



## Liver, Pancreas and Biliary Tract

# Epigenomic derangement of hepatic glucose metabolism by feeding of high fructose diet and its prevention by Rosiglitazone in rats

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## Abstract

**Background.** The high consumption of fructose leads to the increasing incidence of insulin resistance by several unknown mechanisms. Hepatic glucose metabolism may also be an important target of fructose-induced-metabolic alterations.

**Aim.** The aim of present study was to investigate alterations in hepatic glycogenolysis, glycogenesis and gluconeogenic fluxes by feeding of 21% high fructose diet and the effects of Rosiglitazone treatment to prevent these derangements in rats.

**Methods.** Rats were maintained on normal chow and high fructose diet with or without Rosiglitazone for 8 weeks and various biochemical and gene expression measures were estimated.

**Results.** The feeding of high fructose diet impaired glucose, insulin and pyruvate tolerance tests and increased blood HbA<sub>1c</sub>, insulin, triglyceride, free fatty acids and homeostasis model assessment after 8 weeks. In addition, high fructose diet feeding increased expression of phosphoenol-pyruvatecarboxykinase, glucose-6-phosphatase, sterol regulatory element binding proteins-1 and fatty acid synthase through enhanced expression of fork-head receptor, peroxisome proliferator activated receptor-γ-co-activator 1 and cAMP reactive element binding protein. The treatment with Rosiglitazone inhibited all these derangements, i.e. hepato-lipogenic and gluconeogenic effects of high fructose diet feeding in rats.

**Conclusions.** Together these findings suggest that high fructose diet induced hepatic gluconeogenic and lipogenic rate, and increased circulating triglycerides and free fatty acids, which may be the major risk factors for glucose intolerance, hyperglycemia and insulin resistance in rats. In such situations high fructose flux also induces transcriptional cascade of gluconeogenic enzymes through the modulation of various associated transcriptional factors.

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**Keywords:** Gluconeogenesis; High fructose diet; Hepatic steatosis; Insulin resistance; NAFLD; Rosiglitazone; Type 2 diabetes

## 1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver function tests among adults in the developed and developing world [1]. Obesity, type

2 (non-insulin-dependent) diabetes mellitus, and hyperlipidemia are coexisting conditions frequently associated with NAFLD [2]. Dramatic increase in the prevalence of obesity, type 2 diabetes and associated NAFLD strongly suggest the role of life style and dietary factors in disease pathogenesis [3,4]. Due to these reasons, it is taken seriously as a clinical target for the prevention of these health threats. The increase in consumption of high calorie diets, specifically through refined carbohydrates and/or fructose positively correlates with an alarming increase in obesity, type 2 diabetes and NAFLD [5,6]. The feeding of a high fructose diet (HFD) induces metabolic derangements such as hyperinsulinemia

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with glucose and insulin intolerance, dyslipidemia, hypertension and endothelial dysfunction in humans and rats [7]. The precise molecular mechanisms through which diet high in fructose induces the abnormalities in liver carbohydrate metabolism are not fully understood. The liver plays a central role in regulation of glucose metabolism and maintenance of blood glucose homeostasis by regulating glycogen storage and breakdown as well as the synthesis of glucose from lactate, amino acids, and glycerol (gluconeogenesis). The rate of gluconeogenesis is controlled principally by the activities of certain unidirectional enzymes such as PEPCK, FBPase and G-6-Pase. The genes encoding these proteins are powerfully controlled at the transcriptional level by key hormones particularly insulin, glucagon and glucocorticoids [8], mediated by various transcriptional factors and co-activators, i.e. peroxisome proliferator activated receptor- $\gamma$  (PPAR- $\gamma$ ), PPAR- $\gamma$ -co-activator 1 (PGC-1), etc. [9]. In obesity and type 2 diabetes, the rate of the gluconeogenesis pathway failed to be regulated by insulin due to insulin resistance; this results in decreased utilisation of glucose in liver and muscles, and accumulation in the form of glycogen and/or utilisation of energy sources, which lead to increased blood glucose (hyperglycemia) [10].

The recent developments of thiazolidinediones (TZDs) as insulin sensitisers present a new line of therapy for treatment of type 2 diabetes. TZDs enhance insulin action and improve glycemic control by increasing peripheral glucose disposal and reducing hepatic glucose output through activation of PPAR- $\gamma$  [11]. The exact mechanisms by which these agents exert their antidiabetic effects on the liver are still obscure. The aim of this study was to investigate the biochemical steps responsible for enhanced gluconeogenesis in liver as a result of increased dietary fructose intake and site(s) at which Rosiglitazone acts to inhibit these processes.

## 2. Materials and methods

### 2.1. Animals and feeding schedule

Male albino Wistar rats (160–200 g) were housed (3 animals per cage) at 20–25 °C room temperature and 12/12 light/dark cycle in the small animal house of the institute. Animals were grouped ( $n = 6$ ) according to diet and Rosiglitazone administration: (1) *normal control group (NCG)*; fed with standard chow (Table 1), (2) *high fructose fed control group (HFCCG)*; fed with high fructose diet (HFD; 21%), and (3) *high fructose fed and Rosiglitazone treated group (HFRG)*; fed with HFD and Rosiglitazone administered with drinking water at a dose of 10 mg/kg body weight and maintained for 8 weeks. The dietary intake and body weight were recorded on alternate day during the experimental period. The study protocols were approved by the Animal Ethics Committee of the institute and animals were maintained as per the rules and regulations for the care of small animals as sug-

Table 1

Composition of standard (STD) and high fructose diet (HFD) given to the rats.

