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Effects of Inhibition of Angiotensin Converting Enzyme, Blocking Angiotensin Type 1 (AT1) Receptor, and Blocking Aldosterone Receptor on High Fat Fructose Diet Feeding Induced-Changes in Body Weight, Insulin Resistance, and Hepatic Steatosis

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Abstract

Objective: The food industry has evolved, providing a supply of increasingly rich diet in terms of fatty acids and fructose. This shift is not without consequences on general health including increase risk of developing obesity, insulin resistance, hepatic steatosis and diabetes. Expression of all components of the renin-angiotensin-aldosterone-system has (RAAS) been shown in adipose tissue of human and rodent models. The present work aimed at studying the effects of inhibition of angiotensin converting enzyme, blocking angiotensin II type 1 receptor, and blocking aldosterone receptor on high fat fructose diet (HFFD) feeding induced-changes in body weight, insulin resistance, and hepatic steatosis in male albino rats.

Material and Methods: 50 male albino rats with body weight 100-115gm were randomly divided into 5 groups. Each group contained 10 rats and with equal average body weights: Group I (control group) in which rats were fed standard rat chow for the entire study duration, Group II (HFFD) in which rats were fed HFFD for the entire study duration, Group III (HFFD+ACE inhibitor) in which rats were fed (HFFD) and treated orally with angiotensin converting enzyme inhibitor, captopril, at a dose of 40mg/kg/day, for the entire study duration, Group III (HFFD+AT1 blocker) in which rats were fed HFFD and treated orally with angiotensin II type 1 (AT 1) receptor blocker, losartan, at a dose of 10mg/kg/day for the entire study duration, Group V (HFFD+Aldosterone receptor blocker) in which rats were fed HFFD and treated orally with Aldosterone receptor blocker, spironolactone, at a dose of 16mg/kg/day for the entire study duration. At the end of study period (10 weeks), rats were weighted then sacrificed. Serum was prepared for fasting glucose and insulin levels, insulin resistance was assessed using HOMA-IR, liver was removed, weighted, separated into 2 parts for real-time quantitative polymerase chain reaction for tumor necrosis alpha (TNF-a), Interleukin 6 (IL-6), Carbohydrate Response element binding protein (ChREBP), and Sterol response element binding protein-1c (SREBP) gene expression and for histopathological examination for non-alcoholic steatohepatitis called the NAFLD activity score (NAS) and fibrotic changes.

Results: Feeding rats with HFFD (group II) for 10 weeks produced significant increase in the mean values of body weight, fasting serum glucose and insulin levels. HOMA-IR, liver weight, % liver weight/body weight ratio, hepatic gene expression of TNF-α, (IL-6), and SREBP while hepatic gene expression of ChREBP significantly decreased. Moreover, NAS score and fibrotic changes significantly increased compared to group I.

Oral administration of angiotensin converting enzyme inhibitor (group III), angiotensin II type 1 receptor blocker (group IV), and aldosterone receptors blocker (group V) in rats fed HFFD can attenuate HFFD-induced changes as evident by significant decreases in mean values of body weight, fasting serum glucose and insulin levels, HOMA-IR, liver weight, hepatic gene expression of TNF-a, IL-6, SREBP, NAS score and fibrotic liver changes compared to group II. However, the mean values of % liver weight/body weight ratio significantly decreased in group III and IV but insignificantly changed in group V. Hepatic gene expression of ChREBP insignificantly changed in group III, IV, and V compared group II. The ability of interference with RAAS to modify HFFD-induced changes is partial as evident by significant increases in body weight, fasting serum glucose and insulin levels, HOMA-IR, liver weight, hepatic gene expression of TNF- a, IL-6, SREBP, and NAS score compared to control group. However % liver weight/body weight ratio insignificantly changed in group III and IV but significantly increased in group V compared to group I. Hepatic gene expression of ChREBP significantly decreased which is not related to interference with RAAS. Hepatic gene expression of ChREBP significantly decreased also in HFFD compared to control group and insignificantly changed with interference with RAAS.

Summary and Conclusion: High fat fructose diet participates in the development of obesity, insulin resistance, hepatic steatosis, steatohepatitis, and even hepatic fibrosis. Interference with RAAS system by inhibiting angiotensin converting enzyme, blocking angiotensin II type 1 receptor, or blocking aldosterone receptor can partially improve HFFD induced changes. In conclusion, the established role of both circulating and local RAAS on the pathogenesis of obesity induced insulin resistance, NAFLD, and NASH created considerable interest on the effect of RAAS inhibitors since they are widely used, reasonably inexpensive, and with excellent safety profile.

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Despite the encouraging evidence from animal studies, data from human studies are limited and contradictory and most data are from retrospective studies. Accordingly, more studies are needed to directly assess the effectiveness of ACE inhibitor, ARBs, and mineralocorticoid receptor blockers re in obese subjects.

Key Words: *Angiotensin converting enzyme – High fat fructose diet (HFFD) – Renin-angiotensin-aldosterone-system (RAAS).*

Introduction

WESTERNIZED diet, prevalent in modern society, is characterized by the increased consumption of high fat diet (HFD) along with fructose as a common sweetener. This type of food, together with the slothful lifestyle, participates in the amplification of the global wave of obesity, which is invariably associated with a constellation of pathological perturbations, among which is insulin resistance **[1]**. Moreover, this type of diet frequently induces hepatic steatosis, which accounts for hepatic insulin resistance **[2]**, and favors muscular fat storage, as well as visceral fat deposition **[1]**, facilitating thus, the development of peripheral insulin resistance **[3]**.

Expression of all components of the RAAS has been shown in adipose tissue of human and rodent models **[4]**. The activity of the RAAS appears to be regulated by food intake, and overfeeding has been reported to increase formation of angiotensin II in adipocytes in rodents **[5]**. Plasma aldosterone levels, likely driven by elevated Ang II, are increased in obese hypertensive patients, especially in patients with excess visceral fat. Clinical studies show a positive correlation between plasma aldosterone levels and body mass index **[6]**.

The present work aimed at studying the effects of inhibition of angiotensin converting enzyme, blocking angiotensin II type 1 (AT 1) receptor, and blocking aldosterone receptor on high fat fructose feeding induced changes in body weight, insulin resistance, and hepatic steatosis. The study was conducted using rats fed high fat chow and fructose in drinking water.

Material and Methods

Experimental animals:

The present study was carried out in the Physiology, Biochemistry, Pathology departments, Faculty of Medicine, Cairo University. The study was conducted between January and October 2013. Fifty male albino rats belonging to local strain were used in this study with body weights ranged from 105-115 grams. Animals had free access to food and water for the entire period of the study. The duration of the study was 10 weeks for all groups. Rats were placed under ordinary living conditions (e.g. humidity, temperature, and dark/light cycles) in the Animal House. Rats were divided into 5 groups with equal average body weights:

Group I (control group):

This group consists of 10 rats fed standard rat chow for the entire study duration.

Group II (HFFD group):

This group consists of 10 rats fed high fat content in rat chow and fructose in drinking water (HFFD).

Group III (HFFD+ACE inhibitor group):

This group consists of 10 rats fed on HFFD and receives daily oral dose of angiotensin-converting enzyme inhibitor (ACE), Captopril, at a dose of 40mg/kg/day **[7]** for the entire study duration.

Group IV (HFFD+AT1 blocker):

This group consists of 10 rats fed on HFFD and receives daily oral dose of angiotensin receptor AT1 blocker (Losartan) at a dose of 10mg/kg/day **[8]** for the entire study duration.

Group V (HFFD+Aldosterone receptor blocker):

This group consists of 10 rats fed on HFFD and receives daily oral dose of aldosterone receptor blocker (Spironolactone) at a dose of 16mg/kg/day **[13]** for the entire study duration.

Diet:

Standard rat chow: Contains 4% fat representing 14% of the caloric value of chow.

High fat diet: Was prepared by adding 40 grams of saturated fat [mutton tallow (leya)] to 100 grams of rat chow so that fat represents 60% of the total caloric value of the mixture **[10]**.

Fructose: Was added to drinking water presented to animals of groups II, III, IV and V at a concentration of 10% for the entire study duration. This concentration of fructose was chosen to be similar to its concentration in common beverages available in the market **[11]**.

Drugs preparation and administration:

Captopril: Capoten tablets were used (purchased from Smith Kline Beecham, Egypt, contain 25mg captopril). Each tablet was dissolved in 2 ml distilled water. The solution was filtered and suitable volume was given to each animal in group III to deliver the daily dose.

Losartan: Losartan tablets were used (purchased from Amriya Pharm. IND., Egypt, contain 50mg losartan). Each tablet was dissolved in 15 ml distilled water. The solution was filtered and suitable volume was given to each animal in group IV to deliver the daily dose.

Spironolactone: Aldactone tablets (purchased from Kahira Pharm. & Chem. IND. CO , Egypt, contain 25mg Spironolactone). It was dissolved in 2ml distilled water. The solution was filtered and suitable volume was given to each animal in group V to deliver the daily dose.

