

Hyperglycemic-dependent LXR-alpha Gene Regulation within Blood Mononuclear Cells of CHD Patients

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Abstract

Keeping in view the fact that Liver X receptor alpha (LXR- α) is a glucose sensor, the present study was designed to explore the dose dependent synergistic action of glucose and fructose on the regulation of LXR- α transcriptional activity in peripheral blood mononuclear cellular model. Gene expression study revealed that under normal physiological range of glucose and fructose, LXR- α is able to modulate its effector genes whereas at high concentration of glucose and fructose the transcriptional activity of LXR- α becomes redundant. The redundant activity of LXR- α was further precipitated by the increased intracellular cholesterol as well as foam cell formation in response to high dose of glucose and fructose. Further, LXR- α ligand was able to restore glucose induced impaired transcriptional activity of LXR- α . Thus use of LXR- α ligands may be beneficial for the treatment of diabetes induced Coronary Artery Disease.

Keywords: Glucose; Fructose; LXR-alpha; 22(R) OH cholesterol

Introduction

Accumulating data has established a tight regulation and coordination between the glucose and lipid homeostasis at cellular and molecular levels [1-5]. Glucose and fructose are ubiquitous molecules in biology and have been found to be associated with a wide range of fundamental cellular processes to various patho-physiological situations including metabolic syndrome [6,7]. In the last decade studies from invitro to invivo model system have established a pivotal role of ligand activated nuclear receptor Liver X Receptor (LXR) for its ability to regulate two cellular processes i.e. lipid metabolism and inflammation [8,9]. In addition recent study has identified LXRs as a glucose sensor, where D-glucose has been found to be a direct agonist of LXR and increases its transcriptional activity that integrates hepatic glucose metabolism and fatty acid synthesis [10]. Keeping in view LXRs as a glucose sensor, the present study was designed to explore the synergistic action of glucose and fructose on the regulation of LXR- α transcriptional activity in peripheral blood mononuclear cellular model. For the study we incorporated the LXR- α target genes PPAR γ , Low density lipoprotein receptor (LDLR) and Prostate apoptosis response 4 which are positively regulated by LXR- α [11,12] as well as Interferon γ and Apoptosis antagonizing transcription factor (AATF) which are negatively regulated by LXR- α [13,14].

Material and Methods

Cellular model employed

Peripheral blood mononuclear cells (PBMCs) were isolated from normal healthy subjects (Age 18-55 years), having normal serum lipid profile, by employing Ficoll-Hypaque density gradient method [15] with their prior informed consent as per the guidelines of our institute ethics committee. The investigation conforms the principles outlined in the declaration of Helsinki [16]. These subjects were fasting for 12 hours as well as abstained from any medication for 2 weeks before blood withdrawal. Cells were maintained in invitro glucose free culture medium DMEM containing antibiotics and 10% fetal calf serum. The cell viability was assayed using trypan-blue dye exclusion method.

Treatment with glucose and fructose

Cultured PBMCs were exposed with dose dependent glucose (0-200mg/dl) along with fructose (0-12 μ M) in triplicate for 24 hours at 37°C and 5% CO₂ incubator. The cultured PBMCs were also exposed

time dependent with 200mg/dl glucose and 12 μ M fructose from 0 to 72 hours in triplicate and subsequently processed for RNA isolation using standard method [17].

Gene expression study

The cDNA was synthesized from isolated RNA using Revert Aid™ first strand synthesis kit. Transcriptional expression of LXR- α and its effector genes PPAR, Low density lipoprotein receptor (LDLR), Interferon γ (IFN γ), Apoptosis antagonizing transcription factor (AATF) and Prostate apoptosis response-4 (Par-4) gene was studied using gene specific primers and standard RT-PCR method. β actin gene expression was used as invariant control for RNA loading and efficiency of reverse transcription. The PCR amplicons were resolved on ethidium bromide stained 2.5% agarose gels followed by densitometric scanning of each band using Scion Image Analysis software. The level of mRNA expression of each gene was given by normalizing RT-PCR band intensity of the gene in question to β actin band intensity.

Intracellular total cholesterol measurement

The cell lysate of the PBMCs exposed with glucose and fructose (dose dependent and time dependent) were subjected for the intracellular total cholesterol measurement using standard commercial kit.

