in group II were fed high fat diet and water ad libitum for three weeks duration to make diet induced obese model

Table-I: Body weight, naso-anal length, BMI and Lee index of normal control, obese control and obese treated rats.

Parameters / Groups	Group I	Group II	Group III	p-value (ANOVA)	
Body weight	Initial (g)	215 ± 6.5			0.013*
	Final (g)	230 ± 17.01	312 ± 25.20	309 ± 18.67	
	Difference (g)	15	97	94	
Naso-anal length (cm)	18 ± 1.48	20 ± 1.25	20.5 ± 1.16	0.211¥	
BMI	7.22 ± 1.23	7.81 ± 1.01	7.70 ± 1.05	0.098¥	
Lee index	0.338 ± 0.02	0.338 ± 0.02	0.334 ± 0.02	0.753¥	

All values are expressed as Mean ± SD

* Significant difference (p<0.05)

¥ No significant difference (p>0.05)

Table-II: Intra group comparison of body weight at the end of study using post-hoc tukey's test.

Parameters / Groups	Body weight
Group I vs II	0.001 *
Group II vs III	0.271 ¥
Group I vs III	0.001 *

¥ No significant difference (p>0.05)

* Significant difference (p<0.05)

present study was to observe the effect of obesity and subsequent statin administration on various anthropometrical parameters including body weight, BMI and Lee index.

(obese control). Rats in group III were first made obese by giving high fat diet and water ad libitum for three weeks duration and then given 10 mg/kg/day of atorvastatin orally by gavage

method for another three weeks along with continuation of high fat diet (obese treated).

Body weight of all rats in each group was recorded on every alternate day by using a digital balance. The criterion for development of diet induced obese rat model was 20% or more weight gain as compared to the normal control group¹³.

Distance between the nose and anus was measured on ventral side in cm using an inch tape to calculate naso-anal heights of all the rats in each group twice weekly to calculate body mass index and Lee index by using the following formulas⁴:

significantly ($p < 0.05$) greater than that of healthy controls (230 ± 17.019 g) at the end of three weeks. The weight gain in both the groups (II and III) revealed 35.6 and 34.3 percent increase respectively as compared to the healthy controls. Weight of obese treated rats increased after three weeks of atorvastatin administration, but the increase was not significant.

Naso-anal length (cm)

A significant increase was found in naso-anal length of group II and III rats during the study period.

Body mass index

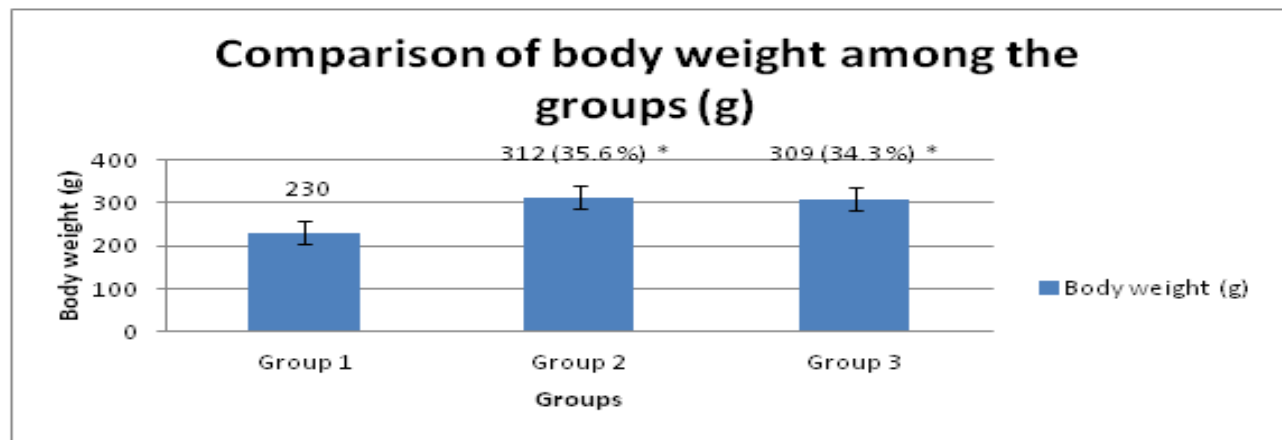


Figure: Comparison of body weight of normal control (Group I), obese control (Group II) and obese treated (Group III) Sprague Dawley rats.

Body mass index (BMI) = body weight (g)/length² (cm²)

Lee index = cube root of body weight (g)/nose-to-anus length (cm)

Data were analyzed by using SPSS version 21. Quantitative variables were expressed as mean \pm standard deviation. Comparison of quantitative parameters among the groups was done by One Way Analysis of Variance (ANOVA) followed by post hoc tukey's t-test for individual comparisons. A p-value less than 0.05 was considered statistically significant.

