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## Reversal of High dietary fructose-induced PPAR $\alpha$ suppression by oral administration of lipoxygenase/cyclooxygenase inhibitors

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### Abstract

High fructose feeding causes diet-induced alterations of lipid metabolism and decreased insulin sensitivity, hallmark of which is a rapid and profound hypertriglyceridemia. One of the mechanisms that contribute to serum hypertriglyceridemia in this model is suppression of hepatic PPAR $\alpha$ . HMG-CoA inhibitors, which reduce serum triglycerides in these animals, also elevate/restore hepatic PPAR $\alpha$ . Previously we demonstrated that two known lipoxygenase/cyclooxygenase inhibitors reversed diet-induced hypertriglyceridemia in this model and that reversal of certain inflammatory markers in the liver correlated with the metabolic benefit. In this paper we extended these studies by examining the impact of these compounds on expression of PPAR $\alpha$ , both at the level of transcription and expression. Our data show that diet-induced suppression of hepatic PPAR $\alpha$  is reversed upon treatment with lipoxygenase/cyclooxygenase compounds. We then tested one of these compounds, BW-755c, over a range of doses from 10 mg/kg to 100 mg/kg to establish a dose-response relationship with the reduction of serum hypertriglyceridemia in this model. These experiments support the concept of using anti-inflammatory medications as one method to correct metabolic dysfunction.

### Background

Recent epidemiological studies have shown that almost a quarter of adults in the United States have metabolic syndrome or syndrome X and prevalence of this syndrome is increasing world-wide owing to lifestyle changes leading to obesity [1-3]. A cluster of abnormalities define metabolic syndrome including insulin resistance, hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol, obesity and hypertension [4-6]; individuals with syndrome have an increased risk of developing cardiovascular disease [5,6]. Insulin resistance is now considered a central factor among these various abnormalities associated with the metabolic syndrome. With this in mind, much effort is being invested in improving the

insulin resistance through lifestyle modification (e.g., weight reduction, dietary interventions, and increased physical activity) and development of new therapeutic agents that sensitize insulin action, ameliorate hypertriglyceridemia, raise HDL levels and improve hypertension [2,7-12].

In an animal model, high fructose fed (HFF) diets induce metabolic dysfunction typically resulting in a rapid elevation of serum triglycerides with a corresponding increase in blood pressure within two weeks of diet initiation. Animals maintained on this diet for longer periods of time develop elevated free fatty acids and hyperinsulinemia at the expense of glycemic control. If HFF animals are

subjected to an exercise regimen, the diet-induced effects can be ameliorated [13]. Thus this animal model exhibits an early stage of the Metabolic Syndrome in which a combination of physical inactivity and diet results in cardiovascular disease and metabolic complications.

High fructose corn sweeteners began widespread use in the food industry in 1967. Since that time the amount of fructose consumption has steadily risen and now accounts for about 9% of daily caloric intake in the United States. Unlike glucose, which is widely utilized by tissues throughout the body, fructose is primarily metabolized in the liver [14,15]. Recent epidemiological data suggests that high fructose corn sweeteners may be contributing to the overall epidemic of obesity and metabolic disease in the US [16].

We initiated studies to test the effects of lipoxygenase/cyclooxygenase inhibitors on the metabolic and hepatic status of HFF rats. We examined two structurally different LOX/COX inhibitors because NDGA, which has previously demonstrated effects in this model [17], also exhibits numerous other biological effects. In an initial report we showed that high levels of dietary fructose induced the JNK/AP-1 stress response pathway. This observation was confirmed by Wei and Pagliassotti, who correlated fructose-induced JNK activity with hepatic insulin resistance [18]. In our first report, we focused on the correlation between normalization of the stress response pathway and reduction of serum triglycerides. In this report, we expanded these studies to examine diet-induced effects on hepatic peroxisome proliferators-activated receptor  $\alpha$  (PPAR $\alpha$ ) activity and the relationship between LOX/COX inhibitor treatment and of PPAR $\alpha$  expression.

PPAR $\alpha$  (NR1C1) is a ligand (lipid)-activated transcription factor that belongs to the superfamily of nuclear receptor [19-22]. In addition to PPAR $\alpha$ , the NR1C subset of receptors includes two closely related members, PPAR $\beta$  (or  $\delta$ , NR1C2) and PPAR $\gamma$  (NR1C3). PPAR $\alpha$  is highly expressed in the liver, cardiac muscle, intestine and renal cortex tissues [19,23], serves an essential function in the regulation of lipid metabolism and controls the expression of a number of genes involved in mitochondrial and peroxisomal  $\beta$ -oxidation [19-22]. It increases gene transcription by binding as a heterodimer with retinoid X receptor (RXR) to PPAR response elements (PPRE) on the promoter regions of the target gene [22]. Activators of PPAR $\alpha$ , such as fibrates, lower circulating levels of lipid and are commonly used to treat hypertriglyceridemia [21,22,24]. More recent studies have shown an inverse relationship between PPAR activity and abnormalities in fatty acid metabolism leading to the development of insulin resistance and alterations in glucose metabolism [25-27]. In addition, animal studies suggest that the activation of

PPAR $\alpha$  improved the insulin resistance that was triggered by the excessive production and accumulation of lipids [28-32].