Constituent	STD	HFD
Ingredients (g/kg)		
Wheat starch	211	181
Bengal gram	415	385
Groundnut cake	195	100
Refined oil	67	40
Casein	68	40
Salt mixture (USP XIX) <sup>a</sup>	40	40
Vitamin mixture <sup>a</sup>	2	2
Choline chloride mixture <sup>a</sup>	2	2
Fructose <sup>b</sup>	–	210
Nutrient and energy composition <sup>c</sup>		
Carbohydrates	594.87	586.84
Proteins <sup>d</sup>	166.09	152.23
Fats	63.98	54.23
Metabolisable energy (kJ/g)	15.90	15.57

<sup>a</sup> Prepared and mixed according to AOAC [35].

<sup>b</sup> From Japan Fructose India (P) Ltd., Bangalore India.

<sup>c</sup> Calculated from food tables and excel based developed programme [36].

<sup>d</sup> Determined by macro-Kjeldahl method [35].

gested by National Institute of Nutrition, Hyderabad, India and CPCSEA [12].

### 2.2. Oral glucose and intravenous insulin and pyruvate tolerance tests

After a 12 h fast, 2 g/kg body weight glucose solution was administered orally and blood samples were collected via a tail nick at 0, 15, 30, 60, 90 and 120 min after glucose administration. The blood glucose was monitored using glucometer (Roche Diagnostics Pvt. Ltd., Mumbai, India). Insulin tolerance test (ITT) was performed in 5 h fasted rats and by injecting 0.75 U/kg body weight and blood glucose measured at 0, 15, 30, 60, 90 and 120 min after glucose injection. Similarly, pyruvate tolerance test (PTT) was also performed on 12 h fasted rats by injecting 2 g/kg body weight and blood glucose was monitored at the above time intervals.

### 2.3. Blood and tissue collection

At the end of the experiment, blood samples were collected by puncture of the venous orbital plexus in heparinised (2 U/ $\mu$ l) vials. Fifty microlitre whole blood samples were used for the determination of glycosylated haemoglobin (HbA<sub>1c</sub>) and remaining samples were centrifuged at 4000  $\times$  g for 10 min at 4 °C and plasma was used for determination of triglycerides and insulin. The animals were killed by cervical dislocation and liver was excised, powdered with liquid nitrogen and stored at –80 °C.

### 2.4. Biochemical assays

HbA<sub>1c</sub> and plasma triglyceride were determined by commercial kits procured from Monozyme Pvt. Ltd., Secun-

derabad, India and Bayers Diagnostic India Pvt. Ltd., Ahamdabad, India, respectively. The plasma insulin was analysed using Mercodia rat insulin ELISA kit (OSB Agencies Pvt. Ltd., Mumbai, India). Homeostasis model assessment (HOMA) was calculated by the formula:  $[(\text{insulin } (\mu\text{U/ml}) \times \text{glucose (mmol/l)})/22.5]$  which is used as an index to measure the degree of insulin resistance. The liver glycogen content was determined as per the method described by Vander-Vries [13] and blood free fatty acids (FFAs) were

measured as method described elsewhere [14]. Liver fat was extracted by method of Folch et al. [15] and hepatic triglycerides were measured as above.

### 2.5. Morphological analysis

Rat liver tissues were fixed in 10% neutral buffered formalin, processed into paraffin blocks, sectioned at 6  $\mu\text{m}$ , and stained with hematoxylin and eosin. Stained sections

Table 2  
PCR primers used for determination of gene expression.

Gene	Primers	Accession no.	Product size
Glycogen synthase			
Forward	5'-TTACACCCAGAATTCCTGTC-3'	J05446	364
Reverse	5'-TCCAGTCCAGAAGATCTGAG-3'		
Glycogen phosphorylase			
Forward	5'-GAAGCAGGAGTACTTTGTGG-3'	BC070901	389
Reverse	5'-CGATGTCTTTAGGAAACAGG-3'		
Glucokinase			
Forward	5'-CCAGTCTACTGTGGAGAGTC-3'	BC079449	302
Reverse	5'-AGAGTTCCTTATCCCATCC-3'		
PEPCK			
Forward	5'-ACAAAGAGTGGAGACCACAG-3'	NM_198780	318
Reverse	5'-GGTACTTGCCGAAGTTGTAG-3'	XM_342593	
FBPase			
Forward	5'-TAATGAGGGCTATGCTAAGG-3'	J04112	321
Reverse	5'-ATGATAACTGGTGCCTCTCG-3'		
G-6-Pase			
Forward	5'-TATGTCCTCTTTCCCATCTG-3'	NM_013098	306
Reverse	5'-CGTTGACTTTTTCTTTCCAC-3'		
PPAR- $\gamma$			
Forward	5'-GGCAAATCTCTGTTTTATGC-3'	NM_013124	338
Reverse	5'-GCACTTTGGTATTCTTGGAG-3'		
HNF-4 $\alpha$			
Forward	5'-CGGATGTGTGTGAGTCTATG-3'	NM_022180	309
Reverse	5'-AGGCGTATTCATTATCATCG-3'		
FKHR			
Forward	5'-ACAATCTGTCCCTACACAGC-3'	AF247812	343
Reverse	5'-GAAAGTCTCCACTGATGG-3'		
PGC-1			
Forward	5'-AGCAGAAAGCAATGAAGAG-3'	AY237127	335
Reverse	5'-ATACTTGCTCTTGGTGGAAG-3'		
CREB			
Forward	5'-AACCAAGTTGTGTTCAGC-3'	NM_134443	308
Reverse	5'-GACTTGTGGCAGTAAAGGTC-3'		
SREBP-1			
Forward	5'-ACCGTTCCTCTATCAATGACAA-3'	L16995	183
Reverse	5'-TGATTTGCTTTTGTGAGCACTT-3'		
FAS			
Forward	5'-GATCGGCAAATTTGATCTTTCT-3'	NM_017332	250
	5'-TTTGCCAATATGTTTCTTGA-3'		
G3PDH			
Forward	5'-GACCCCTTCATTGACCTC-3'	DQ403053	206
Reverse	5'-GTGAAGACGCCAGTAGACT-3'		