In-vitro effect of glucose and fructose followed by + 22(R) OH cholesterol in PBMCs derived from normal and coronary heart disease (CHD) subjects

The PBMCs were isolated from the normolipidemic healthy subjects

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(Age 18-55 years) as well as age matched patients suffering from high severity of coronary heart disease (N=25, N represents number of subjects) by Ficoll-Hypaque density gradient method [15] with their prior informed consent. The inclusion criteria for CHD subjects having a normal lipid profile with unknown origin of chest pain were undergone for coronary angiography. Angiographically confirmed CHD subjects were recruited in the present study. The severity was calculated by the modified Gensini Score [18] which is based upon the position of coronary blockage and the functional signification of the area supplied by that segment. The severity is not based on the single, double or multi vessel disease. The modified Gensini Score more than 30 was considered as most severe Coronary heart disease.

Exclusion criteria for both patients and controls included females, diabetic, cardiomyopathy, serious organ disease, systemic illness, chronic alcohol abuse, serious psychiatric illness and anticonvulsant therapy. The isolated PBMCs were cultured on glucose free DMEM medium containing the antibiotics and 10% fetal calf serum. These cells were exposed with 200mg/dl glucose with 12µM fructose for 36 hours, followed by the exposure of LXR-α ligand +22(R) OH cholesterol (10µM). At 72 hours cells were harvested and subjected for the RNA isolation, followed by the cDNA synthesis. The expression of LXR-α and its effector genes PPARγ, LDLR, IFNγ, AATF and Par-4 were analyzed by RT-PCR method coupled with scion image analysis software. The total intracellular cholesterol was also measured using standard commercial kit.

Cellular apoptosis assay

The PBMCs derived from normal healthy subjects exposed with maximum dose of glucose (200mg/dl) and fructose (12µM) as well as PBMCs exposed with glucose, fructose followed by +22(R) OH cholesterol were subjected to Annexin V- propidium iodide staining for the detection of cellular apoptosis.

Cellular ultra-structure study for foam cell formation

In order to explore the ultra-structural features within the PBMCs derived from normal and CHD subjects exposed with either glucose (200mg/dl) and fructose (12µM) or glucose, fructose followed by

+22(R)OH cholesterol (10µM) were processed for transmission electron microscopic examination using standard method [19].

Statistical analysis

Statistical analysis was performed with the use of SPSS windows version 13. All the data was shown as mean±SD of three independent experiments. One way ANOVA was used to compare the data between control and different dose point. P value less than .05 have been taken as significant (**).

Results

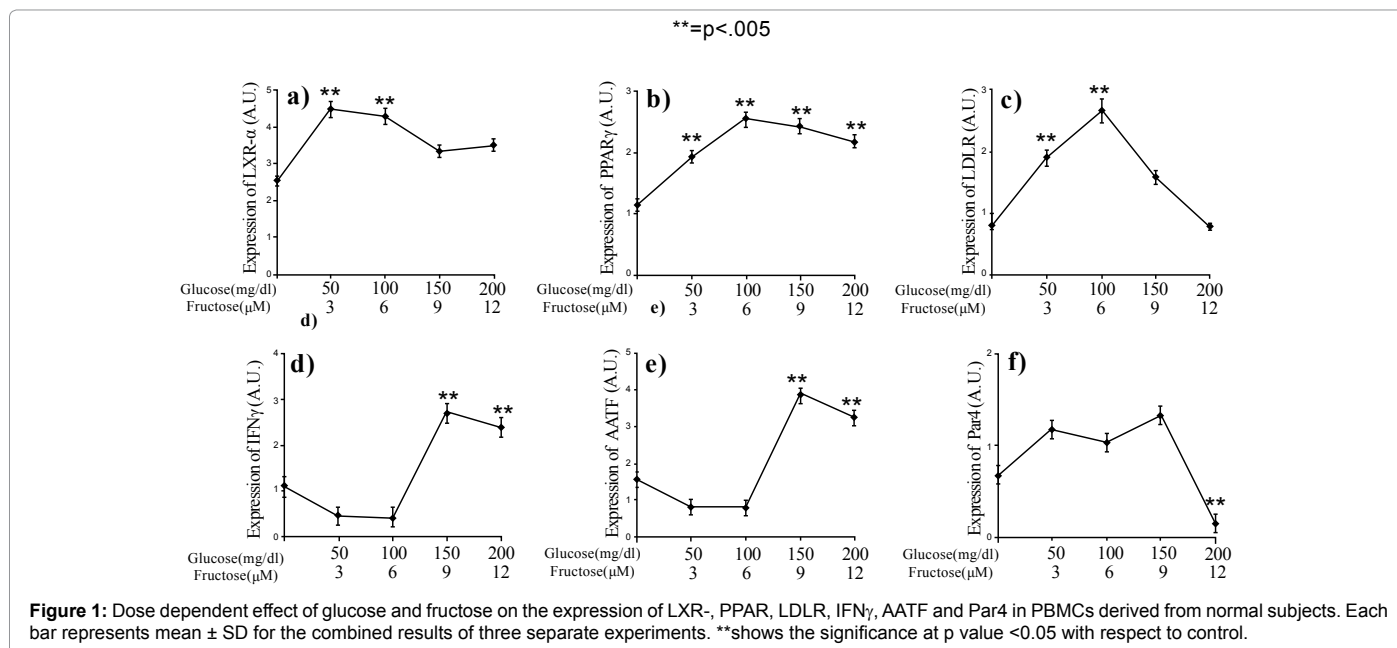
Effect of glucose and fructose on LXR-α and its effector genes