Our studies indicate hypertriglyceridemia induced by fructose feeding greatly reduces the hepatic expression of both PPAR $\alpha$  protein and mRNA levels. We further demonstrate that diet-induced suppression of hepatic PPAR $\alpha$  is reversed upon treatment with lipoxygenase/cyclooxygenase inhibitory compounds. Our findings also suggest that these compounds to some extent also up-regulated the mRNA levels of ubiquitous PPAR $\beta/\delta$ . From these studies, we conclude that anti-inflammatory agents have the potential to prevent or treat multiple aspects of the metabolic syndrome.

## Methods

### Animals and treatments

Male Sprague-Dawley rats weighing approximately 180–200 g first maintained on a rat chow diet and then were divided into 6 groups and were switched to a high-fructose diet (TD89247; Harlan Teklad, Madison, WI) that provided 60% of total calories as a fructose (Day 1). On day 15 of treatment, the rats were fasted for 4 hours and tail vein blood was collected for baseline measurements of serum TG, glucose, insulin and free fatty acids (FFA) as previously described [33]. The groups of rats were then treated with either vehicle (0.5% carboxymethyl cellulose) or BW755c (suspended 0.5% carboxymethyl cellulose) at one of several doses ranging from 5 mg/kg to 100 mg/kg. The vehicle group and most groups receiving BW-755c or NDGA (250 mg/kg) were treated twice a day (b.i.d.) for 4 days, delivered by oral gavage i.e., animals were treated with vehicle or drug at day 0 at 4:00 PM, at day 1 at 9:00 AM and 4:00 PM, at day 2 at 9:00 AM and 4:00 PM, at day 3 at 9:00 AM and 4:00 PM and at day 4 at 9:00 AM. In addition, one group of animals was treated with 50 mg/kg BW-755c once a day (q.d.) at 9:00 AM by oral gavage. During the treatment regimen the animals were maintained on high-fructose diet. On the last day (day 4), animals were fasted for 4 h (8:00 AM-12:00 PM) and blood was collected from the tail vein 3 hours after last dose (i.e., 9:00 AM to 12:00 PM) and serum samples analysed for TG, glucose, insulin, FFA, and total cholesterol [33-35]. After the serum was collected, the animals were sacrificed and tissues removed, snap-frozen in liquid nitrogen and stored at -80°C until analysed. The local committee on animal care approved all animal protocols.

### RNA isolation and reverse transcription

The liver samples used in these studies were derived from animals treated as previously described [36].

Total RNA was extracted from the liver samples (~100–120 mg) using the Trizol reagent (Invitrogen) according

**Table 1: Gene-specific primer used for Real Time RT-PCR assays**

GenBank Accession Number	Primer Sequence (5'→3')	Amplicon Size (bp)	Region of Gene (nt)
<u>NM_013196</u>	<b>Rat PPAR<math>\alpha</math></b>	177	876–895
	<b>Forward</b>		
	TCACACAATGCAATCCGTTT		
	<b>Reverse</b>		
	GGCCTTGACCTTGTTTCATGT		1052–1033
<u>V01279</u>	<b>Rat 18S rRNA</b>	133	1336–1355
	<b>Forward</b>		
	ATGGCCGTTCTTAGTTGGTG		
	<b>Reverse</b>		
	AACGCCACTTGTCCTCTAA		1468–1449

Primer sequences were designed using Primer Express software (Applied Biosystems) using sequences accessed through GenBank. Amplicon size and region of each gene are indicated.

to the protocols recommended by the manufacturer. Subsequently, purified RNA preparations were treated with DNase (to eliminate the possible contamination of the genomic DNA) and further cleaned using Rneasy spin column (Qiagen, Valencia, CA). The integrity of the purified total RNA samples to be used in the Real-time PCR assays was confirmed by 1.2% formaldehyde-agarose gel electrophoresis. No degradation or 28S and 18S rRNA was observed following staining gels with ethidium bromide.