The primers for respective genes were designed with the help of primer 3 software (<http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.www.cgi>) by using the sequences from NCBI database (<http://www.ncbi.nlm.nih.gov/entrez>).

were examined by light microscopy (Olympus BX41). Photographs were taken with an Olympus DP12 digital camera, using the 203 oil immersion lens.

## 2.6. Semi-quantitative RT-PCR

The total RNA was isolated from liver tissues using Trizol reagent (Sigma Chemicals Co. USA) according to the instructions of the manufacturer. Analysis of gene expression of hepatic carbohydrate metabolic enzymes and associated transcription factors was performed using semi-quantitative RT-PCR [16]. The PCR primers were synthesised at Integrated DNA Technology, USA and PCR reactions were performed in T-1 thermocycler (Analytik GmbH, Germany) (Table 2).

For RT-PCR, first strand cDNA was synthesised from 2 µg of total RNA in 20 µl volume using random hexamer and M-MuLV reverse transcriptase (GeneI, Bangalore, India). Reverse transcription reaction mixture (2 µl) was amplified with primers specific for rat PEPCK, fructose-bis-phosphatase (FBPase), G-6-Pase, glucokinase, glycogen synthase, glycogen phosphorylase, PPAR-γ, hepatic nuclear factor-4α (HNF-4α), fork-head receptor (FKHR), PPAR-γ-co-activator-1 (PGC-1), cAMP reactive element binding protein (CREB), sterol regulatory element binding proteins-1 (SREBP-1), fatty acid synthase (FAS) and glyceraldehydes-3-phosphate dehydrogenase (G3PDH) in a total volume of 50 µl starting with a 5-min incubation at 95 °C, followed by a three-step temperature cycling (1 min at 95 °C, 1 min at 52 °C, and 3 min at 72 °C) that was terminated by a 72 °C incubation for 10 min, and then cooling at 4 °C. The cycles were repeated for an optimised number for each transcript (27, 32, 33, 32, 30, 35, 28, 25, 32, 31, 32, 32 and 34 time for PEPCK, FBPase, G-6-Pase, glucokinase, glycogen synthase and glycogen phosphorylase, PPAR-γ, HNF-4α, FKHR, PGC-1, CREB, SREBP-1 and FAS, respectively). G3PDH was used as an internal control for quality and quantity of RNA. The PCR products were subjected to elec-

trophoresis on 1.5% agarose gel and quantified using Gel Quant software (Labnet International Inc., Woodbridge, NJ). Results are presented of triplicate measurements of pooled samples.

## 2.7. Western blotting analyses

Tissue extracts were prepared by homogenisation in the lysis buffer containing 100 mM Tris buffer (pH 8.5), 250 mM NaCl, 1% NP-40, 1 mM EDTA, protease inhibitors and 0.1% phenyl methyl sulfonyl fluoride with a polytron homogeniser following centrifugation at 14,000 × g for 10 min to remove cell debris. Total protein was estimated by Lowry method [17]. Homogenates containing equal amount of total protein from all animals of each group were pooled. Proteins were separated using SDS-PAGE by loading of 20 µg/lane and transferred on nitrocellulose membrane. Non-specific binding sites were blocked by incubating with 5% non-fat milk in TBST buffer (20 mM Tris, 55 mM NaCl and 0.1% Tween 20) for 1 h. The PEPCK, FBPase, G-6-Pase, PGC-1 and CREB were probed with polyclonal antibodies (Santa Cruz Biotechnology, USA) and bands were visualised by using development kit (GeneI Bangalore, India).

## 2.8. Statistical analyses

All the results expressed as means ± standard deviation (S.D.). The data was subjected to analysis of variance and significant differences ( $p < 0.05$ ) were analysed by Bonferroni's test using SPSS (version 10.0).

## 3. Results

### 3.1. Effect on feed intake and body weight

Table 3 depicted that the daily food and energy intake by rats fed with different diets were not altered by devia-

Table 3

Physiological and biochemical parameters in NCG, HFCG and HFRG rats during and/or after 8 weeks of the experimental period\*.