Dose dependent exposure of PBMCs derived from normal subjects with glucose and fructose revealed increase in the expression of LXR-α, PPARγ, LDLR and Par4 upto the 100mg/dl of glucose along with 6µM fructose concentration whereas decreased expression was found at high dose of glucose (200mg/dl) along with Fructose (12µM) (Figure 1a, Figure 1b, Figure 1c and Figure 1f). Further decreased expression of IFNγ and AATF was revealed upto the concentration of 100mg/dl of glucose along with 6µM fructose whereas significant increased expression was noted at high dose of glucose (200mg/dl) and fructose (12µM) (Figure 1d and Figure 1e).

Time dependent exposure of PBMCs derived from normal subjects with high dose of glucose (200mg/dl) along with fructose (12µM) revealed increased in the expression of LXR-α, PPARγ, IFNγ and AATF (Figure 2a, Figure 2b, Figure 2d and Figure 2e) whereas decreased expression of LDLR and Par4 was reported (Figure 2c and Figure 2f).

Intracellular total cholesterol measurement

No change in the intracellular cholesterol of PBMCs was reported at low does of glucose and fructose where as at a significant increased intracellular cholesterol was reported at high dose of glucose (200mg/dl) along with fructose (12µM) (Figure 3a). Similarly at high dose of glucose and fructose a time dependent increased intracellular cholesterol was reported (Figure 3b).



