Original Article

Insulin Reduced Liver

The Effects of Insulin on the Volume, Absolute and Relative Weight of Liver in HFD/Streptozocin Induced Diabetic Rats

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ABSTRACT

Objectives: In present study, the effects of insulin on the volume, absolute and relative weight of liver was studied in Wistar albino rats for a period of 4 weeks.

Study Design: Retrospective / observational study.

Place and duration of study: This study was conducted in the Animal House of DUHS and it took 8 months 1st June 2011 to 1st Feb 2012 to be completed.

Materials and Methods: The Male Wistar albino rats which were randomized into 3 groups; each group containing 10 rats. Group A served as control, Group B as insulin treated and Group E as untreated diabetic rats. All the other rats except the Group A were kept on in-house prepared High Fat Diet (HFD) throughout the study. After 2 weeks of exclusive HFD, diabetes was induced by intraperitoneal (IP) injection of low dose streptozocin (STZ 3.5mg/100gm). After the induction, one group was left untreated (Group E) and one group (Group B) was treated with insulin for 4 weeks. The rats were then, sacrificed, liver was isolated, weighed, and its dimensions were noted.

Results: The mean absolute liver weight (ALW) of rats was observed as 8.60 ± 2.54 gm, 13.18 ± 0.68 gm and 9.40 ± 3.18 gm in control, untreated and insulin treated groups respectively. And the mean percent liver weight (PLW) was calculated as 2.99 ± 0.66%, 5.10 ± 0.73% and 3.99 ± 1.37% in control, untreated and insulin treated groups respectively. Statistically significant difference was noted between ALW, PLW and liver volume of rats of the three groups.

Conclusion: Insulin decreases the volume, absolute and relative weight of liver of diabetic rats when used for a short period.

Key Words: Diabetic rat model, Type 2 diabetes, Diabetes induction, Insulin, Liver.

INTRODUCTION

The day to day researches reveal soaring rates of obesity, non alcoholic fatty liver disease and diabetes mellitus. This emerging prevalence is due to more mechanized life style, unbalanced diet and physical inactivity. Use of excessive fat in diet and no exercise makes a person obese leading to a condition known as insulin resistance (IR)[1] which gives birth to lot more diseases like obesity, diabetes mellitus type 2 (DM2) and fatty liver disease. DM2 is a common, severe and chronic form of metabolic disorder characterized by hyperglycemia due to decline in insulin action i.e. IR followed by the inability of insulin producing beta cells to compensate for IR.[1]Increased morbidity and mortality associated with the disease is due to complex metabolic changes that lead to functional impairment of many organs. About 1.3% population of the world is suffering from DM2[2, 3]and its prevalence in our community as well as in the world is increasing like an epidemic. The Nation (issue 2008)[4], Pakistan occupies 7th position in WHO diabetes prevalence list. "In Pakistan 6.9 million people are affected by diabetes and the International Diabetes Federation has estimated that this number will grow to 11.5 million by 2025 unless measures are taken to control the disease."[4]

Enlarged fatty liver is a well-known complication of DM2 with an established prevalence of 21–78%, [5-7] and usually remains undiagnosed because of its silent course in many of DM2 patients. Type 1 diabetes is not associated with accumulation of fat if blood glucose levels are well controlled, but type 2 diabetes may have a 70% correlation with fat accumulation regardless of blood glucose control.[8]

The fatty liver disease NAFLD is defined as fatty infiltration of liver cells (hepatocytes) in patients are taking no or insignificant alcohol (< 20 g/day).It can be idiopathic or secondary to metabolic syndromes like obesity, hypertension, hyperlipidemia and diabetes.[3] Fatty liver is caused by a number of factors which result in progressive hepatic failure.[9] The factors are summarized as Two hit theory."The "First Hit" to liver occurs when the enzymes involved in lipid metabolism i.e. uptake, synthesis, oxidation, and export are altered. In other words, the uptake of fatty acids by the liver exceeds its oxidation capacity. Lipid synthesis in liver is increased if diet consists of excess of sucrose, fats or fructose. The increase in lipid synthesis and decrease in lipid oxidation leads to the development of insulin resistance, both hepatic and peripheral.[10]The second hit is due to oxidative stress, which causes peroxidation of

lipids in the hepatic cell's membrane and cytokine production.[9, 11]