Parameters	NCG	HFCG	HFRG
Food intake (g/rat/day)	14.98 ± 5.39 <sup>a</sup>	15.12 ± 3.57 <sup>a</sup>	15.17 ± 4.22 <sup>a</sup>
Daily energy intake (kJ/rat)	264.5 ± 1.4 <sup>a</sup>	307.5 ± 5.2 <sup>b</sup>	309.3 ± 3.6 <sup>b</sup>
Water intake (ml/rat/day)	72.2 ± 4.2 <sup>a</sup>	87.3 ± 5.0 <sup>b</sup>	85.3 ± 4.8 <sup>b</sup>
Urine volume (ml/rat/day)	34.59 ± 2.49 <sup>a</sup>	39.68 ± 5.68 <sup>b</sup>	38.22 ± 4.01 <sup>b</sup>
Body weight gain (g)	22.4 ± 5.3 <sup>a</sup>	31.3 ± 4.11 <sup>b</sup>	33.2 ± 6.2 <sup>b</sup>
Liver weight (g)**	10.01 ± 1.78 <sup>a</sup>	10.59 ± 2.01 <sup>a</sup>	9.77 ± 1.56 <sup>a</sup>
Blood glucose (mmol/l)	4.5 ± 0.21 <sup>a</sup>	11.2 ± 2.21 <sup>b</sup>	6.31 ± 0.30 <sup>c</sup>
Plasma insulin (pmol/l)	237.3 ± 32.12 <sup>a</sup>	457.5 ± 56.72 <sup>b</sup>	333.4 ± 32.4 <sup>c</sup>
HbA <sub>1c</sub> (%)	3.49 ± 0.77 <sup>a</sup>	6.31 ± 0.83 <sup>b</sup>	4.11 ± 0.32 <sup>c</sup>
Triglyceride (mg/dl)	57.80 ± 3.48 <sup>a</sup>	103.82 ± 5.38 <sup>b</sup>	68.48 ± 3.37 <sup>c</sup>
Free fatty acids (mg/dl)	28.23 ± 2.74 <sup>a</sup>	65.74 ± 4.31 <sup>b</sup>	39.24 ± 2.37 <sup>c</sup>
HOMA***	40.4 ± 3.48 <sup>a</sup>	230.2 ± 28.32 <sup>b</sup>	110.2 ± 12.3 <sup>c</sup>

Values with different superscripts (a, b, and c) in a row are significantly different ( $p < 0.05$ ).

\* Values are means ± S.D. of 6 animals in each group.

\*\* Values are per 100 g body weight.

\*\*\* HOMA = (insulin (µU/ml) × glucose (mmol/l))/22.5.