All drugs were given daily by oral route using a syringe, for the whole duration of the study.

Throughout the study period, animals of each studied group were housed in a separate wire mesh cage under ordinary living conditions. By the end of the study period (10 weeks) and after an overnight fast, body weight of each rat was recorded before rats were sacrificed. Blood was collected in test tubes and was incubated at 37ºC for 30 minutes to clot. Tubes were centrifuged and serum was separated in clean labeled tubes and stored at –80ºC to be used for measurement of biochemical parameters. Liver was dissected, wiped-dry by tissue paper, then weighed. Liver was divided in two parts; one part was weighted, placed in special tubes supplied in the kit , and was used for quantitative real-time quantitative polymerase chain reaction measurement of gene expression of tumor necrosis alpha (TNF-α), Interleukin 6 (IL6), Carbohydrate Response element binding protein (CHREBP), and Sterol response element binding protein (SREBP). The other part of the liver was placed in 10% formalin solution and was used for histopathological examination.

Measurement of fasting serum glucose:

The serum glucose was assayed using kits supplied by "Diamond Diagnostics" by the method adopted by Trinder **[12]**.

Measurement of fasting serum insulin:

Fasting plasma insulin concentration was estimated using enzyme-linked immunosorbent assay (Dako, Glostrup, Denmark).

HOMA-IR index calculation:

The homeostasis model assessment of insulin resistance (HOMA-IR) method was used to calculate insulin resistance as: HOMA-IR=Fasting serum insulin (µIU/ml) x fasting plasma glucose (mmol/l)/ 22.5 **[13]**.

Real-time quantitative polymerase chain reaction (qPCR):

Total RNA was extracted from liver tissue using SV Total RNA Isolation system (Promega, Madison, WI, USA). The yield of total RNA obtained was determined spectrophotometrically at 260nm. The extracted RNA was reverse transcribed into cDNA using AMV Reverse Transcriptase, Promega, and Madison.VVI, USA (Catalog No.: M5101). Real-Time Quantitative Polymerase Chain Reaction for Tumor necrosis alpha, Interleukin 6, Carbohydrate Response element binding protein, and Sterol response element binding protein-1c were determined. The gene-specific forward and reverse primer pair was normalized (Table 1). Each primer (forward and reverse) concentration in the mixture was 5pmol/µl. At the end of a qPCR running with SYBR Green chemistry, the relative quantification was calculated according to Applied Biosystem soft ware reference **[14]**.

Histopathological examination:

Livers were fixed overnight in 10% neutral buffered formalin. The samples were dehydrated using ethanol and embedded in paraffin. Tissue sections were cut at 6 µm in thickness, stained by Hematoxylin-Eosin staining (H&E), and then examined. Histological scoring was graded by pathologists (blinded to the groups) for hepatic steatosis, lobular inflammation, ballooning, and fibrosis in accordance with the 2011 program for diagnosis and treatment of non-alcoholic steatohepatitis called the NAFLD activity score (NAS), the score of each component of the NAS (steatosis (0-3), lobular inflammation (0-3), ballooning (0-2) **[15]**.

Table (1): Gene-specific forward and reverse primer pairs for TNF- a, IL-6, ChREBP, and SREBP.

	Primer sequence
Tumor necrosis alpha (TNF-a).	Forward:5' CGTCGTAGCAAACCACCAAG 3' Reverse: 5' A CACAGAGCAATGACTCCAAAG3'
Interleukin 6 (IL-6).	Forward:5' GAAACCGCTATGAAGTTCCTCTCTG 3' Reverse:5' TGTTGGGAGTGGTATCCTCTGTGA 3'
Carbohydrate Response element binding protein (ChREBP).	Forward:5' GACCCCTCTCTCAGGGAATACA3' Reverse:5' GGAAAGGTGCTGGGATCCA3'
Sterol response element binding protein (SREBP).	Forward:5' GGAGCCAATGGGGCCGGGG TTAcT-3' Reverse:5' CCTGTCTCACCCCCAGCATA-3'

Statistical analysis:

SPSS, version 12, was used to evaluate the data. The results are expressed as the mean±SD. Comparisons between the mean of different parameters in the different groups were made by 1-way analysis of variance (ANOVA). Significant comparisons where further analysed by post-test to determine the significantly different groups. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. p-values less than 0.05 were considered as statistically significant while p-values more than 0.05 were considered as statistically insignificant.

Results

Feeding rats with HFFD for 10 weeks (group II) resulted in significant (p<0.05) increases in the mean values of body weight, fasting serum glucose and insulin levels, HOMA-IR, liver weight, % liver weight/body weight ratio, hepatic gene expression of TNF-α, IL-6, SREBP while the mean value of hepatic gene expression of ChREBP significantly (p<0.05) decreased. Non alcoholic steatohepatitis score (NAS score) and fibrotic changes significantly (p<0.05) increased compared to the control group (Tables 2-8 and Figs. 1-12).

Oral administration of angiotensin converting enzyme inhibitor (Captopril) to rats fed HFFD for 10 weeks (group III) resulted in significant (p<0.05) decreases in the mean values of body weight, fasting serum glucose and insulin levels, HOMA-IR, liver weight, % liver weight/body weight ratio, significant (p<0.05) decreases in the mean values of hepatic gene expression of TNF-α, IL-6, and SREBP while the mean value of hepatic gene expression of ChREBP insignificantly (p>0.05) changed. NAS score and fibrotic changes significantly (p<0.05) decreased compared to group II (Tables 2-8 and Figs. 1-12).

Oral administration of angiotensin receptor type 1 blocker (Losartan) to rats fed HFFD for 10 weeks (group IV) resulted in significant (p<0.05) decreases in the mean values of body weight, fasting serum glucose and insulin levels, HOMA-IR, liver weight, % liver weight/body weight ratio, significant (p<0.05) decreases in the mean values of hepatic gene expression of TNF-α, IL-6, and SREBP while the mean value of hepatic gene expression of ChREBP insignificantly (p>0.05) changed. NAS score and fibrotic changes significantly (p<0.05) decreased compared to group II (Tables 2-8 and Figs. 1-12).

Oral administration of aldosterone receptor blocker (Spironolactone) to rats fed HFFD for 10 weeks (group V) resulted in significant (p<0.05) decreases in the mean values of body weight, fasting serum glucose and insulin levels, HOMA-IR, liver weight. % Liver weight/body weight ratio insignificantly (p>0.05) changed, hepatic gene expression of TNF-α, IL-6, and SREBP significantly (p<0.05) decreases. The mean value of hepatic gene expression of ChREBP insignificantly (p>0.05) changed while NAS score and fibrotic changes significantly (p<0.05) decreased compared to group II (Tables 2-8 and Figs. 1-12).

Comparing the effects of inhibiting angiotensin converting enzyme (group III), blocking angiotensin II type 1 receptor (group IV) and blocking aldosterone receptor (group V) on HFFD rats, the results of the present work demonstrated that blocking aldosterone receptor produced significant (p<0.05) increase in the mean value of body weight, insignificant (p>0.05) changes in the mean values of fasting serum glucose and insulin levels, HOMA-IR compared to group III and IV, significant (p<0.05) increase in liver weight compared to group III, significant (p<0.05) increase in % liver weight/body weight ratio compared to group IV, and insignificant (p>0.05) changes in the mean values of hepatic gene expression of TNF-α, IL-6, SREBP, ChREBP, NAS score, and fibrotic changes compared to group III and IV (Tables 2-8 and Figs. 1-12).

Comparing the effects of inhibiting angiotensin converting enzyme (group III), blocking angiotensin II type 1 receptor (group IV) and blocking aldosterone receptor (group V) on HFFD rats with control group, the results of the present work demonstrated significant (p<0.05) increases in the mean values of body weight, fasting serum glucose and insulin levels, HOMA-IR, and liver weight in the 3 groups compared to control group. The mean value of % liver weight/body weight ratio insignificantly (p>0.05) changed in group III and IV but significantly (p<0.05) increased in group V compared to control group. The mean values of hepatic gene expression of TNF-α, IL-6, and SREBP significantly (p<0.05) increased in while the mean values of hepatic gene expression of ChREBP significantly (p<0.05) decreased in the 3 groups compared to control group. The mean values of NAS score significantly (p<0.05) increased the 3 groups with insignificant fibrotic changes compared to control group (Tables 2-8 and Figs. 1-12).

Table (2): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II receptor type 1 blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in body weight in male albino rats.

	Control group	High fat fructose feeding			
			Captopril	Losartan	Spironolactone
	Group I	Group II	Group II	Group III	Group V
Body weight (gm)	135.4±4.0	255±17.2 @	163.6±15.9 @#	164.3±7.7 @#	190.1±9.7 @#©

Results are expressed as Mean±S.D.
@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
© : Significant changes (p<0.05) compared to group III and IV.

Table (3): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II receptor type 1 blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in fasting serum glucose and insulin levels and homeostatic model assessment of insulin resistance (HOMA-IR) in male albino rats.