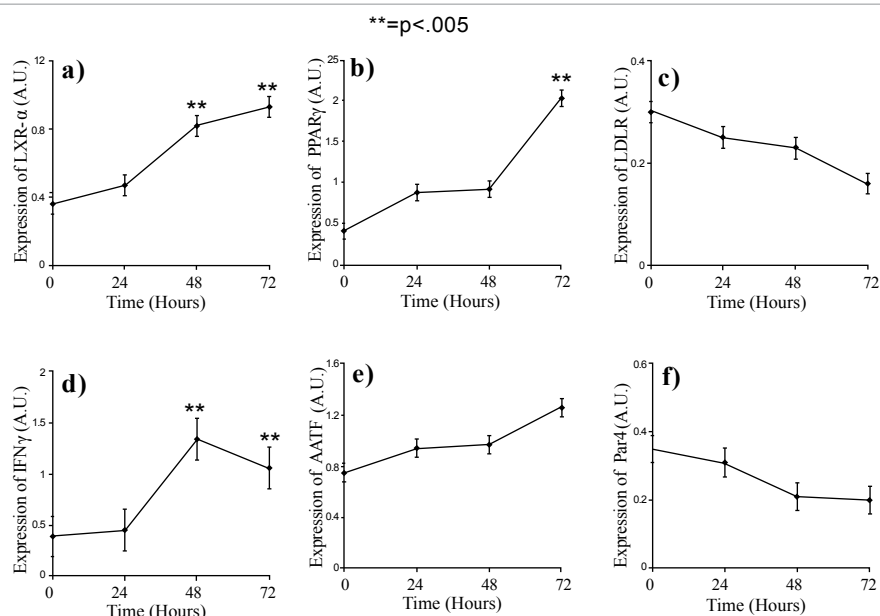


Figure 2: Time dependent effect of glucose (200mg/dl) and fructose (12μM) on the expression of LXR-α, PPARγ, LDLR, IFNγ, AATF and Par4 in PBMCs derived from normal subjects. Each bar represents mean ± SD for the combined results of three separate experiments. **shows the significance at p value <0.05 with respect to control.

***In-vitro* effect of high dose of Glucose and fructose followed by +22(R) OH cholesterol in PBMCs derived from normal and coronary heart disease (CHD) subjects**

Exposure of PBMCs derived from normal subjects with high dose of glucose and fructose followed by LXR-α natural ligand +22(R) OH cholesterol revealed a significant increased expression of LXR-α, PPARγ, LDLR and Par4 which was accompanied by significant decreased expression of IFNγ and AATF (Figure 4). Further we did not found any change in the intracellular total cholesterol (Figure 5).

Further study has revealed the existence of a deregulated LXR-α transcriptome in peripheral blood mononuclear cells derived from CHD patients. This deregulated LXR-α transcriptome was shown to occur as a result of three critical mutations in the ligand binding domain of LXR-α protein comprising of amino acids Asp324, Pro327 and Arg328, which were responsible for inability of this domain to interact with its natural ligands [20]. Exposure of PBMCs derived from CHD subjects with high dose of glucose and fructose followed by LXR-α natural ligand +22(R) OH cholesterol revealed an increased expression of LXR-α, PPARγ and AATF, where as no significant change was reported in the expression of LDLR, IFNγ and Par4 (Figure 4) along with significant high amount of intracellular total cholesterol (Figure 5) accumulation.

Cellular apoptosis assay

No significant change in the apoptosis of PBMCs was observed (Figure 6) after exposure of high dose of glucose (200mg/dl) along with fructose (12μM) as well as followed by +22(R) OH cholesterol (10μM).

