A BACKTRANSLATIONAL STUDY OF THE SELECTIVE 5-HT$_{2C}$ AGONIST LORCASERIN IN TWO RAT OBESITY MODELS

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Introduction

The publication of multiple clinical reports detailing the clinical effects of the recently approved 5-HT$_{2C}$ agonist lorcaserin (LOR) in obese patients, provides opportunity to back-translate to preclinical obesity models. We have investigated the effect of LOR administered twice daily for 28 days in two rat obesity models, (1) the diet induced obesity (DIO) model, and (2) the Zucker rat model. In each study the model was first characterized based on body weight and composition using non-invasive Quantitative Magnetic Resonance (QMR) technology and plasma lipid profile, relative to controls, before drug treatment began. Primary efficacy endpoints were change in body weight, body fat composition (DIO study only), and plasma lipid profile. Secondary efficacy measures of food and water intake were also recorded. At the completion of the treatment phase in the DIO study, the effect of 28 day LOR treatment on cardiac function was assessed using echocardiography.

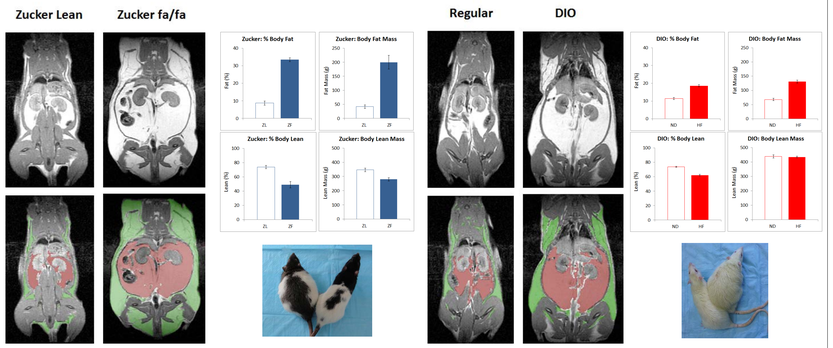
Characterisation of the DIO and Zucker rat as models of obesity

Diet-induced obesity model:: Male, Sprague-Dawley rats, 7 weeks age (body weight range: 170-200g) at the start of the study were randomly allocated into high fat (DIO group; Research diet D12492; 5.24 kcal/g) or regular diet (Lab Diet 5001: 4.07 kcal/g) groups.
Zucker rat obesity model: Male, Zucker rats (Charles River, strain code 185, Crl: ZUC-Lepr fa) and their lean controls (strain code 186) were acquired at 10 weeks age. Animals were fed regular diet (Lab Diet 5001: 4.07 kcal/g) throughout lifespan.

All animals were singly housed in a temperature and humidity controlled environment under a 12h light:dark cycle (lights on 05:00 – 17:00h) throughout the study. A variety of measures including QMR and MRI imaging, behaviour and clinical biomarkers (blood cholesterol, triglycerides, glucose) were made to characterise each obesity model. A comparative summary is presented below.

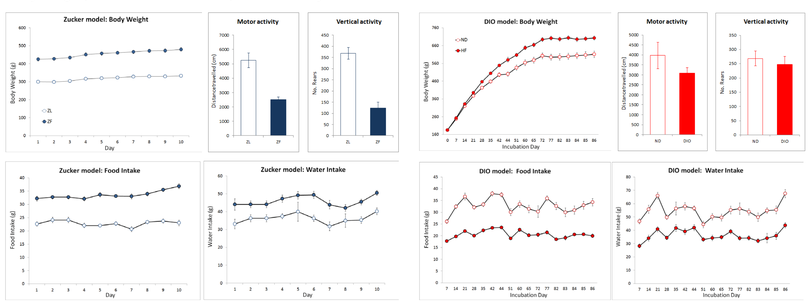
	Zucker model	DIO model
Body mass	~35% > vs. ZL controls	~20% > vs. ND controls
Body composition (QMR)	Fat mass ~35% vs. ~7% ZL controls; Lean mass ~56% vs. 80% ZL controls	Fat mass ~20% vs. ~10% ND controls; Lean mass ~60% vs. 75% ND controls
Food and water intake	Food + water intake elevated vs. ZL controls	Food and water intake reduced vs. ND controls (based on D12492 diet)
Lipid profile	Triglycerides + Cholesterol markedly elevated vs. ZL controls	Triglycerides + Cholesterol modestly elevated vs. ND controls
Blood Insulin levels	Severe hyperinsulinaemia	Normal insulin levels
Blood glucose levels	Hyperglycaemia (fasted)	Normal blood glucose levels (fasted)
Glucose tolerance test	Elevated blood (glucose) following oral load - ~2x AUC vs. ZL controls	Elevated blood (glucose) following oral load - ~1.5x AUC vs. ND controls
Behavioural profile	Sedentary, hypoactive vs. ZL controls	Almost equivalent activity compared to ND controls