First strand cDNAs were generated from total RNA samples as follows: 2.0  $\mu$ g each of total RNA sample was denatured for 5 min at 65 °C in the presence random hexamer, snap cooled in ice water, then reverse transcribed in 100  $\mu$ l using the TaqMan Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

#### Quantitative Real-time PCR measurements

Quantitative real-time PCR (Q-PCR) amplifications were performed in triplicate using the GenAmp Sequence Detection System (Applied Biosystems). The incubation mixture in a final volume of 50- $\mu$ l contained suitable aliquot of template (cDNA or RNA), 300 nM each of forward and reverse primers for PPAR $\alpha$  or 18S ribosomal RNA and 1X SYBR<sup>®</sup> Green PCR Master Mix (Applied Biosystems) in 50- $\mu$ l. Gene-specific primers were designed for rat PPAR $\alpha$  gene and 18S rRNA using Primer Express software (Applied Biosystem) using sequences accessed through GenBank. The primer sequences are as shown in Table 1. Samples were amplified in an ABI Prism<sup>™</sup> 7700 Sequence Detection System (Applied Biosystems). The change in fluorescence of SYBR<sup>®</sup> Green I dye in every cycle was monitored, and threshold cycle ( $C_T$ ) above background for each reaction (i.e., the partial cycle at which statistically significant increases in either the PPAR $\alpha$  or 18S rRNA first detected) was calculated. The calculated  $C_T$  values for

PPAR $\alpha$  in response to various treatments were normalized to the respective  $C_T$  values for 18S rRNA and expressed as 'Relative Values'. Initially, post-amplification melting curves and gel-electrophoresis analyses were performed to confirm that a single PCR product was produced in each reaction. The contribution of contaminating genomic DNA to the observed product in each case was determined from the  $C_T$  given by RNA template. These values usually ranged less than 0.1% of cDNA values.

#### Western Blot analysis of hepatic PPAR $\alpha$

Liver samples (~200 mg) were homogenized using a Potter-Elvehjem homogenizer in 3 volumes of detergent containing lysis buffer [20 mM HEPES, pH 7.4, 1% Triton X-100 (v/v), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 20 mM NaF, 20 mM  $\beta$ -glycerophosphate, 10 mM sodium pyrophosphate, 1 mM sodium vanadate, 10 mM okadaic acid, 1 mM dithiothreitol, 10  $\mu$ g/ml aprotinin, 10  $\mu$ g/ml leupeptin, 10  $\mu$ g/ml pepstatin A, 0.5 mM 4-(2-aminoethyl)benzylsulfonyl fluorid (AEBSF, Roche Molecular Biochemicals), 10  $\mu$ M E-64 and 50  $\mu$ M Bestatin] and incubated for 30 min at 4 °C on an orbital shaker for complete lysis. The lysates were cleared by centrifugation at 15,000  $\times$  g for 10 min, the protein concentration of each solubilized lysate was determined and samples stored frozen until analyzed.

Samples containing an equal amount of protein (50 – 60  $\mu$ g) were fractionated by SDS-polyacrylamide gel electrophoresis (10% polyacrylamide gel with 4% stacking gel) and transferred to polyvinylidene difluoride membrane (Immobilon<sup>™</sup>, Millipore Corp., Bedford, MA). After transfer the membranes were stained with Ponceau S dye (Sigma Chemical Co. St. Louis, MO) to verify loading equivalency and transfer efficiency and then the membrane was washed in TBS containing 0.1% Tween-20 (TTBS) and incubated in blocking buffer (TTBS containing

**Table 2: Dose-responsive effect of BW-755c on hypertriglyceridemia in HFF rats**

	HFF Day 15 n = 24	Vehicle n = 4	5 mg/kg BW (b.i.d.) n = 4	10 mg/kg BW (b.i.d.) n = 4	20 mg/kg BW (b.i.d.) n = 4	50 mg/kg BW (b.i.d.) n = 4	50 mg/kg BW (q.d.) n = 4
Weight (g)	301 ± 2.3	312 ± 8 <sup>a</sup>	312 ± 7 <sup>a</sup>	312 ± 5 <sup>d</sup>	310 ± 5 <sup>a</sup>	316 ± 6 <sup>b</sup>	317 ± 6 <sup>c</sup>
Glucose (mg/dl)	116.0 ± 1.6	113.0 ± 3.4	113.8 ± 6.4	115.3 ± 5.1	112.8 ± 2.9	115.5 ± 8.7	119.3 ± 7.0
Insulin (ng/ml)	1.84 ± 0.06	1.78 ± 0.13	1.83 ± 0.13	1.86 ± 0.15	1.77 ± 0.09	1.82 ± 0.05	1.81 ± 0.13
FFA (μEq/l)	532 ± 17	553 ± 54	552 ± 63	543 ± 92	528 ± 32	562 ± 39	540 ± 52
Cholesterol (mg/dl)	109.6 ± 1.5	106.8 ± 5.8	109.5 ± 6.4	105.0 ± 3.5	107.8 ± 2.8	109.0 ± 4.1	113.3 ± 3.2
TG (mg/dl)	367 ± 16	351 ± 31	319 ± 59	268 ± 18	216 ± 17 <sup>a</sup>	154 ± 33 <sup>a</sup>	215 ± 39 <sup>a</sup>