RESULTS

Body weight

Body weight of HFD obese (312 ± 25.200 g) and obese treated groups (309 ± 18.67 g) was

BMI was not affected significantly either by obesity or after atorvastatin administration. The difference in BMI was insignificant between healthy control and HFD obese rats ($p=0.107$) as well as between HFD obese and atorvastatin treated rats ($p=0.926$).

Lee index

Lee index was also comparable among the three groups. The difference of Lee index between healthy control and HFD obese ($p=0.998$), and HFD obese and obese treated ($p=0.774$) both were insignificant.

DISCUSSION

In the present study various anthropometric parameters were studied in high fat diet induced obese rats and atorvastatin treated obese rats.

Wilkes et al, method for development of diet induced obesity was used. It helped in attaining significant weight gain in a short duration of three weeks¹⁴. The weight gain manifested in two obese groups (II and III) of present study at the end of three weeks was 35.6 and 34.3 percent respectively as compared to the healthy control.

The weight gain by obese rats of present study was less than the weight gain of obese rats in the study conducted by Wilkes et al., They recorded 58.5 percent increase in their weight after three weeks of high fat diet. However, they did not adjust the age related increase in body weight and compared the body weight at the end of three weeks with that of initial body weight of the same group of rats.

The weight gain by obese rats of our study was greater than 20 percent similar to the weight gain suggested by Wang et al, to induce obesity. They administered high fat diet for 12 weeks but the percent contribution of fats in their diet was less than 50 percent as evident from the composition of their diet; 50% normal chow, 12% lard, 5% cane sugar, 8% milk powder, 5% peanut, 10% hen eggs, 3% sesame oil and 2% common salt¹³.

In our study body weight was not affected significantly by atorvastatin administration. This is comparable to the results of Dongdan et al, study, where high fat and high cholesterol diet were given for eight weeks to produce dyslipidemia and obesity. Thereafter, 4 weeks of oral administration of low-dose (5 mg/kg/day) or a high-dose (20 mg/kg body weight/day) atorvastatin did not significantly affect body weight¹⁵.

Body weight significantly decreased in mice after atorvastatin administration in Rinku Rani et al, study. Initial body weights of mice in all the groups were identical ($p>0.05$). Intake of HFD for 28 days significantly increased the body weight and produced hyperlipidemia. Oral treatment with atorvastatin 10mg/kg or 20mg/kg for 14 days significantly decreased the body weight. The difference in results may be due to

discontinuation of high fat diet after obesity induction in their study, whereas in our study the drug was given along with the high fat diet¹⁶.

In our study, BMI did not vary among the three groups. Neither obesity, nor subsequent atorvastatin administration caused a significant change in BMI. This is contrary to the results of study conducted by Malafaia et al, they gave 300 g/l of sucrose supplemented water in addition to the normal chow diet to male Wistar rats for 91 days, and there was increase in both body weight as well as BMI¹⁷. Results of study conducted by Novelli et al, also do not correspond with the results of our study. BMI along with body weight significantly increased in male Wistar rats who were given either ad libitum sucrose diet or high carbohydrate diet for 4 weeks starting at the age of 60 days⁴.

In our study, BMI did not change significantly in obese treated group. This is contrary to the results obtained in other studies. Review of literature has revealed an increase in BMI in statin users. An increase in BMI was observed by Sugiyama et al, in long term statin users as compared to non-statin users who controlled their hyperlipidemia by diet and caloric restriction. The cause found for this increased BMI was lack of dietary control in statin users who consumed high caloric and high fat diet assuming that they are taking a drug and do not require dietary control. This misperception resulted in rather an increase in BMI¹⁸.

Comparison of lipid profile, dietary habits and physical activity by Ingrid Lofgren et al, in statin users and non-users in elderly individuals showed that statin users had a better lipid profile. But they performed less physical activity and their diet composition had significantly less fruits and vegetables as compared to statin non users¹⁹. It is therefore need of the hour to emphasize dietary control and life style modification in statin users.

In our study, high fat diet induced obesity did not affect Lee index significantly. This does not correlate with the results of study conducted by Pooja et al, who developed cafeteria diet

induced obese rat model by administering cafeteria diet for 14 weeks and found significant increase in Lee index. The difference in Lee index, however was observed only at the later stages and end of experiment. At the early stages the Lee index did not increase significantly²⁰.

The results of study conducted by T. Harder et al, also differ from our results. In their study obesity was positively correlated with Lee index but the method of obesity induction was daily subcutaneous injection of long-acting insulin from 8th to 11th day of life. Body weight significantly started increasing from 21st day of life and persisted till adult life. This method interfered with the neuroendocrine development and was not associated with increase in food intake²¹.

In our study atorvastatin administration did not affect Lee index significantly. In literature, not much data is available on statin effect on Lee index. In a study conducted by Poletto et al, atorvastatin administration (0.1% w/w) orally to monosodium glutamate induced obese mice for four weeks did not affect Lee index significantly²².

CONCLUSION

Atorvastatin did not significantly alter the anthropometric parameters in high fat diet induced obese rats.

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CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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