Body composition: QMR and MRI imaging



MRI images taken of both the Zucker and DIO rats compared to their respective controls show an increase in both subcutaneous fat (green) and particularly visceral fat (red). Quantification of fat content was made using QMR imaging which measures total fat and lean mass, using this technology it is not possible to distinguish between the visceral or subcutaneous fat compartments. Zucker rats have substantially higher % body fat by comparison to DIO rats. QMR measures were taken when rats were approximately 5 months (DIO group; 3 months on diet) or 7 months (Zucker group) of age.

Body weight, food and water intake, motor activity



Measurement of daily body weight, food and water intake, motor activity in Zucker rats and controls. Measures were taken from Zucker rats age 3 months. Throughout the course of their lifespan the Zucker rats weighed approximately 30-40% more than their controls, and consumed approximately 30-40% more food (standard lab diet 5001). Zucker rats have significantly lower motor activity relative to controls. DIO rats were assessed weekly over a 3 month span starting from 6 weeks age. Divergence in body weight emerged after 4 weeks and reached plateau by 3 months. Under the high calorific diet used, DIO rats consumed less food and water compared to controls fed regular diet. After 3 months on diet, general activity was only slightly less that of controls.

Blood biomarkers - lipid profile

		Blood glucose (fasted)	OGTT (AUC)	Cholesterol (mmol/l)	Triglycerides (mmol/l)	Insulin (pmol/l)
Zucker lean	3 months	5.5±0.2	1688±42	1.9±0.1	1.5±0.2	662±274
	9 months	7.5±0.4	2063±110	4.0±0.5	2.3±0.4	ND
Zucker fa/fa	3 months	7.7±0.3*	2723±125*	3.5±0.2*	10.5±1.0*	4538±1947*
	9 months	10.6±0.5*	3019±227*	13.1±1.4*	9.9±2.2*	ND

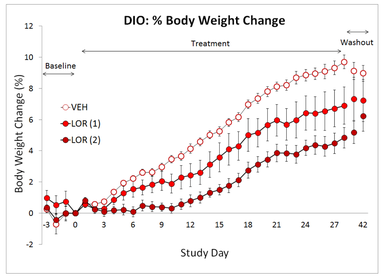
		Blood glucose (fasted)	OGTT (AUC)	Cholesterol (mmol/l)	Triglycerides (mmol/l)	Insulin (pmol/l)
Regular	6 weeks	5.9±0.3	1733±43	2.2±0.1	2.2±0.2	ND
	12 weeks	6.6±0.3	1940±32	2.4±0.1	2.4±0.3	ND
DIO	6 weeks	5.8±0.3	1957±45*	2.9±0.1*	2.0±0.2	ND
	12 weeks	6.3±0.2	2194±79*	3.2±0.1*	2.0±0.2	ND

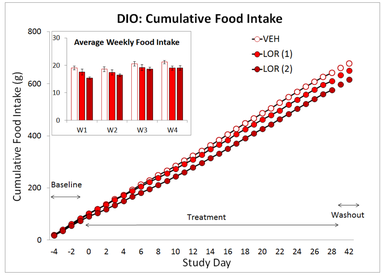
Measurement of various blood biomarkers in Zucker and DIO models. Zucker rats over 3-12 months age have considerably higher levels (3-10x) of cholesterol and triglyceride content compared to their controls. Blood [insulin] and [glucose] also significantly higher consistent with the Zucker being a model of type 2 diabetes. In contrast, equivalent measures in the DIO model are more subtle. No hyperglycaemia was evident although OGTT response elevated. Plasma cholesterol (but not TG) modestly increased.