	Control group	High fat fructose feeding			
			Captopril	Losartan	Spironolactone
	Group I	Group II	Group II	Group III	Group V
Fasting serum glucose (mm/l)	5.75±1.3	11.46±2.3 @	8.03±1.13 @#	8.37±1.2 @#	9.05±1.24 @#†
Fasting insulin (µ IU/ml)	9.53±1.2	18.8±3.4 @	13.62±2.06 @#	12.42±1.9 @#	12.75±1.6 @#†
HOMA-IR	2.42±0.44	9.60±2.83 @	4.86±0.93 @#	4.62±1.02 @#	5.10±0.89 @#†

Results are expressed as Mean±S.D.
@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.

Table (4): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II receptor type 1 blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in liver weight (gm) and % liver weight/body weight ratio in male albino rats.

	Control group	High fat fructose feeding			
			Captopril	Losartan	Spironolactone
	Group I	Group II	Group II	Group III	Group V
Liver wt. (gm)	3.22±0.37	8.0±0.45 @	3.68±0.52 @#	4.23±0.53 @#	4.70±0.98 @#©
% Liver wt./body wt.	2.40±0.26	3.12±0.18 @	2.54±.29 # ❚	2.23±0.40 #	2.81±0.44 @0 ❑

Results are expressed as Mean±S.D.
@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
© : Significant changes (p<0.05) compared to group III.
❑ : Insignificant changes (p>0.05) compared to group I.
0 : Insignificant changes (p>0.05) compared to group II.
❑ : Significant changes (p<0.05) compared to group IV.

Table (5): Effects of angiotensin converting enzyme inhibitor (Capoten), angiotensin type 1 blocker (Losartan), and aldosterone receptor blocker (Aldactone) on high fat fructose diet-induced changes in hepatic gene expression of TNF- a and IL-6 (arbitrary unit/mg tissue) in male albino rats.

	Control group	High fat fructose feeding			
			Captopril	Losartan	Spironolactone
	Group I	Group II	Group II	Group III	Group V
TNF-a	0.21±0.06	1.08±0.31 @	0.65±0.18 @#	0.51±0.11 @#	0.46±0.14 @#†
IL-6	1.21±0.20	8.72±2.49 @	5.34±1.52 @#	5.07±2.11 @#	4.00±1.88 @#†

Results are expressed as Mean±S.D.
@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.

Table (6): Effects of angiotensin converting enzyme inhibitor (Capoten), angiotensin II type 1 receptor blocker (Losartan), and aldosterone receptor blocker (Aldactone) on high fat fructose diet-induced changes in hepatic gene expression of SREBP and ChREBP (arbitrary unit/mg tissue) in male albino rats.

	Control group	High fat fructose feeding			
			Captopril	Losartan	Spironolactone
	Group I	Group II	Group II	Group III	Group V
SREBP	9.77±0.87	64.53±18.88@	39.18±15.7@#	40.04±9.87@#	42.76±16.7@#†
ChREBP	1.95±0.5	0.86±0.3@	1.07±0.40@□	1.178±0.51@□	1.118±0.62@□†

Results are expressed as Mean±S.D.

@ : Significant changes (p<0.05) compared to group I.
□ : Insignificant changes (p>0.05) compared to group II.
: Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.

Table (7): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II type 1 receptor blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced non alcoholic fatty liver disease activity score (NAS).

	Control group	High fat fructose feeding			
			Captopril	Losartan	Spironolactone
	Group I	**Group II**	**Group II**	**Group III**	**Group V**
NAS	0.3±0.48	3.6±0.69@	1.7±0.94@#	2.4±0.96@#	2.1±0.87@#†

Results are expressed as Mean±S.D.

@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.

Table (8): Effects of angiotensin converting enzyme inhibitor (Capoten), angiotensin II type 1 receptor blocker (Losartan), and aldosterone receptor blocker (Aldactone) on the incidence of fibrotic changes induced by high fat fructose diet in male albino rats.

		Control group		High fat fructose feeding							
						Captopril		Losartan		Spironolactone	
		Group I		Group II		Group II		Group III		Group V	
		Count	%	Count	%	Count	%	Count	%	Count	%
Hepatic fibrosis	Yes	0	0	9	90	2	20	3	30	3	30
	No	10	100	1	10	8	80	7	70	7	70
p-value				p<0.05@		p>0.05□ p<0.05#		p>0.05□ p<0.05#		p>0.05□ p<0.05# p>0.05†	

Results are expressed as Mean±S.D.

@ : Significant changes (p<0.05) compared to group I.
□ : Insignificant changes (p>0.05) compared to group I
: Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.

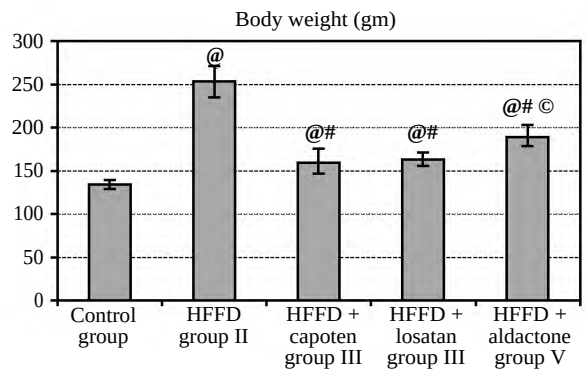


Fig. (1): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II receptor type 1 blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in body weight (gm) in male albino rats.

@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
© : Significant changes (p<0.05) compared to group III and IV.

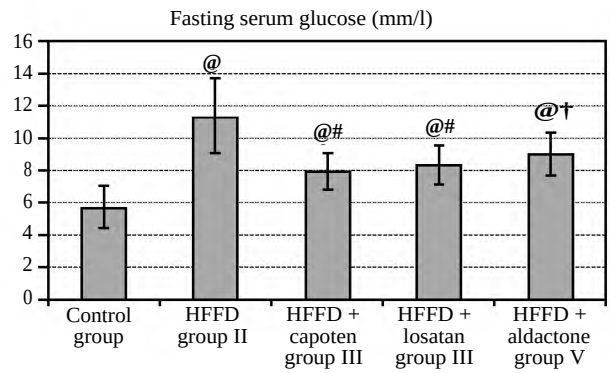


Fig. (2): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II receptor type 1 blocker (Losartan), and aldosterone receptor blocker (Spiron-olactone) on high fat fructose diet-induced changes in fasting serum glucose level.

@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.

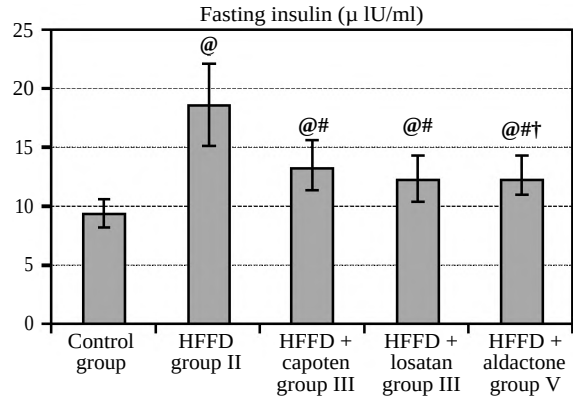


Fig. (3): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II receptor type 1 blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in fasting serum insulin level.

@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.

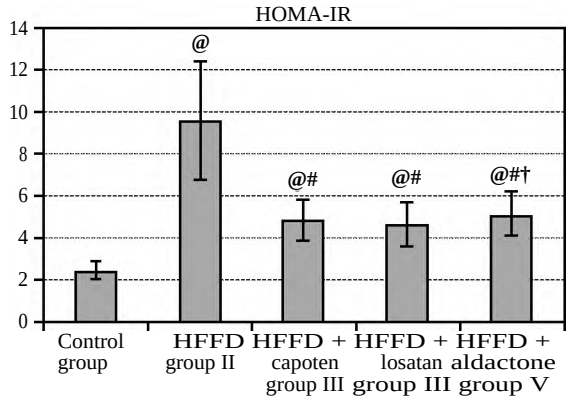


Fig. (4): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II receptor type 1 blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in and homeostatic model assessment of insulin resistance (HOMA-IR).

@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.

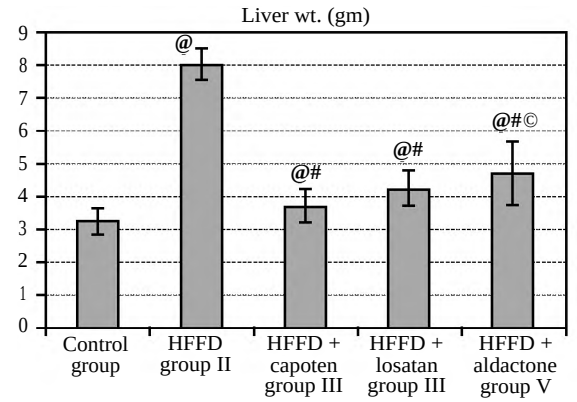


Fig. (5): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II receptor type 1 blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in liver weight in male albino rats.