Cellular ultra-structure study for foam cell formation

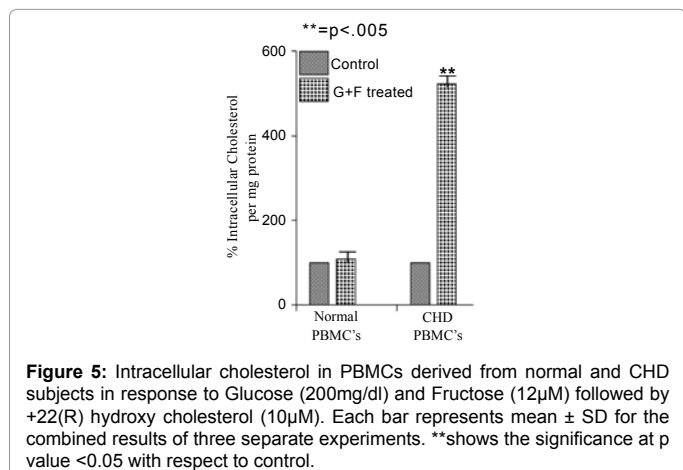
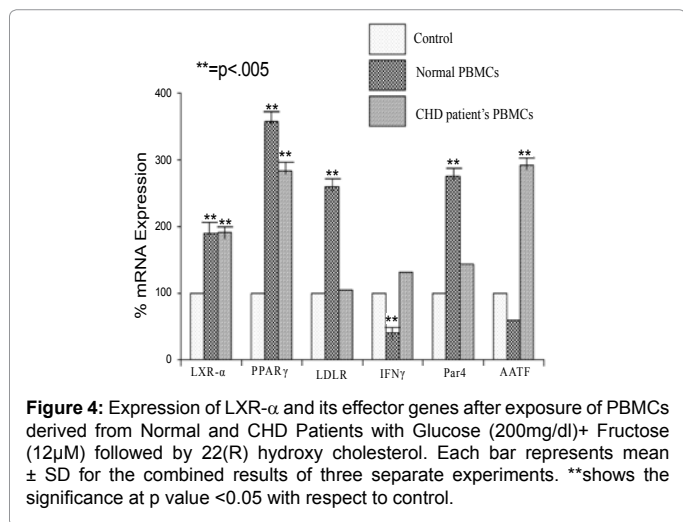
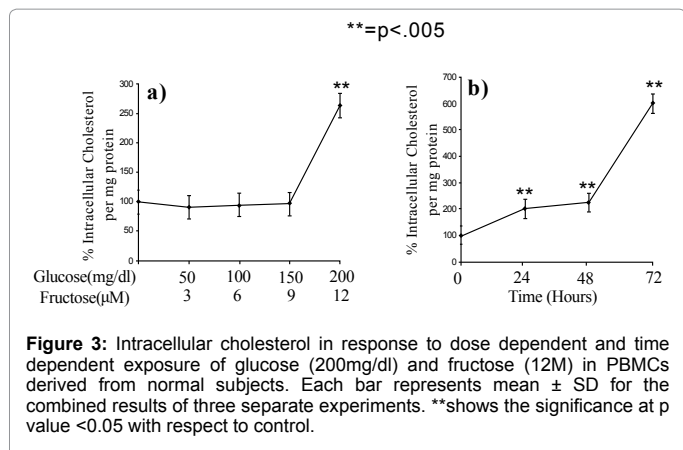
Transmission electron microscopy revealed an accumulation of intracellular cholesterol (foam cell) in the PBMCs derived from normal subjects after exposure of high dose of glucose (200mg/dl) along with fructose (12μM) (Figure 7b) and reversal in the intracellular cholesterol accumulation was observed after treatment with LXR agonist +22(R)OH cholesterol (Figure 7c). Further intracellular cholesterol accumulation

was also observed in the PBMCs derived from CHD subjects after exposure of high dose of glucose and fructose (Figure 7d), as well as no reduction in the accumulated intracellular cholesterol was observed after treatment with +22(R) OH cholesterol (Figure 7e).

Discussion

In addition to well established role of LXRs in lipid homeostasis and inflammation, recent study has identified LXRs as a glucose sensor. Glucose is an endogenous LXR ligand and modulates transcriptional activity of LXRs that acts as a transcriptional switch which integrates hepatic glucose metabolism and fatty acid synthesis [10]. In this regard the present study was designed to explore the synergistic effect of glucose and fructose at physiological to patho-physiological concentrations on the regulation of LXR-α activity in peripheral blood mononuclear cellular model.

Gene expression study revealed that LXR-α can sense the glucose and fructose in the physiological concentration and can modulate the expression of its target genes that is upregulation of LDLR and Par 4 (Figure 1c and Figure 1f) and reciprocal regulation of IFNγ as well as AATF (Figure 1d and Figure 1e), whereas at high dose of glucose (200mg/dl) along with fructose (12μM), transcriptional activity of LXR- become redundant and it is unable to regulate the expression of its target genes. Keeping in view the fact that LXR-α is a cholesterol sensor and maintains the intracellular cholesterol by regulating the expression of ATP binding cassette transporter (ABCG1) [21,22], the results reported here assume importance that within the physiological concentration of glucose along with fructose intracellular cholesterol level is maintained (Figure 3a) whereas at high dose of glucose and fructose LXR-α transcriptional activity becomes redundant which was reflected by significant intracellular cholesterol accumulation (Figure 3a and Figure 3b). The foam cell formation in response to high dose of glucose and fructose was also confirmed by the transmission electron microscopy (Figure 7b). The results obtained were in consistency with the fact that high glucose promotes intracellular lipid accumulation by impairing cholesterol influx and efflux balance [23]. The observation



also supports the fact that ABCG1 expression (an LXR target gene) is decreased in macrophages isolated from patients with Type 2 diabetes, leading to decreased cholesterol efflux to HDL, and resulting in increased macrophage cholesterol content [24].