Lorcaserin: DIO model

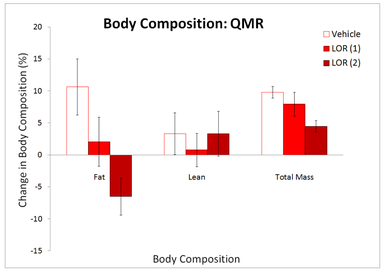
Male Sprague-Dawley rats (approximate age 5 months) were placed on a high fat diet (Research diet D12492; 5.24 kcal/g) for 3 months prior to study. DIO rats were then randomly allocated into 3 groups (VEH: N=10; LOR (1): N=8; LOR (2): N=8). Treatments were administered SC, 2x daily. Daily food/water intake, and body weight was recorded. At the completion of the 28 day treatment phase, blood was collected to determine plasma lipid content. QMR imaging was conducted pre- and post-treatment. Lorcaserin doses were based on previously published work (Higgins et al, 2012; 2013).

A. Efficacy phase





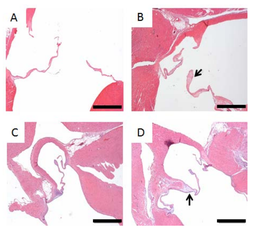
		Blood glucose (fasted)	OGTT (AUC)	Cholesterol (mmol/l)	Triglycerides (mmol/l)
Vehicle	Pre	6.19±0.30	-	3.35±0.16	1.95±0.20
	28 day	6.11±0.24	2036±56	3.13±0.09	2.29±0.34
Lorcaserin 1 mg/kg	Pre	6.46±0.25	-	3.17±0.19	1.91±0.21
	28 day	6.16±0.19	2133±117	2.74±0.11 *	1.68±0.12
Lorcaserin 2 mg/kg	Pre	6.40±0.42	-	3.06±0.26	2.13±0.40
	28 day	5.85±0.17	1992±62	2.71±0.11 *	1.80±0.30

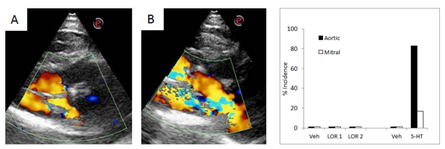


Primary efficacy measures over the 28 day treatment phase were (A) body weight change from pretreatment baseline, (B) cumulative daily food intake, (C) total (visceral and subcutaneous) body fat and lean mass measured by QMR imaging from pretreatment baseline, (D) lipid biomarkers.
Lorcaserin dose dependently reduced body weight change by 3.0-5.2% compared to vehicle controls. This change was through a selective decrease in fat vs. lean mass. Effect on food intake was most pronounced on week 1. Modest effects on plasma lipid biomarkers were also noted.

B. Safety phase

At the completion of the 28 day treatment phase, the rats underwent echocardiography (MyLab Alpha, Esaote Canada, Georgetown, ON). A left parasternal approach was used and long and short axis views of the heart were captured. Colour flow and pulsed Doppler interrogation of the aortic and mitral valves was performed. For comparison a separate group of rats were treated for 28 days with 5-HT (4 days 75mg/kg IP x 1 daily, 24 days 60mg/kg IP x 1 daily; Elangbam et al, 2008). After these studies, blood was collected for clinical chemistry and cardiac tissue collected for histopathology.





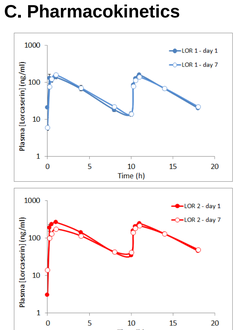
Representative echocardiographic images showing normal flow through the aortic valve with normal antegrade flow represented in yellow (A) and turbulent regurgitant flow in blue in an abnormal valve (B). Image (A) was taken from a vehicle treated rat, (B) from a 28 day 5-HT treated rat. (C) Graph to summarise the percent incidence of regurgitant flow across the aortic and mitral valves from all study animals.