Results are Mean ± SE

<sup>a</sup>p < 0.001; <sup>b</sup>p < 0.002; <sup>c</sup>p < 0.004; <sup>d</sup>p < 0.04

5% non-fat dry milk) for 90 min at room temperature followed by overnight incubation at 4°C with rabbit anti-PPARα IgG (Santa Cruz Biotechnology, Santa Cruz, CA) diluted in blocking buffer. Subsequently, the membrane was washed in TTBS and incubated for 2 hr with horseradish peroxidase conjugated goat anti-rabbit IgG (Sigma Chemical Co. St. Louis, MO) in blocking buffer. Bands were visualized by enhanced chemiluminescence detection as described by the manufacturer (ECL System, Amersham Pharmacia Biotech). Blots were exposed to film for various times (3–10 min), and exposures were subjected to densitometric scanning using Fluor-S™ MultiImager with a built-in computer software (Bio-Rad). Equal loading of proteins was confirmed by staining the membranes with the Ponsceau S.

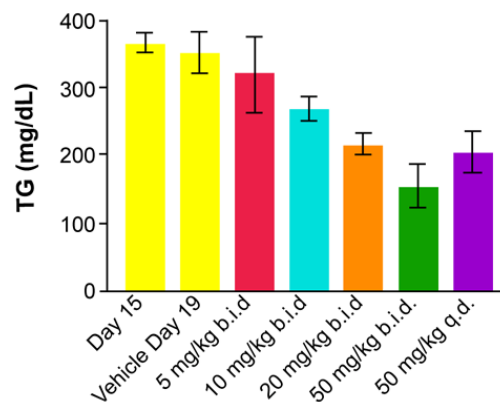
**Statistical Analysis**

Dose response analysis was conducted using paired t-tests. PPARα RT-PCR and Western Blots were analyzed using t-test comparing the drug treatment group to the vehicle control. All statistical analyses were performed using GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego California USA, <http://www.graphpad.com>.

**Results**

**Dose-responsive reduction of diet-induced hypertriglyceridemia**

Table 2 summarizes the effect of 4 consecutive day's treatment using BW-755c on HFF animals. During the treatment phase no obvious toxic effects were noted in the animals. All animals at all dose groups gained weight during the testing period. In these animals there was no discernable hyperinsulinemia or elevated FFA. The only metabolic effect in this group of animals was hypertriglyceridemia. BW-755c lowered serum triglycerides in a dose-related fashion (Figure 1). In addition, there was no difference between dosing the animals once daily versus twice daily as can be appreciated by comparing the 20 mg/kg bid (40 mg/kg daily) results versus the 50 mg/kg qd

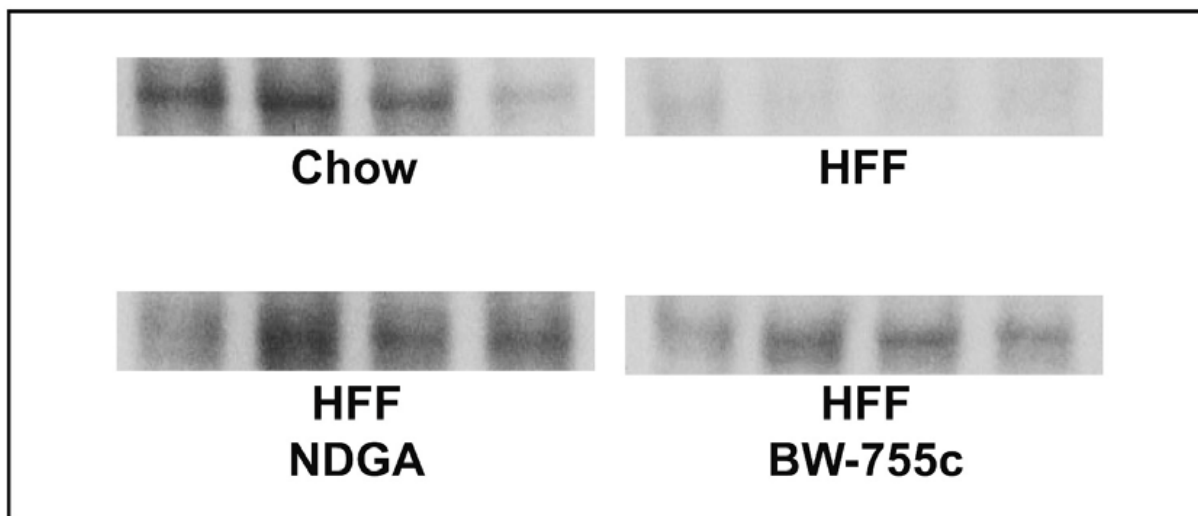


**Figure 1**  
**Dose-responsive reduction of serum triglycerides upon treatment with BW-755c (mean +/- SD).** The statistical significance of each of the dose groups is shown in Table 2.

groups. There were no changes in any of the other measured metabolic parameters during the test period.