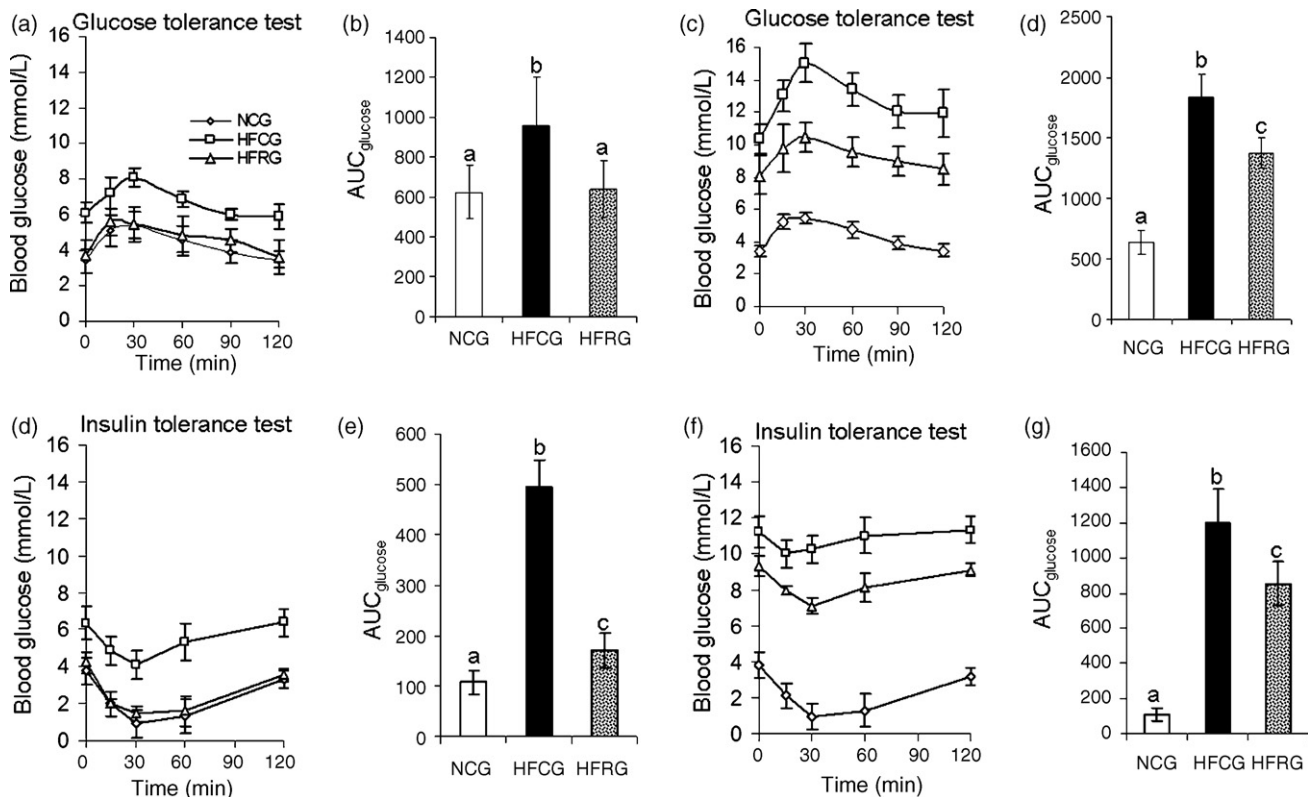


Fig. 1. The oral glucose and intravenous insulin tolerance tests in NCG, HFCG and HFRG rats after 4 weeks (a, b, e, and f) and 8 weeks (c, d, g, and h). Rats ( $n=6$  in each group) were administered 2 g/kg body weight of glucose for OGTT and 0.75 U/kg body weight of insulin for ITT, and blood glucose levels were examined at 0, 15, 30, 60, 90 and 120 min. 'a, b, c' values (mean  $\pm$  S.D.) with different superscript were statistically significant ( $p < 0.05$ ).

tions in diet, however water intake and excreted urine volume was significantly higher in HFCG rats than NCG and HFRG ( $p < 0.05$ ), which are typical characteristics of human diabetes [18]. The body weight gain was higher in HFCG than NCG animals, which was further highest in HFRG animals. On the other hand, no significant differences were observed in liver weight among different group of animals.

### 3.2. Effects on insulin resistance measures

Feeding of HFD induced partial insulin resistance characterised by impaired glucose and insulin tolerance tests after 4 weeks and completely developed insulin resistance after 8 weeks (Fig. 1). Rosiglitazone treatment fully prevented the induction of insulin resistance for up to 4 weeks (Fig. 1a, b, e, and f), and after 8 weeks rats treated with Rosiglitazone became insulin resistant, but it was still less than in the HFD-fed control group (Fig. 1c, d, g, and h). After 8 weeks of HFD-feeding rats became diabetic and presented with increased blood glucose, HbA<sub>1c</sub>, plasma insulin, triglycerides, hepatic triglyceride and FFAs (59, 34, 38, 48, 44 and 57%, respectively) compared to normal rats ( $p < 0.05$ ). Rosiglitazone treatment restrained these metabolic derangements induced by HFD feeding (Table 3). The blood glucose was significantly lower in rosiglitazone-treated rats (43%,  $p < 0.05$ ) when compared with HFCG. Similarly, plasma

insulin, triglycerides and FFAs were also lower in HFRG than HFCG (27, 34, 33 and 40%, respectively;  $p < 0.05$ ). In addition, rosiglitazone treatment also consistently restrained the elevation of HOMA, which was 52% lower than HFCG.

### 3.3. Effects on hepatic glycogen metabolism

The hepatic glycogen was significantly higher (23%) in HFCG than NCG ( $p < 0.05$ ). However, compared to HFCG, rosiglitazone significantly reduced hepatic glycogen content, but could not bring it to normal levels (Fig. 2a). In addition, no significant changes in the mRNA levels of glycogen synthase, glycogen phosphorylase and glucokinase by HFD feeding and rosiglitazone treatment in hepatic tissues of rats (Fig. 2b).

### 3.4. Effects on hepatic gluconeogenesis

We also measured hepatic endogenous glucose production (EGP) in terms of PTT, and results clearly indicate that feeding of HFD impaired pyruvate-responsive hepatic glucose production, and in response to similar pyruvate load, blood glucose levels increased more in HFD than in controls; this is compatible with increased hepatic transformation of pyruvate into glucose through gluconeogenesis. Rosiglitazone treatment prevented the induction of impairment in PTT (Fig. 3a and b). Additionally, we found that the expression of rate-

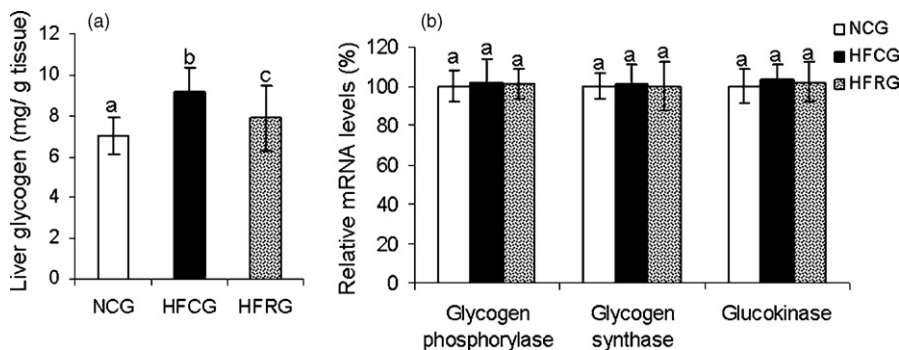


Fig. 2. Effects of HFD and Rosiglitazone treatment on glycogen metabolism in rats. (a) Hepatic glycogen levels and (b) mRNA levels of enzymes involved in glycogen metabolism in liver. The total RNA was isolated from liver tissues collected from 6 animals of each group. cDNA was prepared for amplification with specific primers to the corresponding genes with housekeeping gene (G3PDH). The PCR products visualised by running on 1.5% agarose gel in the presence of ethidium bromide and band densities were quantified and expressed as relative amount (%) as compared with NCG. The results represent means  $\pm$  S.D. of triplicate measurements of pooled samples. Data are expressed as the mean ( $n=3$ ) and 'a' values with similar superscript are not statistically different ( $p < 0.05$ ).