Nonalcoholic steatohepatitis (NASH) is a variant of fatty liver in which fat in the hepatocytes is accompanied by lobular inflammation and steatonecrosis. The diagnosis can only be made in the absence of alcohol abuse or other causes of liver disease, particularly hepatitis C. Nonalcoholic steatohepatitis has been associated most commonly with obese women with diabetes.[12] There is certainly a higher prevalence in type 2 diabetic patients on insulin.[13]

Insulin is an essential hormone which is involved in glucose as well as lipid metabolism.[14] It is synthesized and released by the β cells of pancreas in the blood in response to elevated blood glucose levels. The body cells especially fat, muscle and liver cells have "insulin receptors" on their surface which regulate entrance of glucose into cells. As glucose levels in blood increase, glucose is pushed into the cells where glucose is converted into energy. Other mechanisms by which insulin keeps blood glucose level in normal range is the inhibition of glucose production by the liver.[15] Unfortunately, people who are insulin-resistant cannot utilize insulin efficiently and glucose, therefore, is unable to enter the cells and accumulate in the blood. In order to clear excess glucose from the blood, the body signals the pancreatic β cells to produce excess insulin which results in a condition referred to as hyperinsulinemia (high level of insulin in blood). Hyperinsulinemia, though compensatory, but has various serious side effects on body. It suppresses fatty acid oxidation which leads to high levels of fatty acids in the form of triglycerides (TGs), to accumulate in the blood.[16] In 2002, Mason et al. proved that serum TGs are usually increased after chronic intraperitoneal and subcutaneous insulin infusion[17] which in turn, causes fatty acids to be deposited in the liver which leads to development of HIR.[18, 19] Thus, fatty infiltration and IR can potentiate each other creating vicious cycle of metabolic dysfunction.[9] The underlying mechanism by which obesity induces IR is the fat deposition in liver (HIR) skeletal muscles and adipose tissue (peripheral insulin resistance).[20] The liver compensates for hyperglycemia by decreasing its glucose secretion in the presence of insulin. As the resistance of cells to insulin increases, compensatory insulin secretion fails and either fasting, postprandial or random glucose concentrations increase. IR can progress to DM2 when insufficient insulin is produced by β cells in a condition of hyperglycemia.[21]

The cure for diabetes type 2 is not known yet, but research is focused mainly on the medicines that can halt organ damage or at least slow the process of damage in DM2 patient diabetes. Insulin is the most commonly prescribed drug in patients of diabetes. But, studies reveal that insulin alone is detrimental to liver cells because of its lipogenic effect.[22]

In view of above facts, the current research was designed to see the effects of insulin on the liver of untreated and insulin treated diabetic rats for a short period.

MATERIALS AND METHODS

The study was conducted at Anatomy Department, Dow International Medical College, Dow University of Health Sciences, Karachi.

Duration of Study: The study took 8 months to be completed from 1st June 2011 to 1st Feb 2012.

Animals were obtained from the Animal House of Dow University of Health Science. Thirty adult albino rats (average body weight 160 ± 20 gm) were selected for the study and were acclimatized for one week. They were kept at room temperature 30 ± 1°C with a 12 hour light and dark cycle and were given balanced in-house prepared HFD diet and water ad libitum. The animals were divided into three groups each containing 10 animals. All rats were weighed on a digital scale at the beginning of the experiment and weekly until sacrificed. Also blood glucose levels were done with glucometer (Accucheck Performa) in all rats every week. Under aseptic condition, the blood sample for glucose testing was taken from the base of the tail because of easy visibility of veins at that area.

Experimental induction of diabetes type 2 was done in 2 phases: In the first phase, control rats (n=10) were given free access to water and in-house prepared standard diet while the other rats were kept on in-house prepared HFD for 2 weeks. After 2 weeks of dietary manipulation, all rats except the control group (n=10) were given low dose STZ (manufactured by Bio Plus Fine Chemicals, USA) (3.5mg/ 100 mg) intraperitonelly (IP).[1] STZ was prepared freshly before use by dissolving the powder in cold citrate buffer (pH 4.5) for immediate use within 10 minutes of preparation. Rats were fasted for12-14 hours before IP injection of STZ. The matched control group was given citrate buffer (0.1 ml/100mg).Four day after injection of STZ rats were tested for blood glucose level. Rats having blood glucose levels ≥200mg/dl were considered diabetic and were included in the study.