**BW-755c treatment restores PPARα protein levels in HFF animals**

Figure 2 shows that the amount of hepatic PPARα protein is reduced upon initiation of the HFF diet. Each lane represents a liver sample from a different animal in the dosing group. This was done to control for variation due to unrestricted feeding of the animals and its potential impact on the expression of PPARα and to ensure that the observed responses were generalized to the group and not an animal-specific observation. Liver samples from chow-fed animals were included as a control group because they represent normal expression levels of PPARalpha. Liver samples from the group of vehicle treated HFF animal



**Figure 2**  
**Representative Western blots for PPAR $\alpha$  expression in hepatic tissue from chow-fed (control) rats and high fructose-fed rats that were subsequently treated with vehicle alone, NDGA or BW755c.** Each lane represents hepatic tissue from each of four different animals in each group.

represent the expected degree of diet-induced PPAR $\alpha$  suppression. Liver samples from HFF animals that were treated with either NDGA or BW-755c demonstrate normal or elevated levels of PPAR $\alpha$  protein (Table 3). This data shows that these compounds are able to overcome the diet induced suppression either directly or by a compensatory mechanism.

#### **BW-755c treatment restores PPAR $\alpha$ RNA expression in HFF animals**

In order to determine the mechanism by which the HFF diet suppressed PPAR $\alpha$  we measured the relative amount of PPAR $\alpha$  RNA in the livers of the animal in the various treatment groups. As seen in Table 4, PPAR $\alpha$  RNA is suppressed in HFF animals, indicating that the method of control is related to transcription rather than protein expression. The LOX/COX drug treatment increased PPAR $\alpha$  RNA levels. In the case of BW-755c the relative amounts of RNA were comparable to that of the vehicle control. NDGA treatment resulted in higher levels of PPAR $\alpha$  RNA. This may be a secondary effect of this compound because hepatic RNA levels of PPAR $\beta/\delta$  were also elevated in response to this drug (data not shown).

#### **Discussion**

There is building evidence that improper consumption of metabolic components, including fatty acids and carbohydrates, can lead to systemic inflammation. This systemic inflammation is at least contributory, if not causative, to metabolic disease progression.

The early stage of the HFF model is useful to examine dyslipidemia because the animals lack many confounding factors such as insulin resistance or the impact of elevated FFA or obesity. In the earliest stage of metabolic dysfunction, these animals receive excess dietary fructose and exhibit hypertriglyceridemia. Hepatic fructose metabolism leads to precursors of triglyceride synthesis and thus it is not altogether surprising that dietary fructose leads to generation of triglycerides. Clearly, however the compensatory metabolic regulatory mechanisms are disrupted in these animals because they fail to control triglycerides within normal ranges. In our prior work, we showed a correlation between activation of the JNK stress pathway leading to activation of the transcription factor AP-1 and abnormal triglyceride levels. We also showed that LOX/COX inhibitors inhibited the JNK/AP-1 activation and correspondingly reduced serum triglyceride levels even in the face of excess dietary fructose.

**Table 3: Quantitative Western blot/densitometric analysis of hepatic PPAR $\alpha$  protein levels**

Diet	Treatment	PPAR $\alpha$ level (Mean $\pm$ SE)
Normal Chow	Vehicle	0.149 $\pm$ 0.025
HFF	Vehicle	0.038 $\pm$ 0.005 <sup>a</sup>
HFF	NDGA-250 mg/kg (b.i.d.)	0.290 $\pm$ 0.050 <sup>b</sup>
HFF	BW-755c-100 mg/kg (b.i.d.)	0.190 $\pm$ 0.030 <sup>c</sup>

Results are Mean  $\pm$  SE of four different samples

<sup>a</sup>*p* < 0.004 Chow vs HFF

<sup>b</sup>*p* < 0.003 HFF vs HFF/NDGA treatment

<sup>c</sup>*p* < 0.003 HFF vs HFF/BW-755c treatment

**Table 4: Real-Time RT-PCR analysis of PPAR $\alpha$  gene expression in liver tissue from chow-fed (control), and high fructose-fed animals treated with vehicle, NDGA or BW-755c**

Diet	Treatment	PPAR $\alpha$ normalized to 18S rRNA (Relative value $\pm$ SE)
Normal Chow	Vehicle	2.375 $\pm$ 0.576
HFF	Vehicle	0.425 $\pm$ 0.085 <sup>a</sup>
HFF	NDGA-250 mg/kg (b.i.d.)	3.600 $\pm$ 0.339 <sup>b</sup>
HFF	BW-755c-100 mg/kg (b.i.d.)	2.150 $\pm$ 0.417 <sup>c</sup>