@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
©: Significant changes (p<0.05) compared to group III.

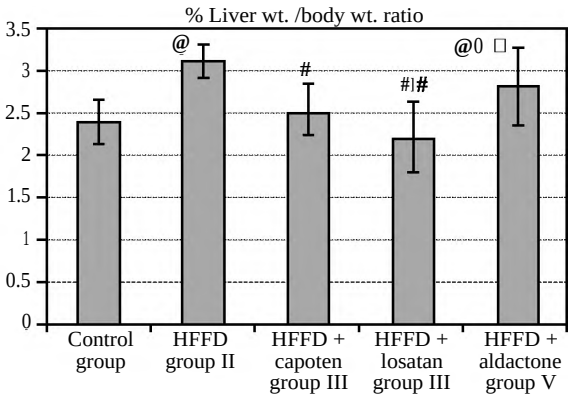


Fig. (6): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II receptor type 1 blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in % liver weight/body weight ratio in male albino rats.

@ : Significant changes (p<0.05) compared to group I.
❑ : Insignificant changes (p>0.05) compared to group I.
: Significant changes (p<0.05) compared to group II.
0 : Insignificant changes (p>0.05) compared to group II.
❑ : Significant changes (p<0.05) compared to group IV.

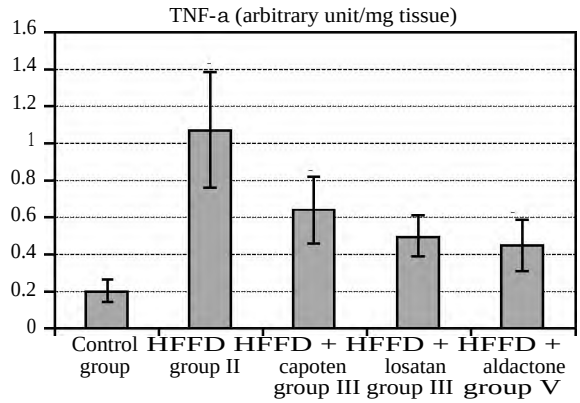


Fig. (7): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II type 1 receptor blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in hepatic gene expression of TNF-a in male albino rats.

@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.

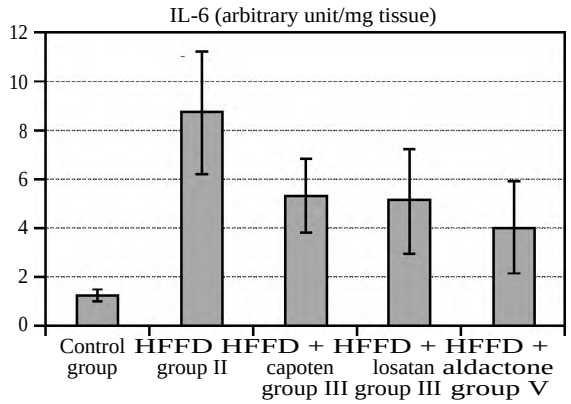


Fig. (8): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II type 1 receptor blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in hepatic gene expression of IL-6 in male albino rats.

@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.

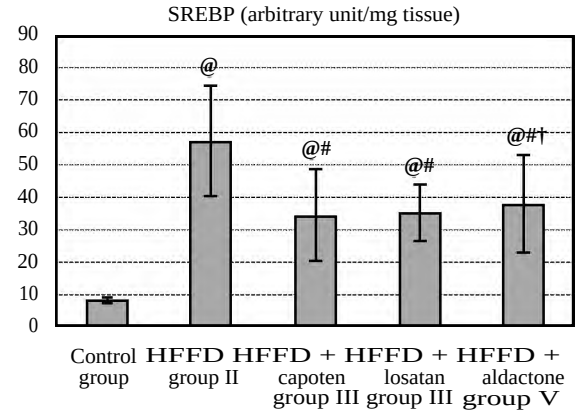


Fig. (9): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II type 1 receptor blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in hepatic gene expression of SREBP (arbitrary unit/mg tissue) in male albino rats.

@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.

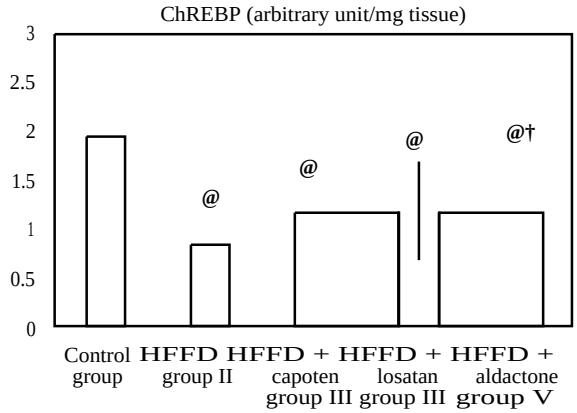


Fig. (10): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II type 1 receptor blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced changes in hepatic gene expression of SREBP (arbitrary unit/mg tissue) in male albino rats.

@ : Significant changes (p<0.05) compared to group I.
▫ : Insignificant changes (p>0.05) compared to group II
† : Insignificant changes (p>0.05) compared to group III and IV

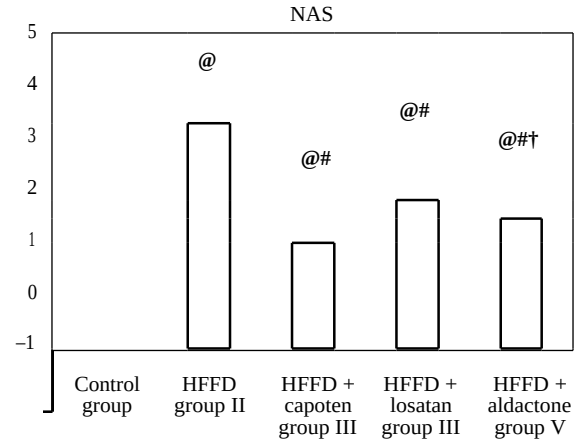
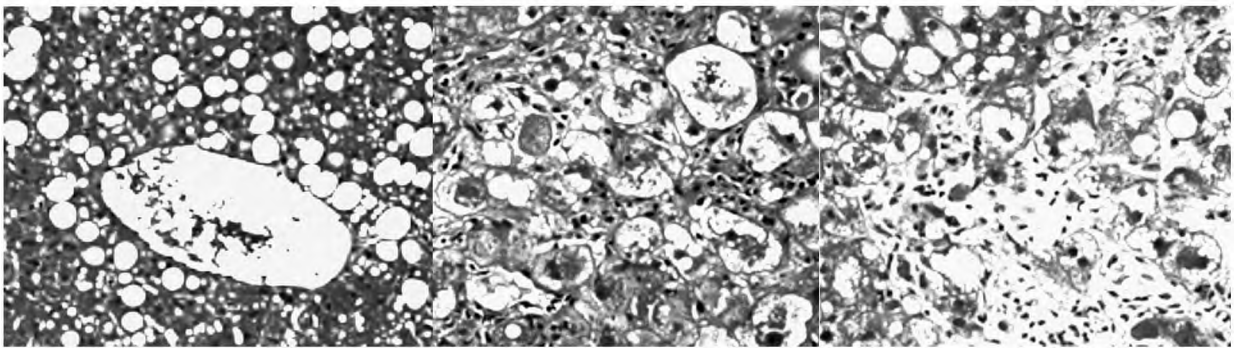


Fig. (11): Effects of angiotensin converting enzyme inhibitor (Captopril), angiotensin II type 1 receptor blocker (Losartan), and aldosterone receptor blocker (Spironolactone) on high fat fructose diet-induced non alcoholic fatty liver disease activity score (NAS).

@ : Significant changes (p<0.05) compared to group I.
# : Significant changes (p<0.05) compared to group II.
† : Insignificant changes (p>0.05) compared to group III and IV.



(A) (B) (C)

Fig. (12): (A) Liver histology demonstrating moderate macrovesicular steatosis around the central vein. Hematoxylin and eosin staining 200x. (B) Liver histology demonstrating active steatohepatitis with steatosis, ballooned hepatocytes, and inflammatory infiltrate. (C) Liver histology demonstrating steatohepatitis with extensive pericellular fibrosis. 400x.

Discussion

Results of this work showed that rats receiving HFFD, compared to control rats, developed obesity (as indicated by increased body weight). Both liver weight and relative liver weight (% liver weight/ body weight ratio) increased as well. Histological examination revealed the accumulation of lipid droplets in the hepatocytes with significance incidence of fibrotic changes. The development of hepatic steatosis was associated with increased gene expression of SREBP1c, while gene expression of ChREBP decreases. Moreover, the expression of genes encoding the synthesis of two key inflammatory mediators TNF-α and IL6 increased.