After experimental induction of diabetes type 2, the rats were divided into 3 groups; Control Group-A (n=10), Untreated diabetic Group-E (n=10) and Insulin treated Group-B (n=10).

HFD/STZ induced rats (Group B) were given IP injection of insulin (0.3U/100 gm/day) [23] for 4 weeks. The rats were allowed to have HFD till the end of the experiment. After 4 weeks of treatment, the animals were weighed and rats were sacrificed. The abdominal cavity was dissected; liver exposed, carefully separated from surrounding tissues and dissected out and placed in a petri dish. Liver was examined for the significant

gross features. The absolute weight of the liver was carefully noted by placing the organ on the Sartorius balance and the relative weight of the liver was obtained by the formula: weight of liver/weight of rat x 100. The volume (length x breadth x height) [24] of the organ was also appropriately measured.

The change in the study variables was statistically analyzed by using ANOVA. Tukey type non-parametric post hoc test was utilized if tests showed the significant difference. P-value of ≤ 0.05 was considered as statistically significant.

RESULTS

1. **Gross appearance of liver:**

Untreated HFD/STZ induced diabetic rats displayed a dark brown coloration of the liver. While insignificant difference was seen in the gross appearance of liver (contour and consistency) of the insulin-treated group when they were compared with the control rats.

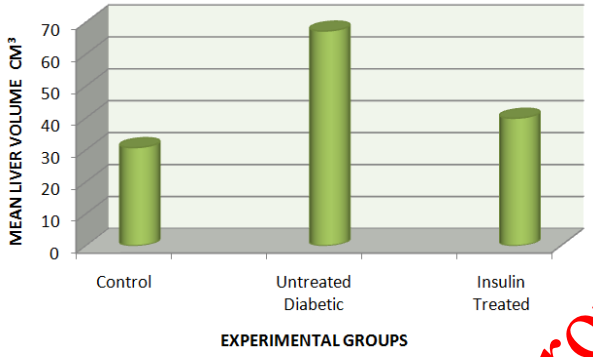

Figure No.I: Graphical representation showing comparative analysis of mean liver Volume (cms³) in experimental groups

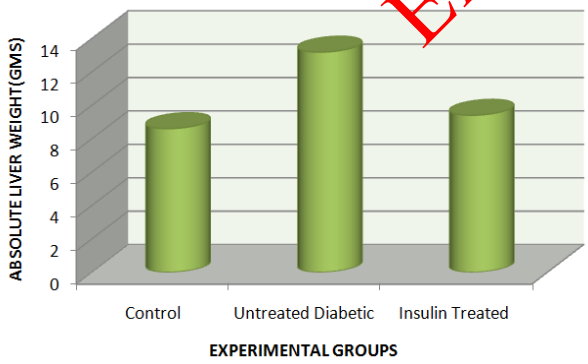

Figure No.2: Graphical representation displaying comparative analysis of mean absolute liver weight (gm) in experimental groups

2. **Liver volume(length x breadth x height) cm^3:**

Liver volume showed significant changes in insulin treated and untreated diabetic groups in comparison to control group. Mean Liver Volume observed in group A, E and B was 30.72 ± 3.85 cm^3, 67.24 ± 13.32 cm^3 and 39.93 ± 3.82 cm^3 respectively. Significant increase (P Value =< 0.05) in liver volume was found in Group E while comparing with group A. Similarly significant increase (P Value =< 0.05) in liver volume was seen in group B when compared to group A. Graphical presentation of variation in liver volume is shown in Figure-I.

3. **Absolute liver weight (ALW) gm:**

Mean absolute liver weight (ALW) of rats was observed as 8.60 ± 2.54 gm, 9.40 ± 3.18 gm, and 13.18 ± 0.68 gm in control, insulin treated and untreated groups respectively. On comparison of group A with E, there was significant increase (P Value =<0.05)in ALW as shown in figure II. On comparison of group A with B, insignificant increase (P-value=0.48) in ALW is noted. On comparing group E with B, there was significant increase (P-value=< 0.05) in ALW was observed.