Results are Mean  $\pm$  SE of four different samples

<sup>a</sup>*p* < 0.01 Chow vs HFF

<sup>b</sup>*p* < 0.001 HFF vs HFF/NDGA treatment

<sup>c</sup>*p* < 0.001 HFF vs HFF/BW-755c treatment

In this paper we extend this correlation to the expression of PPAR $\alpha$ , in which excess dietary fructose suppresses PPAR $\alpha$  but such suppression can be overcome by administration of these LOX/COX inhibitory compounds. The PPAR $\alpha$  is lipid activated transcription factor that plays a pivotal role in the transcription regulation of genes involved in lipid catabolism and lipoprotein metabolism. In hepatocytes and other tissues (e.g., heart) natural long chain fatty acids (ligand) activated PPAR $\alpha$  binds to peroxisome proliferators response element (PPRE) of DNA and increases the transcription of genes encoding enzymes involved in fatty acid oxidation (e.g., acyl-CoA oxidase and carnitine palmitoyltransferase) and lipoprotein (HDL and VLDL/TG) metabolism (e.g., apo-AI, AII, AV, CIII, and PTP and LPL) [19,37,38]. The outcome is an increase in hepatic fatty acid oxidation and ketogenesis, decreased tissue levels of lipids and protection against lipotoxicity. Our present data suggest that expression of PPAR $\alpha$  activity is primarily regulated at the level of transcription as determined by comparing RNA levels of hepatic PPAR $\alpha$  in chow-fed versus HFF animals. The fact that the LOX/COX inhibitors resulted in restored levels of hepatic PPAR $\alpha$  RNA suggests that these compounds are influencing sig-

nalling pathways that regulate PPAR $\alpha$  transcription. Beier et al have shown that TNF $\alpha$  downregulates expression of hepatic PPAR $\alpha$  RNA [39]. We and others have demonstrated that the HFF diet induces a TNF $\alpha$ -like stress response through the JNK pathway [18,36]. Therefore a likely mechanism by which hypertriglyceridemia is elicited in the HFF model is as follows:

1. Consumption of high levels of dietary fructose leads to activation of the JNK/AP-1 stress response pathway.
2. The activation of the stress response suppresses PPAR $\alpha$  expression in the liver;
3. Suppression of PPAR $\alpha$  disrupts normal lipid homeostasis and metabolism;
4. The metabolism of fructose leads to an abundance of TG precursors that provide a source for TG synthesis; and
5. The fructose-mediated stress response may increase the expression of sterol regulatory element-binding protein-1c (SREBP-1c) [40,41], which activates the genes involved

in this seems to be unlikely possibility given the fact the cytokine TNF $\alpha$  is known to negatively regulate SREBP-1c expression [42,43] and that expression of lipogenic enzymes is also achieved through an SREBP-1c independent mechanism [44].

With this as a working model, the mechanism by which the LOX/COX inhibitors could act would be by their anti-inflammatory actions in the TNF $\alpha$  pathway. By preventing activation of the stress response pathways, PPAR $\alpha$  levels are not suppressed and therefore lipid homeostasis would not be disrupted. Therefore the excess fructose can be normally metabolized in other ways, such as being shunted into gluconeogenesis pathways.

## Conclusion

The current studies indicate that hypertriglyceridemia induced by high fructose feeding leads to major reduction in the steady-state levels of both PPAR $\alpha$  protein and mRNA. Treatment with lipoyxygenase/cyclooxygenase inhibitor compounds reversed the fructose-induced suppression of hepatic PPAR $\alpha$  expression. These compounds also up-regulated mRNA levels of ubiquitous PPAR isoform, PPAR $\beta/\delta$ . These anti-inflammatory agents have the therapeutic potential in the prevention and/or management of various aspects of the metabolic syndrome.

## List of Abbreviations

AP-1 – Activator Protein-1

BID – Twice daily

BW-755c – 4,5-Dihydro-1-(3-(trifluoromethyl)phenyl)-1H-pyrazol-3-amine

COX – cyclooxygenase

HFF – high fructose-fed diet

JNK – Janus Kinase

LO – lipoyxygenase

NDGA – nordihydroguaiaretic acid

PPAR – peroxidase proliferators-activated receptor

TG – triglyceride

TNF – Tumor Necrosis Factor

SREBP-1c – sterol regulatory element-binding protein-1c

## Competing interests

The author(s) declare that they have no competing interests.

## Authors' contributions

Both authors declare that they have made contributions to experimental design and analysis/interpretation of the data. Both authors have been involved in drafting the manuscript and have given their approval for the publication of this manuscript.