Dietary fat is deposited in adipose tissue. Absorbed fatty acids reach the liver as chylomicron particles [16]. Assembly of free fatty acids (FFAs) into very-low-density lipoprotein (VLDL), which is liberated into the circulation, occurs in the liver, [17]. In adipose tissue, FFAs are released from VLDL and chylomicrons by lipolysis under the effect of lipoprotein lipase enzyme. They are then taken up by adipocytes and re-synthesized into triglycerides [18]. Fructose feeding may accentuate obesity by affecting appetite and food intake. Ingestion of fructose-containing meals elicits a lesser suppression of the appetite-stimulating hormone ghrelin and a lower increase in leptin than meals containing an equivalent amount of glucose [19]. Although prolonged ingestion of fructose increases leptin secretion, it simultaneously causes a state of leptin resistance [20]. Fructose in diet not only contributes to the development of obesity, but it also favors the deposition of excess fat in visceral depot. Thus fructose contributes to both general and selective visceral adiposity [21]. Several reports [9,22,23] have also demonstrated that chronic high fat and fructose consumption, in terms of increased energy intake, produced a significant increase in body weight.

Together with development of obesity, HFFD-fed animals in the present work developed insulin resistance as indicated by high fasting serum glucose and insulin levels, and high HOMA-IR index compared to control rats. These findings agree with previous reports [24].

Insulin receptor substrates (IRS) contains several serine residues that are phosphorylated by kinases such as extracellular signal regulated kinase (ERK), c Jun N-terminal kinase (JNK), protein kinase Cζ (PKCζ), and p70S6 Kinase [25]. The phosphorylation of IRS on Ser-307 is a typical inhibitory signal to suppress insulin signaling [26].

Several mechanisms could have contributed to the development of this insulin resistance state in rats kept on HFFD.

Low-grade chronic inflammation is an important factor in the pathogenesis of type II diabetes mellitus in humans and rodent animal models [27]. Excess nutrition overload initiates adipocytes hypertrophy and hyperplasia resulting in cellular stress that in turn initiates oxidative stress and inflammatory responses in adipose tissue that eventually leads to increased local and systemic levels of various proinflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1ß, that are causative for insulin resistance [28].

Obesity-related hyperlipidemia, hyperglycemia, hypoxia, oxidative stress, and endoplasmic reticulum (ER) stress can also induce insulin resistance in peripheral tissues and can induce activation of inflammatory signalling cascades in adipose tissues [29]. Increased TNF-α in obese individuals activate c Jun N-terminal kinase (JNK), protein kinase and inhibitor of nuclear factor κB kinase ß (IKKß) to phosphorylate Ser-307 of insulin receptor substrate-1 (IRS-1). In addition, extracellular signal regulated kinase (ERK) activated by insulin also phosphorylates IRS 1 on Ser-612 to attenuate inositol 3-kinase (PI3K)-AKT/protein kinase B (PKB) (AKT) activation [30].

Badin et al., [31] reported that high fat diet induced whole-body insulin resistance and glucose intolerance and reduced skeletal muscle glucose uptake compared with normal chow diet. High fat diet increased skeletal muscle total diacylglycerol (DAG) content, protein kinase C θ and protein kinase C ε membrane translocation, and impaired insulin signaling as reflected by a robust increase of basal Ser1 101 insulin receptor substrate 1 phosphorylation.

Accumulation of bioactive lipids such as diacylglycerol (DAG) and/or ceramide is actually responsible for the insulin resistance [32]. DAG can activate several isoforms of protein kinase C (PKC), which can impair insulin signal transduction via serine phosphorylation of insulin receptor substrate (IRS)-1 [33]. Ceramides can cause insulin resistance by preventing insulin- stimulated Akt phosphorylation and activation and translocation of Akt to its substrate [34]. In addition, ceramide initiates inflammatory signaling pathways, leading to the activation of both c jun NH_2-terminal kinase (JNK) and nuclear factor κB/inducer of κ kinase [35], which have been implicated in the development of insulin resistance [36].

Fructose delivery to and metabolism in the hepatocyte generates a signal that culminates in the activation of JNK, phosphorylation of serine307 of IRS-1 and reduced insulin- stimulated tyrosine phosphorylation of IRS-1 and IRS-2 **[37]**. This response to fructose delivery results from the burden of fructose metabolism **[38]**. Wei et al., **[39]** examined three potential intrahepatic signals that can promote JNK activation and modulation of IRS-1 phosphorylation; ceramide, oxidative stress and methylglyoxal **[40-43]**. Although fructose delivery increased all three of these signals, inhibition of the increase in ceramide, or oxidative stress, did not reduce fructose-mediated effects on stress or insulin signaling. In contrast, experiments in which cellular methylglyoxal concentrations were selectively elevated to levels observed with fructose delivery resulted in the activation of stress signaling and reductions in insulin signaling **[39]**.

In the present work, HFFD produced significant increase in liver weight, both absolute and relative to body weight, compared to control rats fed standard chow. The expression of hepatic sterol response element binding protein (SREBP- 1 c) was also increased significantly while the expression of hepatic carbohydrate response element binding protein (ChREBP) significantly decreased. Histological examination also revealed evidence of NAFLD in HFFD groups of rats. NAS score (collectively evaluating steatosis, ballooning degeneration and lobular inflammation) was significantly higher in HFFD rats. Moreover, livers of HFFD-fed rats show significant fibrosis. The expression of genes encoding the synthesis of two key inflammatory mediators TNF-a and IL6 increased significantly compared to control group.

The increased liver weight can be explained by increased hepatic lipid content. Arunkumar et al., **[44]** reported that HFFD-fed mice showed a 2.5-fold increase of hepatic lipid compared to control chow fed mice.

SREBPs are membrane-bound members of the basic helix-loop-helix leucine zipper family of transcription factors. Two SREBP genes produce three separate proteins (SREBP-1a and-1c from one and SREBP-2 from the other) with SREBP-1c and SREBP-2 being the predominant isoforms in the liver **[45]**. SREBPs function as master regulators of cholesterol and fatty acid synthesis. SREBP-2 upregulates expression of most cholesterol biosynthetic enzymes and the low density lipoprotein (LDL) receptor, whereas SREBP-1c stimulates transcription of genes required for fatty acid synthesis, such as acetyl-CoA carboxylase and fatty acid synthase. SREBPs cooperate with other DNA-binding transcription factors and coactivators **[46]**.

ChREBP is a transcription factor responsible for inducing liver lipogenesis after carbohydrate ingestion. ChREBP controls about half of hepatic lipogenesis by regulating glycolytic and lipogenic gene transcription **[47]**. ChREBP is the main factor responsible for the increase in rat liver lipogenesis following fructose supplementation **[11]**. ChREBP controls the expression of regulator of G protein signaling (RGS) 16, a regulator of G protein signaling that inhibits hepatic fatty acid oxidation **[48]**.

Fatty liver (excessive accumulation of triglyceride in hepatocytes) is the hallmark of non alcoholic fatty liver disease (NAFLD), which has become an important public health problem due to its high prevalence, potential progression to severe liver disease, and association with cardio metabolic abnormalities **[49]**. NAFLD and non alcoholic steatohepatitis (NASH) are increasingly recognized causes of liver disease and liver-related morbidity and mortality **[50]**.Obesity, insulin resistance and diabetes type II are considered risk factors for the development of the more severe manifestations of NAFLD, like NASH and cirrhosis **[51]**.

The key events that contribute to the initiation and progression of NAFLD are summarized in a “multi-hit” model of NASH pathogenesis by Polyzos et al., **[52]**. According to this model, dysregulated metabolism of FFAs is considered to be the “first-hit” of NAFLD pathogenesis, which leads to insulin resistance and fat accumulation in the liver. Steatosis develops when the rate of fatty acids input (uptake and synthesis with subsequent esterification to triglycerides) is greater than the rate of fatty acids output (oxidation and secretion). When fat intake in diet is high, adipocytes become unable to uptake all FFAs released by lipolysis and the excess FFAs is released to the circulation (spill over) **[53]**. This will increase hepatic FFAs input causing excessive deposition of triglycerides droplets in hepatocytes, i.e. steatosis **[54]**.

SREBP-1c has been shown to be responsible for steatosis that develops in mice fed lard based high fat diet **[55]**. SREBP-1c is mainly stimulated by insulin. High insulin level observed in the present study in HFFD rats is therefore an important underlying mechanism responsible for development of steatosis. Continued activation of SREBP-1c due to elevated insulin signaling in these rats in presence of insulin resistance, forms the basis of

the paradox of "mixed insulin resistance" where insulin activation of SREBP-1c and lipogenesis is maintained, whereas insulin dependent suppression of hepatic glucose production is impaired [56]. Most type 2 diabetic patients exhibit hepatic insulin resistance along with varying degrees of steatosis. In diabetes/obesity, hepatic insulin resistance can be selective; i.e. insulin's ability to inhibit gluconeogenesis is impaired, whereas insulin stimulated lipogenesis is not.

Insulin induces de novo lipogenesis by activating the key transcription factor SREBP-1 c through a PI3K/Akt signaling pathway [55]. Liver-specific insulin receptor knock-out mice exhibit global hepatic insulin resistance with no steatosis, confirming the key role of insulin signaling in fat accumulation in liver [57], suggesting the presence of divergent intra hepatocyte insulin signaling pathways, allowing enhanced hepatic lipogenesis and gluconeogenesis in insulin-resistant states.