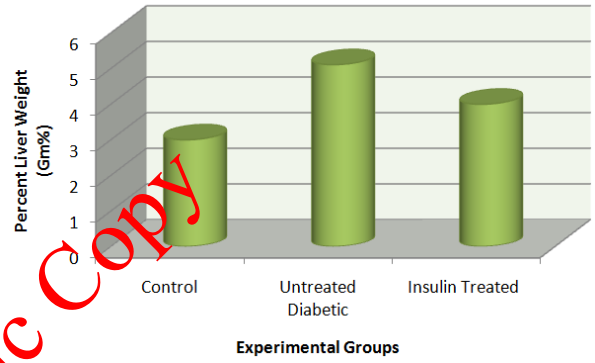

Figure No.3: Graphical representation showing comparative analysis of mean percent liver weight (%) in experimental groups

4. **Percent liver weight (PLW) %:**

The mean percent liver weight (PLW) was calculated as 2.99 ± 0.66%, 3.99 ±1.37% and 5.10 ± 0.73% in control, insulin treated and untreated groups respectively. Insignificant increase in PLW was seen when Group A is compared with Group B (P-value = 0.73). While a significant increase was seen in PLW while comparing group A with E (P-value = < 0.05). The comparison between Group B and E depicts significant increase in PLW (P-value = 0.44) as shown in Figure 3.

DISCUSSION

Increasing prevalence of diabetes in our community, has focused our research to learn natural course, triggering factors and effects of therapeutic interventions in diabetes. Hepatomegaly in DM2 patients was overlooked for a long time because of its indolent course. But, with the rise of DM2 and associated liver related morbidities and mortalities in these patients, hepatomegaly and fatty liver disease in diabetics has become the hub of research. An increase in liver volume, absolute and relative weight of liver points to the accumulation of fat. There is strong

association of type 2 diabetes with IR and fatty liver disease. A lot of work has been done on fatty liver disease associated with obesity, but less work is done on increased liver span, its causes and consequences in diabetes. It might be because human livers are not easily available.

In the present study, liver weight and volume was analyzed in untreated diabetic rats and insulin treated diabetic rats. The blend of dietary manipulation and chemicals was used to make a perfect experimental model of DM2 as done previously by many renowned scientists.[21, 22, 24] It is known the fact that HFD creates an animal model with glucose intolerance and IR.[22,24,25] The rats were fed HFD to induce IR followed by a low dose of STZ (diabetogenic agent) which specifically targets insulin secreting cells of pancreas and is used by many researchers to create diabetic model as it is simple, time saving and economical. [21, 24, 26] Therats developed diabetic symptoms, increased blood glucose levels and body weights which is comparable with the studies of Ikebukuro 2002 and Srinivasan K 2005, with no significant difference in them. [22, 27]

As far as the gross features of liver are concerned, no significant changes were noted when compared to control rats. Significant changes were observed in liver volume, absolute and percent liver weight of treated and untreated diabetic rats when compared with control rats. The absolute and percent liver weight of untreated and insulin treated rats increased despite of the facts that mean body weight of these rats decreased. It might be because of the fact that lipogenesis is decreased in peripheral tissues while these lipogenic pathways are activated in liver causing build up of fat in liver leading to enlarged liver.[28] The present observations are consistent with other study. [29]

The study variables showed a significant increase in untreated diabetic rats which can be a consequence of sustained increase in blood glucose levels as they were not given any medicine to lower blood sugar. Increased blood glucose is an another doubtful but, potential risk factor for the development of fatty liver disease because fat deposition is independent of blood glucose levels[30, 31]

Insignificant increase is noted in the study variables of insulin treated diabetic rats. The likely explanation for the insignificant increase could be the IP administration of insulin which by passes liver and therefore, less insulin passes through liver to stimulate lipogenesis in liver. The IP injection of insulin reduced high blood glucose levels and therefore, insulin was no longer deficient. Thus, the peripheral lipogenic pathways were activated in the presence of insulin and the fat transiently deposited in ectopic sites (i.e. liver) was now redirected to its natural storage place, the peripheral adipose tissue.[22] These results suggest that short term treatment with IP insulin does not increase absolute and percent liver weight in diabetic rats.

Regardless of the true origin of fatty liver in diabetics, be it our unhealthy life style, increase insulin resistance or uncontrolled glucose, lipid dysmetabolism seems to be the culprit. Despite its obvious importance, pathogenesis of fatty liver is poorly understood mainly because of ethical limitations. But, the questions need to be addressed as the condition is increasing with the increase of diabetes.

CONCLUSION

It is concluded from the study that short-term (4 weeks) use of intraperitoneal injection of insulin decrease absolute and relative weight as well as volume of the liver in HFD/STZ induced diabetic rats.

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