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## References

- Ginsberg H: **Treatment for patients with the metabolic syndrome.** *Am J Cardiol* 2003, **91**:29E-39E.
- Aude Y, Mego P, Mehta J: **Metabolic Syndrome: dietary interventions.** *Curr Opin Cardiol* 2004, **19**:473-479.
- Deen D: **Metabolic syndrome: time for action.** *Am Fam Physician* 2004, **69**:2875-2882.
- Reaven G: **Banting Lecture 1988: role for insulin resistance in human diseases.** *Diabetes* 1988, **37**:1595-1607.
- Moller D: **Metabolic Syndrome: a clinical and molecular perspective.** *Annu Rev Med* 2005, **56**:45-62.
- Reaven G: **Why syndrome X?: from Harold Himsworth to the insulin resistance syndrome.** *Cell Metabolism* 2005, **1**:9-14.
- Daskalopoulou S, Mikhailidis D, Elisaf M: **Prevention and treatment of the metabolic syndrome.** *Angiology* 2004, **55**:589-612.
- Davidson M: **Emerging therapeutic strategies for the management of dyslipidemia in patients with the metabolic syndrome.** *Am J Cardiol* 2004, **93**:3C-11C.
- Hawley J, Houmard J: **Introduction-preventing insulin resistance through exercise: a cellular approach.** *Med Sci Sports Exerc* 2004, **36**:1187-1190.
- Mazzone T: **Strategies in ongoing clinical trials to reduce cardiovascular disease in patients with diabetes mellitus and insulin resistance.** *Am J Cardiol* 2004, **93**:27C-31C.
- Sowers J, Frohlich E: **Insulin and insulin resistance: impact on blood pressure and cardiovascular disease.** *Med Clin North Am* 2004, **88**:63-82.
- Stone N: **Focus on lifestyle change and the metabolic syndrome.** *Endocrinol Metab Clin North Am* 2004, **33**:4983-4508.
- Zavaroni I, Chen Y, Reaven GM: **Studies of the mechanism of fructose-induced hypertriglyceridemia in the rat.** *Metabolism* 1982, **31**:1077-83.
- Hallfrisch J: **Metabolic effects of dietary fructose.** *FASEB J* 1990, **4**:2652-2660.
- Bantle JP, Raatz SK, Thomas W, Georgopoulos A: **Effects of dietary fructose on plasma lipids in healthy subjects.** *Am J Clin Nutr* 2000, **72**:1128-1134.
- Gross LS, Li L, Ford ES, Liu S: **Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment.** *Am J Clin Nutr* 2004, **79**:711-712.
- Scribner KA, Gadbois TM, Gowri M, Azhar S, Reaven GM: **Masoprol decreases serum triglyceride concentrations in rats with fructose-induced hypertriglyceridemia.** *Metabolism* 2000, **49**:1106-1110.
- Wei Y, Pagliassotti MJ: **Hepatospecific effects of fructose on c-jun NH2-terminal kinase: implications for hepatic insulin resistance.** *Am J Physiol Endocrinol Metab* 2004, **287**:E926-E933.
- Desvergne B, Wahli W: **Peroxisome proliferators-activated receptors: nuclear control of metabolism.** *Endocr Rev* 1999, **20**:649-688.
- Chawla A, Repa J, Evans R: **Nuclear receptors and lipid physiology: opening the x-files.** *Science* 2001, **294**:1866-1870.