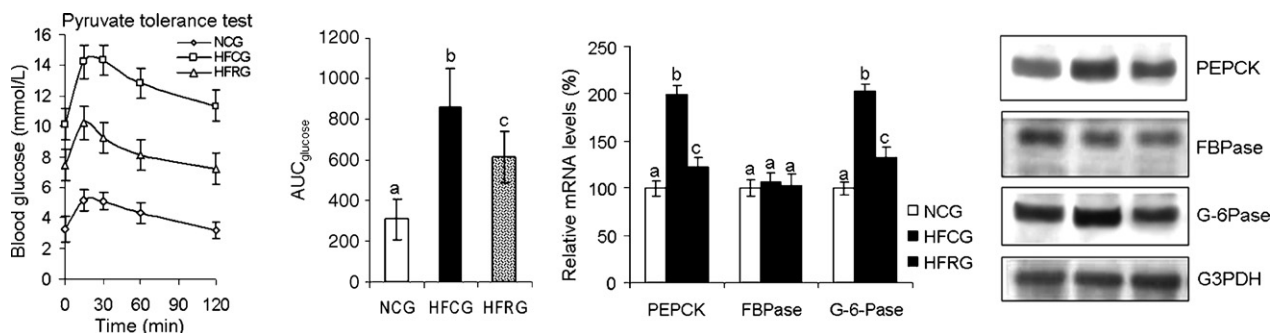


Fig. 3. Effects of HFD and Rosiglitazone on gluconeogenesis in hepatic tissues of rats. Rats ( $n=6$  each group) were administered with 2 g/kg body weight of pyruvate solution and blood glucose levels were examined at 0, 15, 30, 60, 90 and 120 min (a). The RNA analysis was performed as described in Fig. 2 (b). For western blotting (c), the tissue homogenate was prepared in Tris buffer and pooled from 6 animals of each group. Proteins were separated by running on 10% SDS-PAGE by loading 20  $\mu$ g/lane. The protein bands were transferred on nitrocellulose membrane and targeted by specific antibodies and visualised by western blot development kit. The results represent means  $\pm$  S.D. of triplicate measurements. Data are expressed as the mean  $\pm$  S.D. and 'a, b, c' values with different superscript are statistically different ( $p < 0.05$ ).

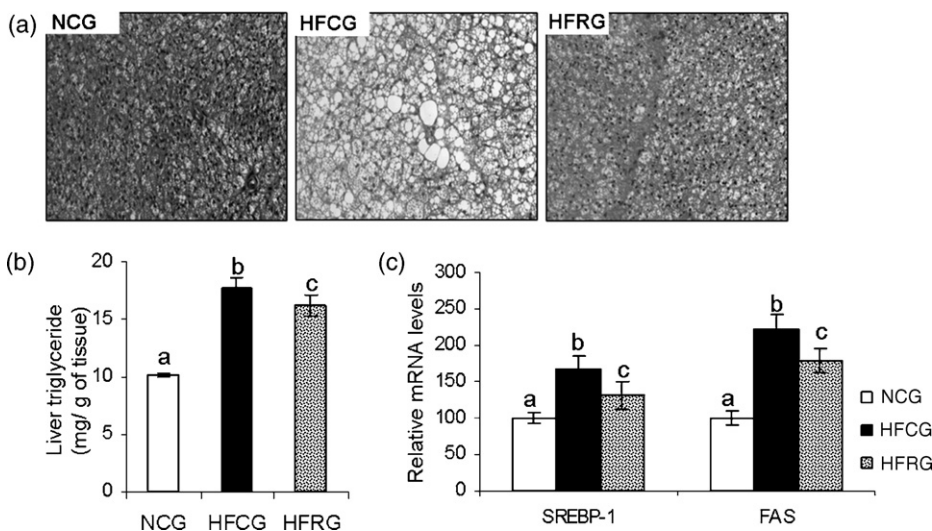


Fig. 4. Effects of HFD and Rosiglitazone treatment on hepatic steatosis in rats. Hematoxyline and Eosin staining of liver sections (a), hepatic triglyceride content (b) and mRNA levels of lipogenic factors (c) in liver. The analysis of morphological characteristics, hepatic triglyceride content and mRNA levels were described in the text.