Further exposure of PBMCs derived from normal subjects with high dose of glucose (200mg/dl) with fructose (12μM) followed by the treatment with high affinity LXR ligand +22(R) OH cholesterol revealed a significant increase in the expression of LXR-α target gene PPARγ,

LDLR and Par4 and decrease in the expression of IFNγ and AATF (Figure 4) suggest that LXR agonist can be used to restore the high dose glucose and fructose induced impaired LXR-α transcriptional activity. This fact was also confirmed by the unaltered intracellular cholesterol after exposing the PBMCs with high dose of glucose and fructose followed by +22(R) OH treatment (Figure 5 and Figure 7c).

Study has revealed the existence of a deregulated LXR-α transcriptome in peripheral blood mononuclear cells derived from CHD patients. This deregulated LXR-α transcriptome was shown to occur as a result of three critical mutations in the ligand binding domain of LXR-α protein comprising of amino acids Asp324, Pro327 and Arg328, which were responsible for inability of this domain to interact with its natural physiological ligands [20]. Exposure of PBMCs derived from CHD subjects with high dose of glucose along with fructose followed by the treatment with +22(R) OH cholesterol did not restore the high dose glucose and fructose induced impaired LXR-α transcriptional activity (Figure 4) as well as revealed massive accumulation of intracellular cholesterol (Figure 5, Figure 7d and Figure 7e).

Although the precise binding mode of glucose with LXR awaits the resolution of a crystal structure, results of the study suggest that under physiological concentration of glucose and fructose, LXR-α can act as a glucose sensor and can modulate the expression of its target genes, whereas high dose of glucose and fructose leads to impaired transcriptional activity of LXR-α which can be restored with the use of

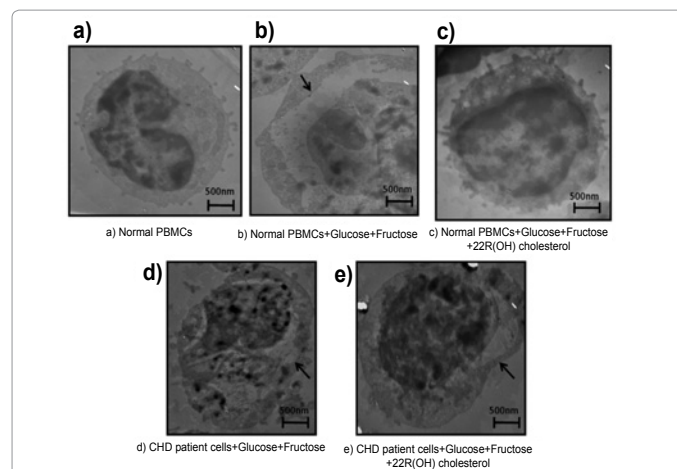
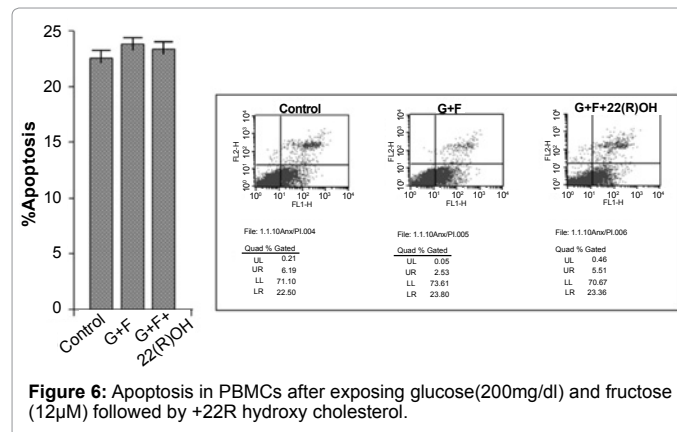


Figure 7: Representative ultra structure TEM analysis for the foam cell formation in PBMCs derived from normal and CHD subjects after exposing with glucose + Fructose and Glucose + Fructose + 22(R) OH Cholesterol.

high affinity LXR ligands. Thus use of LXR- α ligands may be beneficial for the treatment of various glucose and fructose associated metabolic syndrome like impaired glucose tolerance, type II diabetes and obesity.

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