21. Kliewer SA, Xu HE, Lambert MH, Willson TM: **Peroxisome proliferators-activated receptors: from genes to physiology.** *Recent Prog Horm Res* 2001, **56**:239-263.
22. Berger JJ, Moller DE: **The mechanism of action of the PPARs.** *Annu Rev Med* 2002, **53**:409-435.
23. Corton J, Andersen S, Stauber A: **Central role of peroxisome proliferators-activated receptors in the actions of peroxisome proliferators.** *Annu Rev Pharmacol Toxicol* 2000, **40**:491-518.
24. Schoonjans J, Staels B, Auwerx J: **Role of the peroxisome proliferators-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression.** *J Lipid Res* 1996, **37**:907-925.
25. Lee C-H, Olson P, Evans R: **Minireview: lipid metabolism, metabolic disease, and peroxisome proliferators-activated receptors.** *Endocrinology* 2003, **144**:2201-2207.
26. Ferre P: **The biology of peroxisome proliferators-activated receptors: Relationship with lipid metabolism and insulin sensitivity.** *Diabetes* 2004, **53**:S43-S50.
27. Li A, Glass C: **PPAR- and LXR-dependent pathways controlling lipid metabolism and development of atherosclerosis.** *J Lipid Res* 2004, **45**:2161-2173.
28. Matsui H, Okumura K, Kawakami K, Hibino M, Toki Y, Ito T: **Improved insulin sensitivity by bezafibrate in rats: relationship to fatty acid composition of skeletal muscle triglycerides.** *Diabetes* 1997, **46**:348-353.
29. Guerre-Millo M, Gervois P, Raspe E, Madsen L, Poulin P, Derudas B, Herbert JM, Winegar DA, Wilson TM, Fruchart JC, Berge RK, Staels B: **Peroxisome proliferators-activated receptor alpha activators improve insulin sensitivity and reduce adiposity.** *J Biol Chem* 2000, **278**:16638-16642.
30. Nagai Y, Nishio Y, Nakamura T, Maegawa H, Kikkawa R, Kashiwagi A: **Amelioration of high fructose-induced metabolic derangements by activation of PPAR $\alpha$ .** *Am J Physiol Endocrinol Metab* 2001, **282**:E1180-E1190.
31. Ye J-M, Doyle PJ, Iglesias MA, Watson DG, Cooney GJ, Kraegen EW: **Peroxisome proliferators-activated receptor (PPAR)-alpha activation lowers muscle lipids and improves muscle sensitivity in high fat fed rats: comparison with PPAR-gamma activation.** *Diabetes* 2001, **50**:411-417.
32. Yajima K, Hirose H, Fujita H, Seto Y, Fujita H, Ukeda K, Miyashita K, Kawai T, Yamamoto Y, Ogawa T, Yamada T, Saruta T: **Combination therapy with PPAR gamma and PPAR alpha agonists increases glucose-stimulated insulin secretion in db/db mice.** *Am J Physiol Endocrinol Metab* 2003, **284**:E966-E971.
33. Gowri MS, Reaven GM, Azhar S: **Masoprocol lowers blood pressure in rats with fructose-induced hypertension.** *Am J Hypertens* 1999, **12**:744-746.
34. Tercyak AM: **Determination of cholesterol and cholesterol esters.** *J Nutr Biochem* 1991, **2**:181-192.
35. Kraemer FB, Shen W-J, Natu V, Patel S, Osuga J-I, Ishibashi S, Azhar S: **Adrenal neutral cholesteryl ester hydrolase: Identification, subcellular distribution, and sex differences.** *Endocrinology* 2002, **143**:801-806.
36. Kelley G, Allan G, Azhar S: **High dietary fructose induces a hepatic stress response resulting in cholesterol and lipid dysregulation.** *Endocrinology* 2004, **145**:548-555.
37. Mandard S, Muller M, Kersten S: **Peroxisome proliferators-activated receptor alpha target genes.** *Cell Mol Life Sci* 2004, **61**:393-416.
38. Fatehi-Hassanabad Z, Chan C: **Transcriptional regulation of lipid metabolism by fatty acids: a key determinant of pancreatic beta-cell function.** *Nutr Metab* 2005, **2**:1-12.
39. Beier K, Volkl A, Fahimi H: **TNF-alpha downregulates the peroxisome proliferator activated receptor-alpha and the mRNAs encoding peroxisomal proteins in rat liver.** *FEBS Lett* 1997, **412**:385-387.
40. Shimomura I, Matsuda M, Hammer RE, Bashmakov Y, Brown MS, Goldstein JL: **Decreased IRS-2 and increased SREBP-1C lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice.** *Mol Cell* 2000, **6**:77-86.
41. Taniguchi C, Ueki K, Kahn C: **Complementary roles of IRS-1 and IRS-2 in the hepatic regulation of metabolism.** *J Clin Invest* 2005, **115**:718-727.
42. Roche H, Noone E, Sewter C, Mc Bennett S, Savage D, Gibney MJ, O'Rahilly S, Vidal-Puig AJ: **Isomer-dependent metabolic effects of conjugated linoleic acid: insights from molecular markers sterol regulatory element-binding protein-1C and LXR alpha.** *Diabetes* 2002, **51**:2037-2044.
43. Sewter C, Berger D, Considine RV, Medina G, Rochford J, Ciaraldi T, Henry R, Dohm L, Flier JS, O'Rahilly S, Vidal-puig AJ: **Human obesity and type 2 diabetes are associated with alteration in SREBP1 isoform expression that are reproduced ex vivo by tumor necrosis factor alpha.** *Diabetes* 2002, **51**:1035-1041.
44. Matsuzaka T, Shimano H, Yahagi N, Amemiya-Kudo M, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Tomita S, Sekiya M, Hasty A, Nakagawa Y, Sone H, Toyoshima H, Ishibashi S, Osuga J-I, Yamada N: **Insulin-independent induction of sterol regulatory element-binding protein-1c expression in the livers of streptozotocin-treated mice.** *Diabetes* 2004, **53**:650-659.

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