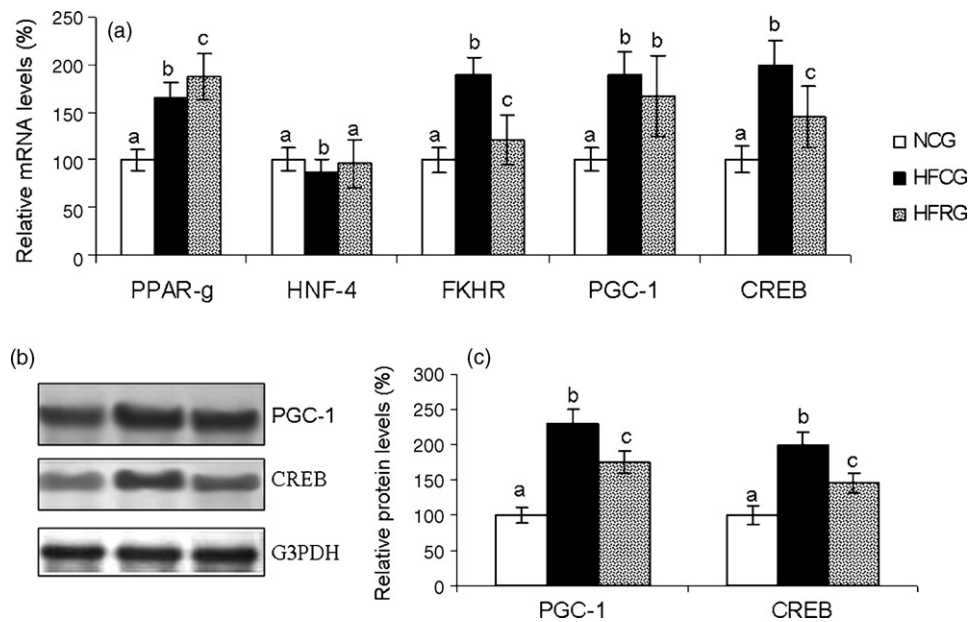


Fig. 5. Effects of HFD and rosiglitazone treatment various transcriptional factors liver tissues of rats. The mRNA (a) and protein (b and c) were analysed as per the method described in text of materials and methods.

limiting enzymes, i.e. PEPCK and G-6-Pase was significantly increased by HFD feeding, while expression of FBPase was not significantly changed (Fig. 3c and d). However, rosiglitazone treatment significantly depleted the elevation of PEPCK and G-6-Pase mRNA and protein levels in hepatic tissues of HFRG animals compared to HFCG (Fig. 3c and d).

### 3.5. Effects on hepatic steatosis

The histological analysis of hepatic tissues clearly indicated that the feeding of HFD significantly increased ectopic fat deposition in the liver (hepatic steatosis), while rosiglitazone significantly ameliorated the ectopic fat deposition in liver tissues of rats (Fig. 4a). This was further confirmed by the increased triglyceride content in HFD fed rats and decreased triglyceride levels in hepatic tissues (Fig. 4b). Moreover, rosiglitazone significantly suppressed HFD-induced increased expression of SREBP-1 and FAS (fatty acid biosynthesis regulators).

### 3.6. Effects on expression of PPAR- $\gamma$ , HNF-4 $\alpha$ , FKHR, PGC-1 and CREB in liver

As shown in Fig. 5, the mRNA levels of PPAR- $\gamma$  significantly increased in the liver tissues collected from rats fed with HFD, which was further increased by rosiglitazone treatment, while HNF-4 $\alpha$  mRNA levels significantly decreased in HFD-fed rats and rosiglitazone treatment prevented this decrease of HNF-4 $\alpha$  expression. The mRNA levels of FKHR were significantly higher in liver tissues of HFCG rats than NCG ( $p < 0.05$ ), and that was decreased in HFRG rats. Moreover, mRNA and protein levels of PGC-1 and CREB were

also significantly increased in HFCG rats compared to NCG; however, the treatment with rosiglitazone slightly suppressed the elevation of PGC-1 and CREB expressions, but still remained higher than in NCG (Fig. 5b and c).

## 4. Discussion

The objective of present study was to investigate the effect of HFD on hepatic glycogen metabolism and gluconeogenesis, to ascertain the enzymatic steps and transcriptional machinery responsible for these changes, and to determine whether rosiglitazone can ameliorate the HFD-induced hepatic metabolic derangements. It is well known that fructose is a highly lipogenic sugar molecule, which triggers the accumulation of triglyceride and FFAs into the hepatic tissues as well as in circulating blood, and leads to insulin resistance [7]. The feeding of HFD impaired glucose tolerance test within 3 weeks and increased plasma insulin, triglycerides, FFAs and HOMA, which are the prominent manifestations of insulin resistance. The treatment with rosiglitazone delayed the induction of these alterations in HFD fed rats. This indicates that rosiglitazone ameliorated the metabolic derangements induced by HFD and the presumed insulin action in liver. It has also been established that the increase in circulatory triglyceride and FFAs results in the impaired insulin mediated suppression of hepatic EGP [19,20] and gluconeogenesis which contribute to the pathogenesis of hyperglycemia in type 2 diabetes [21]. Paquot et al. [22] reported that, the administration of fructose increases EGP in type 2 diabetic subjects. However, whether this glucose production was due to increased gluconeogenesis or glycogenolysis was not clear.