Fructose is well-known for its lipogenic effect. Fructose, per se, in drinking water combined with standard rat chow activates lipogenesis partly through inducing expression of SREBP-1c and consequently lipogenic enzymes. This activation is achieved by either high (60%) fructose [58] or low (10%) fructose [59]. Fructose feeding has also been shown to induce the activation of ChREBP and increase the expression of lipogenic genes such as fatty acid synthase (FAS), acyl coenzyme-A carboxylase (ACC), and stearoyl coenzyme-A desaturase-1 [60].

The present work demonstrated that HFFD increased the expression of SREBP-1c in the liver. However, the finding that the expression of ChREBP in the liver of HFFD rats in the present work was lower than control rats fed standard rat chow may be due to the lower level of carbohydrates consumed by the rats. Adding 40gm fat to each 100gm chow resulted in lowering carbohydrate concentration in the mixture by ~ 28%. The amount of fructose taken in drinking water apparently did not compensate for that. Moreover, ChREBP is activated by a high carbohydrate diet and inhibited by a high fat diet. The DNA binding activity of ChREBP in nuclear extract of livers from rats fed a high fat diet is inhibited compared with those of control rats fed laboratory chow or a high carbohydrate diet [61]. The mechanism of ChREBP inhibition by fatty acids appeared to be mediated by phosphorylation of Ser^{568} of ChREBP by 5' adenosine monophosphate-activated protein kinase (AMPK), which was activated by increased 5' adenosine monophosphate (AMP) that was produced by the activation of fatty acids catalyzed by acyl-CoA synthetas [62].

Inflammatory response, oxidative stress, and apoptosis, serve as "following-hits" that contribute to the ongoing inflammation that leads to non-alcoholic steatohepatitis (NASH). Oxidative stress and apoptosis plays a critical role in NAFLD-induced liver injury and in the progression from steatosis to NASH and then to cirrhosis [63].

NASH is the result of complex hepatocellular metabolic dysfunction in which insulin action is deranged. In NASH pathophysiology, fat accumulation is considered as the "first hit", which increases the generation of reactive oxygen species (ROS) that promote lipid peroxidation [64], early mitochondrial dysfunction, endoplasmic reticulum stress, iron accumulation [65]. Damage due to "first hit" intensifies the susceptibility of the liver to inflammation and fibrosis by the "second hit", fostered by the production of inflammatory cytokines and fibrogenic factors, activation of hepatic stellate cells (HSC) and apoptosis of the hepatocytes [66]. Induction of proinflammatory cytokines, in particular, TNF-α, is thought to represent the first step towards the subsequent development of liver fibrosis [66], supporting the finding that hepatic expression of TNF-α and IL-6 are increased in HFFD-fed rats in the present study. In line with this, serum concentrations [67] and liver m-RNA [68] of both TNF-α and IL-6 have been reported to increase in NASH patients. TNF-α and other factors activate the hepatic stellate cells (HSC) and induce the expression of transforming growth factor ß1 (TGF -ß1) [69].

Liver fibrosis is a complicated crosstalking of various cell types such as hepatocytes, Kupffer cells and HSC mediated by different cytokines, ROS and soluble factors bringing about excessive fibrogenesis with disruption of intercellular contacts and interactions and of extracellular matrix composition [70]. Damaged liver cells generate ROS which stimulate the release of proinflammatory cytokines like TNF-α from hepatocytes and Kupffer cells thereby increasing the severity of inflammatory stress in the liver [71]. TNF-α exerts its biological functions via interaction with two membrane receptors [72] and activate cell death through TNF-α related apoptosis [73]. In agreement of findings of the present work, Jaya et al., [74] reported enhanced hepatic TNF-α mRNA expression in rats fed with HFFD. The increased expression of TNF-α in HFFD-fed rat, clearly indicates that chronic hepatic inflammation correlates the severity of disease progression.

TNF-α mRNA upregulation has been detected in the liver in both dietary and genetic models of NASH [75]. Elevated Transforming growth factor ß1 (TGF-ß1) production by HFFD animals is trigger for collagen biosynthesis. TGF-ß1 binds to its receptors on HSC and activates the transcription of pro-collagen genes causing deposition of collagen type I and III [76]. During the development of fibrosis, quiescent HSC are transformed to a myo-fibroblast like cells that start secreting α-smooth muscle actin (α-SMA), a cytoskeletal protein [77]. Increased expression of α-SMA is considered a marker of HSC activation. Although α-SMA positivity in a few stellate cells of the liver is normal, significant increase in α-SMA expression suggests HSC proliferation.

Hepatic IL-6 expression is upregulated and correlates positively with the degree of liver fibrosis in non-alcoholic steatohepatitis [78]. Because IL-6 receptors are expressed ubiquitously on all types of liver cells, it is plausible to speculate that IL-6 may positively and negatively regulate liver fibrosis by targeting different types of liver cells. IL-6 protects against hepatocellular damage, thereby reducing injury-driven liver fibrosis, while it may also directly promote HSC survival and proliferation, followed by enhanced liver fibrosis. The final effect of IL-6 on liver fibrosis is likely determined by the balance between these inhibitory and stimulatory effects and is dependent on the stage and etiology of liver fibrosis [79].

Renin-Angiotensin-Aldosterone-system (RAAS) is classically known for its role in the regulation of blood pressure, fluid, and electrolyte balance. In addition, it is involved in many physiologic and pathophysiologic processes such as tissue growth and hypertrophy, inflammation, and interference with glucose, lipid, and energy metabolism [80].

Angiotensinogen (AGT), the obligate precursor of all bioactive angiotensin peptides, undergoes 2 enzymatic cleavages by renin and angiotensin converting enzyme (ACE) to produce angiotensin I (Ang I) and angiotensin II (Ang II), respectively. Moreover, there are additional angiotensin degradation pathways such as the ACE2. Ang II as the final effector of RAAS itself has emerged as a multifunctional cytokine showing many non hemodynamic properties such as acting as a growth factor and profibrogenic and proinflammatory cytokine [81].

ACE2, a homolog of classic ACE, forms Ang (1-7) directly from Ang II and indirectly from Ang I; the ACE2/Ang (1-7)/Mas receptor axis opposes the vascular and proliferative effects of Ang II [82].

Expression of all components of the RAAS including AGT, ACE, renin, and Ang II type-1 (AT 1) and type 2- (AT2) receptors have been shown in adipose tissue of human and rodent models [4].

The activity of the RAAS appears to be regulated by food intake, and overfeeding has been reported to increase formation of Ang II in adipocytes in rodents. Plasma aldosterone levels, likely driven by elevated Ang II, are increased in obese hypertensive patients, especially in patients with excess visceral fat [5].

Clinical studies show a positive correlation between plasma aldosterone levels and body mass index. An increased prevalence of hyperaldosteronism has been associated with obesity, suggesting that aldosterone may be a mechanistic link between adiposity and cardiovascular risk factors [6].

In addition to Ang II, adipocytes secrete mineralocorticoid releasing factors, which stimulate steroidogenesis in human adrenocortical cells [83]. Adipocytes are a secondary source of aldosterone [84]. This locally produced aldosterone might have an autocrine (patho) physiological role by influencing, among other processes, adipocyte differentiation [85].

In the present work, rats fed HFFD and treated with ACE inhibitor (Captopril), AT 1 receptor blocker (Losartan), or mineralocorticoid receptors (MR) blocker (Spironolactone) demonstrated significant ($p<0.05$) decrease in the body weight gain compared to untreated rats fed HFFD. Moreover, the reduction of weight gain was more pronounced in rats treated with captopril and losartan than rats treated with spironolactone.

De kloet et al., [7] reported that captopril decreased weight gain in rats fed high or low fat chow. This was accompanied by a decrease in food intake. Rosselli et al., [86] reported that losartan treatment caused a reduction in body weight. Müller-Fielitz et al., [87] reported that chronic administration of AT1R blocker telmisartan caused marked reduction of body weight when given to already diet-induced obese rats. However, Oliveira-Junior et al., [88] reported no effect of losartan treatment on body weight in obese rats and explained the failure of losartan treatment to decrease weigh gain by the short duration of that study (5 weeks) which may has not given enough time for body weight reduction despite the higher dose of losartan used (30mg/kg body weight).

Oh et al., [89] reported that chronic intake of captopril for 4 wks decreased body weight gain and the amount of adipose tissue and increased plasma Ang-(1 -7) level. These effects were attenuated by administration of a Mas receptor blocker. These results suggest that captopril intake decreased body weight gain partly through potentiation of Ang-(1-7)/Mas receptor/PI3K pathway [90]. In line with this, peripheral administration of angiotensin II receptor blockers (ARBs) was shown to produce compensatory increase of circulating Ang II and Ang-(1-7) [91], providing a possible mechanism for reduction of body weight gain by losartan. de Kloet et al., [7] provided evidence that the peripherally-acting ACE inhibitor, captopril, that does not itself readily access the brain, increases circulating angiotensin-I that does enter the brain where it is converted to Ang-II by local ACE, and that the locally-generated Ang-II then results in hypophagia and weight loss.