(A) Normal aortic valve, taken from a lorcaserin vehicle control rat fed high fat diet. (B) Aortic valve, taken from a lorcaserin vehicle control rat fed high fat diet. Note the mild multifocal thickening of valve spongiosa by moderately cellular myxomatous and collagenous matrix. (C) Normal aortic valve, taken from a vehicle control rat fed regular diet. (D) Aortic valve, taken from a 28 day 5-HT treated rat fed regular diet. The arrow denotes expansion of spongiosa at base of valve cusp by myxomatous stroma. Scale bar = 1mm.

	Clinical chemistry								Percent organ weights			
	ALB	GLOB	AST	ALT	ALP	TBIL	BUN	CRE	Brain	Kidneys	Heart	Whole Liver
Vehicle	40±2	40± 2	117±20	59±13	176±15	3.2±0.8	5.9±0.5	50.6±5.7	0.30±0.01	0.46±0.02	0.26±0.01	2.64±0.09
Lorcaserin 1 mg/kg	40±2	37±2	106±10	30±3	175±13	3.4±0.6	5.4±0.5	42.0±2.4	0.29±0.01	0.49±0.01	0.25±0.01	2.79±0.12
Lorcaserin 2 mg/kg	33±4	39±3	90±16	31±7	160±24	2.6±0.6	5.1±0.4	39.6±8.7	0.30±0.02	0.54±0.03	0.27±0.01	2.61±0.09

Summary of safety markers. No measures from lorcaserin treated rats were different to controls. All values within normal range for Sprague-Dawley rats.

C. Pharmacokinetics



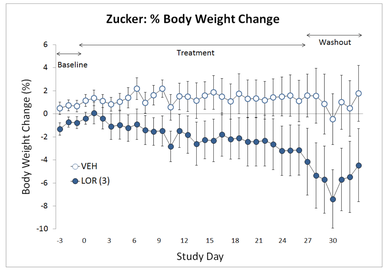
A cohort of DIO rats were treated 2x daily with either lorcaserin at 1 mg/kg SC or 2 mg/kg SC for one week (N=4 rats per treatment group), i.e to match the treatment regimen used in the DIO study. On DAY 1 and DAY 7, blood was collected at multiple timepoints (0.25h, 0.5h, 1h, 4h, 8h postdose) from the saphenous vein for determination of lorcaserin concentration. Bioanalytical analyses were conducted using an LC-MS/MS system.

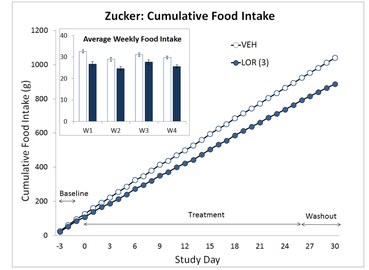
		C_{max} (ng/ml)		C_{max} (ng/ml)		$T_{1/2}$ (h)		AUC_{0-8h} (h*ng/ml)	
		AM	PM	AM	PM	AM	PM	AM	PM
Lorcaserin 1 mg/kg SC	Day 1	-	14±4	143±35	158±17	2.6±0.2	2.3±0.2	492±67	582±61
	Day 7	6+1	14±3	159±15	139±18	2.4±0.2	2.5±0.1	599±50	560±51
Lorcaserin 2 mg/kg SC	Day 1	-	34±5	264±22	244±21	2.6±0.1	2.6±0.2	1105±96	1069±50
	Day 7	14+4	54±12	227±17	212±19	2.8±0.2	2.9±0.3	1089±92	952±116

Lorcaserin: Zucker model

Male Zucker rats (approximate age 7 months) were randomly allocated into 2 groups, one designated vehicle control (N=6 rats), the other lorcaserin (LOR 3 mg/kg SC; N=6). Following a 3 day baseline period, the rats were treated 2x daily for 28 days. Daily food and water intake, and body weight was recorded. At the completion of the 28 treatment phase, blood was collected to determine plasma lipid content. No QMR studies were conducted in these rats.