In present study we found that, feeding of HFD significantly increased EGP indicated by impaired pyruvate tolerance and increased expression of PEPCK and G-6-Pase (Fig. 3), while no changes were observed in the expression of glycogen phosphorylase and glycogen synthase in HFD fed rats. This indicates that, HFD-induced hyperglycemia in rats might be due to increased EGP that was due to increased gluconeogenesis [23]. But surprisingly, we also observed that the glycogen content in liver tissues was significantly increased (Fig. 2a) [24]. The exact reason of increased hepatic glycogen accumulation in HFD fed rats is not known, but one explanation for this might be the higher consumption of fructose might have increased hepatic glycogen accumulation due to inhibition of glycogen breakdown rather than enhancement of synthesis [25], because fructose 1-phosphate; an intermediate of fructose metabolism in liver, inhibits the glycogen phosphorylase activity by depletion of inorganic phosphate [26]. Rosiglitazone treatment ameliorated the hepatic glycogen deposition without affecting mRNA levels of glycogen phosphorylase and synthase (Fig. 2b), which depicts that rosiglitazone may regulate glycogen accumulation by affecting the protein levels and/or enzymatic activities of glycogen synthase and glycogen phosphorylase, instead of their increased transcriptional program.

Deposition of ectopic fat in liver tissues (hepatic steatosis) is a prominent factor for induction of hepatic insulin resistance [27]. In the present study we found that feeding of HFD significantly increased ectopic fat deposition in liver tissues of rats, which was further confirmed by increased hepatic triglyceride content (Fig. 4a and b). The increased ectopic fat deposition in hepatic tissues of HFD fed rats might be due to increased lipogenesis in liver, which was confirmed by increased expression of SREBP-1 and FAS (Fig. 4c). Interestingly, treatment with rosiglitazone significantly ameliorated the hepatic steatosis in HFD-fed rats, which indicates that rosiglitazone can modulate the lipogenic cascade in liver under fructose-induced energy overloaded conditions.

The mechanism of increased hepatic gluconeogenesis, lipogenesis and induction of hepatic insulin resistance-mediated hyperglycemia has not been fully elucidated in HFD fed rats. To provide a mechanism for the increase of these effects in response to HFD, we measured the expression of various transcription factors, i.e. PPAR- $\gamma$ , HNF-4 $\alpha$ , FKHR, PGC-1 and CREB, involved in the regulation of hepatic glucose homeostasis [28]. PPAR- $\gamma$  is a crosstalk transcription factor, contributes to the regulation of genes involved in glucose, lipid and protein metabolism, and insulin sensitivity [29]. TZDs activate PPAR- $\gamma$  in adipose tissues and improve diabetic condition by increasing insulin sensitivity [30]. The effects of TZDs in adipose tissues have been extensively studied, but crosstalk between TZDs and PPAR- $\gamma$  in hepatic tissues is obscure. In this study, we observed that the mRNA levels of PPAR- $\gamma$  by feeding of HFD significantly increased and treatment with rosiglitazone further increased its mRNA levels. However a previous study conducted in our laboratory

[31], depicted that the protein levels of PPAR- $\gamma$  in muscle tissues slightly decreased by feeding of HFD and rosiglitazone treatment inhibited the depletion of PPAR- $\gamma$  proteins; this indicates that the regulation of transcriptional and translational machinery of PPAR- $\gamma$  by rosiglitazone might be different in different tissues. This mechanism warrants to be explored in further detail.

PGC-1 is an important transcription co-activator that regulates hepatic gluconeogenic rate by targeting the gene expression of PEPCK and G-6-Pase. PGC-1 expression in liver dramatically increased in streptozotocin-injected and ob/ob mice, models of type 1 and 2 diabetes, respectively. Moreover, PGC-1 was highly elevated in LIRKO (liver insulin receptor knockout) mice, which is an ideal model of hepatic insulin resistance [9]. In present study, the feeding of HFD increased the mRNA and protein levels of PGC-1 in liver. However, the treatments with rosiglitazone maintained lower levels of PGC-1 expression. But the exact mechanism of alteration of PGC-1 expression by HFD and rosiglitazone is not fully elucidated. Furthermore, CREB mRNA and protein levels were also higher in HFCG rats than NCG, which indicates that the expression of PGC-1 was stimulated by the increased levels of the CREB [32]. On the other hand, HFD feeding also depleted the HNF-4 $\alpha$  and stimulated FKHR expression. HNF-4 $\alpha$  interacts with PGC-1 and forms a complex, which facilitates the target gene (i.e. PEPCK, etc.) transcription by providing histone-acetyl-transferase activity [33]. FKHR is a mediator of insulin action to regulate PGC-1 expression through protein kinase B-mediated dephosphorylation and phosphorylation cycle to attaching and displacing on IRSs, respectively [34], but the exact mechanism of this scheme is not fully understood. The rosiglitazone treatment markedly ameliorated the elevation of mRNAs of HNF-4 $\alpha$  and depletion FKHR in liver tissues from rats fed with HFD.

The data of the present work together with our previous study [31] clearly indicates the importance of HFD feeding to induce a type 2 diabetes model with the characteristics of glucose intolerance, hyperinsulinemia, hypertriglyceridemia, increased FFAs, gluconeogenic rate and hepatic steatosis. We showed that feeding of HFD increased EGP due to an increase in hepatic gluconeogenesis rate. The expression of PEPCK and G-6-Pase was increased by feeding of HFD, and the elevated expression of PGC-1 counteracting with HNF-4 $\alpha$ , FKHR and CREB. The antidiabetic drug rosiglitazone suppressed all these derangements induced by HFD.

#### Conflict of interest

There is no conflict of interest among authors.

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