Machado et al., [92] reported that spironolactone treatment decreased body weight gain in high fat fed rats. Wada et al., [93] reported that the MR blocker eplerenone ameliorated adipose tissue hypertrophy due to HFFD feeding. This finding can be attributed to the inhibition of clonal expansion, differentiation, and accumulation of triglycerides in the adipocytes and by decreased expression of the peroxisome proliferator- activated receptor-gamma due to MR blockade [94].

In the present work, rats fed HFFD and treated with ACE inhibitor (Captopril), AT1 receptor blocker (Losartan), or mineralocorticoid receptors (MR) blocker (Spironolactone) produced significant (p<0.05) decrease in insulin resistance as indicated by decreased fasting blood glucose level, insulin level and HOMA-IR index compared to rats fed HFFD. However, decreased fasting blood glucose level, insulin level and HOMA-IR index remain significantly higher compared to control group. These results indicate partial improvement of insulin resistance in HFFD rats treated by inhibitors of components of RAAS. No difference was detected between the effects of the three types of treatments on insulin resistance parameters.

In accordance to the results of the present work, angiotensin converting enzyme inhibitors have been shown by to improve insulin resistance [7,95]. AT 1 R blockers have also been reported to improve insulin resistance [96]. Many reports confirm the finding that losartan treatment improves insulin resistance in obese rats [97-99]. Oliveira-Junior et al., [88] reported that in rats with diet-induced obesity, losartan treatment resulted in minimization of disorders observed with obesity namely; glucose metabolism, insulin resistance, and systolic blood pressure. The result of the present work that spironolactone treatment in HFFD-fed rats improves insulin resistance is in accordance with previous reports [100,101].

Excessive RAAS activation occurs in conditions such as obesity and hypertension, and has been linked to the development of insulin resistance and type 2 diabetes [102-104]. Blockade of the RAAS at any of several stages leads to amelioration of insulin-resistant states in rodents characterized by RAAS over activation, including direct renin inhibition [105,106], ACE inhibition [103], Ang II type 1 receptor (AT1R) blockers (ARBs) [107], and mineralocorticoid receptor blockade [108]. In the clinical setting, ARBs may have a beneficial impact on insulin resistance and glucose dysregulation [104,109].

The mechanism whereby ACE-1 inhibitors improve glucose metabolism and protect against the development of clinical diabetes may involve at least two processes: 1- The improvement of blood flow through the microcirculation to adipose tissue and skeletal muscle and/or 2- The improvement of insulin action at the cellular level by interfering with the Ang II-induced alteration of insulin signaling [110].

Ang II negatively modulates insulin signaling by stimulating multiple serine phosphorylation events in the early components of the insulin signalling cascade [111]. However, other results in rats strongly suggest that Ang II-induced insulin resistance cannot be attributed to impairment of early insulin- signaling steps and that oxidative stress, possibly through impaired insulin signaling located downstream from PI 3-kinase activation, is involved in Ang II-induced insulin resistance [112].

Ang II stimulates increased production of superoxide radicals via activation of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase), and consequent production of reactive oxygen species (ROS) generation in skeletal muscle cells [113]. Diamond-Stanic and Henriksen [114] demonstrated, in skeletal muscle from lean Zucker rats, that Ang II caused significant inhibition of insulin-stimulated glucose transport, which partly could be reversed by the application of superoxide dismutase mimetic tempol, indicating that AngII inhibits distal insulin signaling and insulin-stimulated glucose transport in isolated mammalian skeletal muscle, and that this effect is mediated partly by ROS. Ang II activates NADPH oxidase

and increases the ROS in skeletal muscle, which inhibits phosphatidyl inositol 3-kinase (PI3K) recruitment of glucose transporter-4 (GLUT4) [115].

ACE inhibitors and angiotensin-receptor blockers lead to an increase in GLUT4 translocation into the cell membrane and improves skeletal glucose uptake in animal models [116].

Bradykinin can potentiate the insulin responsiveness of both adipocytes and muscle fibers; this effect may be mediated by a reduction in the activity of a tyrosine phosphatase that targets the insulin receptor. ACE inhibitors, in turn, increase the availability of bradykinin by suppressing its proteolytic degradation [117].

Treatment hypertensive patients with enalapril or losartan was associated with improved endothelial dysfunction correlating with increased insulin sensitivity and increased nitric oxide (NO) metabolite levels [118]. Increased NO has been reported to contribute to the favorable effects of ACE inhibition and AngII-receptor blockade on muscle glucose uptake [119]. In contrast, in another study in patients with obesity and insulin resistance, losartan treatment did not improve skeletal glucose metabolism or changes in NO production [120].

The angiotensin-receptor blocker-related improvement in insulin-stimulated glucose uptake has been documented not only in muscle and hepatic cells, but in adipose tissue as well [96].

In a rodent model of obesity and metabolic syndrome, angiotensin-receptor blocker therapy resulted in improvement of glucose intolerance that was not dependent on muscle insulin signalling, but rather was caused by improved glucose stimulated insulin secretion associated with decreased pancreatic AT1-receptor activation and increased glucagon-like peptide-1 signalling [121].

AT 1 R blockers increase insulin sensitivity not only by blocking Ang II actions, but other mechanisms also exist. Some ARBs, such as telmisartan, have weak but significant peroxisome proliferator-activated receptor (PPAR) ψ agonistic activity, as they enhance adipose differentiation of 3T3-L1 cells [122]. However, PPARψ agonists also have an anti-inflammatory role, as shown by their inhibitory effects on the production of inflammatory cytokines such as TNF-α, in turn promoting the production of adiponectin and thereby normalizing obesity-related insulin resistance [123]. However, Müller-Fielitz et al., [124] demonstrated that despite the PPARψ agonist effect of relatively short term treatment with telmisartan, chronic treatment with the drug improved insulin resistance by a mechanism which is not PPARψ-dependent. Valsartan has been shown to increase insulin sensitivity by suppressing the inflammatory response of macrophages and thereby restore impaired insulin signalling and reduce abnormal gene expressions of adipocytes. This action of Valsartan occurs independent of AT1R or PPAR agonist action [125].

Clinical use of ACE inhibitors and ARBs show conflicting results regarding their effect on insulin resistance. ACE inhibitors [126] and ARBs [127] have been shown to improve insulin resistance in obese hypertensive patients. However, other reports did not find beneficial effect of ARBs on insulin resistance [128].

Plasma aldosterone levels predicted the development of insulin resistance in the general population [129] Hannemann et al., [130] documented an association of plasma aldosterone with the metabolic syndrome.

Increased aldosterone secretion is observed in high fat-fed rats [131]. Mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) are present in adipocytes, whereby aldosterone influences their function [132]. Accumulating evidences reveal the existence of a vital role of aldosterone in the development of insulin resistance. In rats, excess aldosterone has an adverse effect on glucose uptake in liver and skeletal muscle [133] and on skeletal muscle insulin metabolic signaling [108]. Hitomi et al., [134] reported that aldosterone attenuates glucose metabolism in vascular smooth muscle cells. Sowers et al., [135] reported that increased plasma aldosterone levels are associated with insulin resistance independent of other components of the metabolic syndrome.

Selvaraj et al., [136] demonstrated that aldosterone treatment impaired the rate of glucose uptake, oxidation, and insulin signal transduction in the gastrocnemius muscle through defective expression of IR, IRS-1, Akt, and GLUT4 genes. Phosphorylation of IRS-1 and Akt was also reduced in a dose-dependent manner. Aldosterone induces oxidative damage and causes gene repression of adiponectin and peroxisome proliferator-activated receptor-ψ (PPARψ) [100-101]. Urbanet et al., [137] observed no effect of physiological or supra physiological concentrations of aldosterone on insulin sensitivity in visceral adipose tissue, whereas pharmacologic concentration of aldosterone decreased insulin sensitivity through a glucocorticoid receptor-dependent but mineralocorticoid receptor-independent mechanism.

Wada et al., [9] hypothesized that administration of MR antagonist might ameliorate hepatic insulin resistance, hepatic steatosis, and systemic glucose and lipid metabolism by suppressing hepatic inflammation and ROS production induced by excess ingestion of the dietary nutrient.

Results of the present work demonstrated the ability of ACE inhibitor (Captopril), AT1 receptor blocker (Losartan), and MR blocker (Spironolactone) to significantly decrease liver weight, % liver/weight body weight ratio, and the development of hepatic steatohepatitis and fibrosis in HFFD-fed rats. This work also points out, by demonstrating a decrease in gene expression of proinflammatory cytokines TNF-a and IL-6 in hepatic tissue, to causal possible link to suppression of local inflammation in hepatic tissue. Moreover, systemic improvement of insulin resistance by these drug interventions with consequent reduction of gene expression of the transcriptional factor SREBP-1c in the liver shown in the present work could be an additional mechanism.