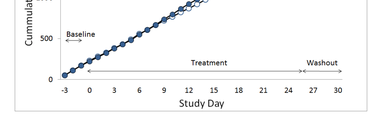
A. Efficacy phase





		Blood glucose (fasted)	OGTT (AUC)	Cholesterol (mmol/l)	Triglycerides (mmol/l)	Insulin (pmol/l)
Vehicle	Pre	8.6±0.8	2676±216	15.3±1.8	36.1±5.0	369±134
	28 day	11.3±1.5	3091±388	16.5±2.8	37.9±7.4	700±196
Lorcaserin 3 mg/kg	Pre	7.9±0.5	3108±313	13.8±3.1	36.4±10.1	632±147
	28 day	10.2±0.6	3011±209	10.3±1.4*	15.2±4.1*	1013±362

Primary efficacy measures over the 28 day treatment phase were (A) body weight change from pretreatment baseline, (B) daily food intake, (C) daily water intake, (D) lipid biomarkers.
Lorcaserin dose dependently reduced body weight change by ~4% compared to vehicle controls. The effect of lorcaserin to reduce food intake was consistent throughout the 4 week treatment period. Water intake showed a trend to increase over this period. Lorcaserin significantly reduced plasma triglyceride and cholesterol levels compared to vehicle treated rats at 28 days. No clear trends were seen on glucose levels or OGTT.



Summary and conclusions

1. **In the DIO rat model, lorcaserin administered at a dose regimen of 1-2mg/kg SC x2daily (equivalent to plasma [drug] 14-160ng/ml [1mg/kg], 30-260ng/ml [2mg/kg]) produced a decline in % body weight change of 3.0-5.2% compared to vehicle controls.**
2. **Lorcaserin produced this weight change through reduction in fat, not lean mass. Modest effects on lipid biomarkers (blood cholesterol) were found.**
3. **In the Zucker rat model, lorcaserin at a dose regimen of 3mg/kg SC x2daily produced a decline in % body weight of ~4% compared to vehicle controls. Effects on food intake and lipid biomarkers were also noted.**
4. **In sum, these studies show an effect of lorcaserin in both the DIO and Zucker models, which in the context of the primary endpoint measure of % body weight change was similar to that reported clinically (i.e. 3.2%; see Chan et al (2013) Lorcaserin Phase III obesity trial meta-analysis).**
5. **No evidence for cardiac valvulopathy was evident following this dose regimen of lorcaserin. 28 day treatment with 5-HT did produce a detectable effect.**
6. **The present studies highlight the translational value of obesity models such as the DIO model, and suggest that assuming consideration is paid to non-specific drug effects such as malaise, the DIO and Zucker models show reasonable forward translational value to help predict clinical outcomes of a new chemical entity (see also Vickers et al, 2012). The chronic nature of these study designs provide opportunity to combine with safety assessments.**

References

Chan EW, He Y, Chui CS, Wong AY, Lau WC, Wong IC (2012) Efficacy and safety of lorcaserin in obese adults: a meta-analysis of 1-year randomized controlled trials (RCTs) and narrative review on short-term RCTs. Obesity Rev. 14: 383-392.

Elangbam CS, Job LE, Zadrozny LM, Barton JC, Yoon LW, Gates LD, Slocum N. (2008) 5-hydroxytryptamine (5HT)-induced valvulopathy: compositional valvular alterations are associated with 5HT2B receptor and 5HT transporter transcript changes in Sprague-Dawley rats. Exp Toxicol Pathol. 60: 253-262.

Higgins GA, Silenieks LB, Rossmann A, Rizos Z, Noble K, Soko AD, Fletcher PJ (2012) The 5-HT$_{2C}$ receptor agonist lorcaserin reduces nicotine self-administration, discrimination, and reinstatement: relationship to feeding behavior and impulse control. Neuropsychopharmacology 37:1177-1191

Higgins GA, Silenieks LB, Lau W, de Lannoy IA, Lee DK, Izhakova J, Coen K, Le AD, Fletcher PJ (2013) Evaluation of chemically diverse 5-HT$_{2C}$ receptor agonists on behaviours motivated by food and nicotine and on side effect profiles. Psychopharmacology 226:475-490.

Vickers SP, Jackson HC, Cheetham SC (2011) The utility of animal models to evaluate novel anti-obesity agents. Br J Pharmacol. 164:1248-1262.