Activated hepatic stellate cells express renin and ACE and synthesize Ang II. Ang II activates HSC, promoting their differentiation into myofibroblasts and stimulates cellular proliferation contraction [138]. Moreover, Ang II up-regulates tissue inhibitor of metalloproteinases- 1 (TIMP-1) mRNA expression and increases collagen and protein deposition in the extracellular matrix. The profibrogenic effect of Ang II is also mediated via NADPH oxidase, which produces ROS. AT 1 R activation also increases the expression of vascular endothelial growth factor, promoting neoangiogenesis. Ang II also exerts proinflammatory effects by up-regulating the synthesis of the pro-inflammatory cytokines IL-1 and IL-6 and the expression of the transcription factor nuclear factor kappa B. In addition, Ang II stimulates the production of growth factors including transforming growth factor (TGF)-ß1 and connective tissue growth factor. Moreover, Ang II stimulates cell migration and concentration of activated HSCs at the site of hepatic injury [139-141].

Toblli et al., [142] studied the effect of perindopril (ACE inhibitor) and irbesartan (AT1 blocker) on steatohepatitis and fibrosis in obese Zucker rats. Both drugs resulted in decreased production of TNF-a, IL-6 and transforming growth factor-beta 1 with improvement of hepatic steatosis. Zhang et al., [143] also showed that ACE inhibitor fosinopril significantly reduced the degree of hepatic steatosis, serum fasting glucose, insulin, TNF-a and IL-6 concentrations and hepatic TNF-a and IL-6 mRNA expression.

Candesartan (AT1 blocker)-treated obese mice with type 2 diabetes mellitus demonstrated, in agreement with our findings using losartan, significantly lower liver weights and reduced lipid droplets in hepatic cells compared with control mice. The circulating adiponectin levels and hepatic expression of adiponectin mRNA were significantly higher in candesartan-treated mice than control mice [144].

Frantz et al., [145] compared the effect of aliskiren (renin antagonist), enalapril (ACE inhibitor) and losartan on the development of hepatic steatosis in rats fed with high fat diet. Only enalapril was able to improve glucose intolerance, insulin resistance and hepatic steatosis. Enalapril treatment was associated with up regulating signal transduction through the insulin receptor substrate (IRS) 1/protein kinase B (Akt) pathway, as well as down regulating the protein levels and mRNA expression of sterol regulatory element-binding protein-1c (SREBP-1c), and fatty acid synthase [145]. Failure of renin inhibition in this study may point out to the possibility that Ang 1-7/Mas receptor pathway may be involved. Inhibition of ACE is known to increase Ang 1-7 level [146]. Angiotensin (1-7) has been shown to possess actions which generally antagonize those of Ang II through AT 1 R (vasodilatation, anti-proliferative, anti-inflammatory, and anti-fibrotic actions) [147]. It may be speculated that renin inhibition, by inhibiting the generation of angiotensin I, would decrease the production of both Ang II and Ang 1-7 by decreasing their precursor peptide. However, ACE inhibition will decrease AngII and potentiate the Ang 1-7/Mas receptor mediated actions. However, this argument cannot explain failure of losartan to affect the development of NASH in this study in view of the similar increase in Ang 1-7 in response to AT 1 R blocker treatment [148].

Telmisartan (AT 1R blocker) has been reported to improve dietary-induced obesity, insulin resistance and fatty liver in mice by a mechanism not involving its action on AT 1 R [149]. Telmisartan has been shown to possess partial PPAR-gamma agonist effect [122]. Telmisartan showed a potential action to improve non alcoholic steatohepatitis in Wistar rats, possibly due to increased hepatocyte growth factor production through its partial PPAR-gamma agonist action [150]. Telmisartan has also been shown to reduce steatohepatitis by inhibiting hepatic macrophage infiltration in mice [151].

AT1R, which is localized in hepatocytes, bile duct cells, hepatic stellate cells, myofibroblasts, Kupffer cells and vascular endothelial cells, mediates most of the actions of Ang II in the liver [139].

However, studies have reported AT2R gene expression in liver tissue [152], suggesting that AT2R might have anti-fibrogenic effects in the liver [153]. Thus, a possibility also exists that AT 1 R blockers by attenuating AT 1 R-mediated actions of Ang II will at the same time exaggerate AT2R-mediated effects in the liver.

Paschalis and Konstantinos [154] reported that there are no studies that evaluated the effects of ACE inhibitors in patients with NAFLD or nonalcoholic steatohepatitis (NASH). However, regarding angiotensin receptor blockers, some clinical trials exploring the potential benefit of these blockers in NASH patients have been published which show discrepancy in results.

A study in 12 patients with NASH showed that losartan (50mg/d) can improve biochemical parameters, liver steatosis and inflammation but had no effect on fibrosis [155]. In a larger study, 54 hypertensive patients with NASH were randomly assigned to either telmisartan (20mg/d) or valsartan (80mg/d). Both ARBs reduced transaminase levels and improved insulin resistance but this improvement was more profound in the telmisartan group, which also showed a significant decrease of NASH activity score and fibrosis. Valsartan did not improve liver histology except steatosis [156]. The difference between the two drugs may be attributed to PPAR-y-activating properties of telmisartan [157].

Mineralocorticoid receptor (MR) blockers have also been investigated in treatment of steatohepatitis and fibrosis. Clinical observations point out to a link between NAFLD and aldosterone level. Fallo et al., [158] reported an increased incidence of NAFLD and insulin resistance in patients with primary aldosteronism. The results of the present work demonstrated that spironolactone can attenuate the development of NASH and fibrosis in HFFD-fed rats. These results are supported by the work of Wada et al., [9] who found that treatment with spironolactone effectively ameliorated impaired glucose and lipid metabolism, reduced epididymal fat weight, and improved histological changes of fatty liver in HFFD mice. Thus, an MR antagonist might be a beneficial novel option for the treatment of patients with diet-induced metabolic syndrome and/or NAFLD, in addition to its wide usage as an antihypertensive agent and diuretic.

Wada et al., [93] reported that in a mouse model reflecting metabolic syndrome in humans, treatment with MR blocker eplerenone significantly ameliorated the histological picture of steatosis and fibrosis observed in the liver of the HFFD-fed animals. These findings were also accompanied by reduction of body and epididymal fat weight gain, reduction of blood pressure and amelioration of dyslipidemia. These results indicated that blocking aldosterone action by eplerenone inhibited the development of NASH and metabolic abnormalities in mice by inhibiting inflammatory responses in both Kupffer cells and macrophages [93]. These findings agree with findings in the present work that spironolactone treatment suppresses hepatic steatosis and fibrosis and decreased the gene expression of TNF-a and IL6 in the liver. In addition, the present work shows that spironolactone treatment in HFFD-fed rats resulted in decreased expression of SREBP-1c in the liver, another mechanism explaining the observed decrease in steatosis and fibrosis. Wada et al., [9] also reported that, in C57BL/6 mice receiving HFFD, spironolactone inhibited fat accumulation in hepatocytes and down regulated the hepatic mRNA expression of proinflammatory cytokines (TNF-(x, IL-6, and monocyte chemo attractant protein-1). These data also agree with the results of the present work. Noguchi et al., [159] reported that eplerenone, a selective aldosterone receptor antagonist, attenuated the pro-gression of liver fibrosis in a rat model with NASH, possibly through the suppression of the activated hepatic stellate cells and neovascularisation.

Other investigators have reported results against an effect of MR blockers on NASH and fibrosis. Gamliel-Lazarovich et al., [160] reported that in male C57BL/6 mice fed with a 60% fat diet for 20 weeks, eplerenone did not affect fatty liver formation. The reason for the discrepancy between the results of Gamliel-Lazarovich et al., [160] and those of Wada et al., [93], and Wada et al., [9] is not clear since in these three studies the same species of mice (C57BL/6) was used. However, Wada et al., [93] and Wada et al., [9] induced NAFLD using both high fat and high fructose diet; whereas Gamliel-Lazarovich et al., [160] used high fat only diet. Whether the difference in diet used to induce NAFLD can explain this discrepancy is not clear.

Summary and Conclusion:

High fat fructose diet participates in the development of obesity, insulin resistance, hepatic steatosis, steatohepatitis, and even hepatic fibrosis. Interference with RAAS system by inhibiting angiotensin converting enzyme, blocking angiotensin II type 1 receptor, or blocking aldosterone receptor can partially improve HFFD induced changes. In conclusion, the established role of both circulating and local RAAS on the pathogenesis of obesity induced insulin resistance and NAFLD, created

considerable interest on the effect of RAAS inhibitors since they are widely used, reasonably inexpensive, and with excellent safety profile. Despite the encouraging evidence from animal studies, data from human studies are limited and contradictory and most data are from retrospective studies. Accordingly, more and larger studies are needed to directly assess the effectiveness of ACE inhibitor, ARBs, and mineralocorticoid receptors blockers.

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