

The dual PPAR α/γ agonist, ragaglitazar, improves insulin sensitivity and metabolic profile equally with pioglitazone in diabetic and dietary obese ZDF rats

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1 In 6- and 10-week-old obesity-prone (*fa/fa*) Zucker diabetic fatty (ZDF) rats, effects of prevention and intervention therapies, respectively, were compared between PPAR α/γ agonist, ragaglitazar (RAGA) and separate PPAR γ and α agonists, pioglitazone (PIO) and bezafibrate (BF).

2 In a separate study, lean (+ / +) ZDF rats fed highly palatable chow to induce dietary obesity and insulin resistance were treated similarly. To test insulin-secretory capacity, all animals underwent a hyperglycaemic clamp.

3 Insulin sensitivity was improved equally by RAGA and PIO in *fa/fa* rats subjected to both prevention and intervention treatments (e.g., prevention HOMA-IR: –71 and –72%, respectively), as was hyperglycaemia (both –68%). BF had no effect on either parameter in any study. Plasma lipids were markedly reduced (by 48–77%) by RAGA in all studies, equivalent to PIO, but to a greater extent than BF.

4 RAGA improved β -cell function (HOMA- β) more than three-fold with prevention and intervention therapies, whereas PIO showed improvement only in intervention therapy. Consistent with improved insulin sensitivity, glucose infusion rate during the clamp was 60% higher in RAGA-treated animals subjected to prevention therapy, but there was little additional insulin-secretory response, suggesting that insulin secretion was already maximal.

5 Thus, RAGA and PIO equally improve metabolic profile in ZDF rats, particularly when administered early in the course of diabetes. They also improve β -cell function, although this is better demonstrated through indices incorporating fasting insulin and glucose concentrations than through the hyperglycaemic clamp technique in this model.

British Journal of Pharmacology (2005) **144**, 308–316. doi:10.1038/sj.bjp.0706041

Published online 17 January 2005

Keywords: Dietary obesity; hyperglycaemic clamp; insulin secretion; insulin resistance; PPAR α/γ agonist; pioglitazone; ragaglitazar; Zucker diabetic fatty rat

Abbreviations: BF, bezafibrate; DIO, dietary obese; DIO-CON, dietary obese control; FFA, free fatty acid; GIR, glucose infusion rate; HOMA, homeostasis model assessment; HPD, highly palatable diet; PIO, pioglitazone; PPAR, peroxisome proliferator-activated receptor; RAGA, ragaglitazar; SC, subcutaneous; S-S, steady state; TG, triglyceride; TZD, thiazolidinedione

Introduction

Drugs which treat type II diabetes should preferably improve insulin resistance, correct dyslipidaemia and preserve pancreatic β -cell function, the main factors characterizing this progressive syndrome (Reaven, 1988; Unger, 1995). Agonists of the γ isoform of the peroxisome proliferator-activated receptor (PPAR) family, such as the thiazolidinediones (TZDs), stimulate genes which favour storage of triglycerides (TGs), thus lowering circulating free fatty acid (FFA) concentrations (Moore *et al.*, 2001). This shift from FFAs to glucose as a fuel substrate is one mechanism whereby these drugs improve insulin sensitivity in peripheral tissues (Spiegelman, 1998). Furthermore, TZDs have been shown to improve β -cell survival and function *in vitro* and prevent TG

accumulation in islets (Shimabukuro *et al.*, 1998; Murakami *et al.*, 1999; Finegood *et al.*, 2001; Brand *et al.*, 2003; Pickavance *et al.*, 2003; Yajima *et al.*, 2003).

PPAR isoforms exhibit distinct tissue distributions, with PPAR γ being expressed predominantly in adipose tissue, and PPAR α , the target for the fibrate group of lipid-lowering drugs, being most abundant in liver (Braissant *et al.*, 1996; Fajas *et al.*, 1997). Drugs with activity at both PPAR α and γ are able to combine insulin-sensitising and lipid-lowering effects (Murakami *et al.*, 1998; 1999; Etgen *et al.*, 2002; Ljung *et al.*, 2002; Brand *et al.*, 2003; Ye *et al.*, 2003), but whether this is associated with better *in vivo* preservation of β -cell function than obtained with γ agonists alone is unknown.

Both ZDF and Zucker obese rats have an identical homozygote mutation (*fa/fa*) in the gene encoding for the leptin receptor that leads to obesity and insulin resistance. In

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Published online 17 January 2005

addition, the ZDF strain has a genetic defect in β -cell gene expression (Griffen *et al.*, 2001) that leads to the development of diabetes in this animal model. The ZDF rat is prone to β -cell failure in response to weight gain and is a useful model of type II diabetes (Unger, 1997). Therefore, we used this ZDF rat model to test the effects of treatments with ragaglitazar (RAGA), a dual PPAR α/γ agonist, pioglitazone (PIO), a PPAR γ agonist, and bezafibrate (BF), a PPAR α agonist, early and late in the development of diabetes. A third group of lean (+/+) ZDF rats, genetically equivalent to their obese counterparts, albeit without the leptin receptor mutation (Peterson, 1994), were treated with the above-mentioned compounds after making them obese by feeding them a highly palatable diet (HPD). This had the secondary aim of investigating whether obesity induced by such a diet might reveal the genetic susceptibility to diabetes in the ZDF rat.

Methods

Animals and treatment

The prevention and intervention studies were initiated in 6-week-old pre-diabetic and in 10-week-old diabetic (*fa/fa*) ZDF rats, respectively. Rats were singly housed, with free access to pelleted chow (CRM (P), Biosure, Cambridge, U.K.) and water. They were randomly assigned to treatment groups ($n=8$ per group) and gavaged once daily for 28 days with either vehicle (CON, 1% carboxymethylcellulose, 1 ml kg⁻¹), RAGA (Novo Nordisk A/S, Denmark; 1.5 mg kg⁻¹), PIO (Actos[®], Takeda, Japan; 30 mg kg⁻¹) or BF (Sigma-Aldrich Co., Ltd, Poole, Dorset, U.K.; 100 mg kg⁻¹). Dosages were selected on the basis of previous findings on efficacy (Haubenwallner *et al.*, 1995; Matsui *et al.*, 1997; Arakawa *et al.*, 1998; Kawamori *et al.*, 1998; Cabrero *et al.*, 1999; Lohray *et al.*, 2001; Sauerberg *et al.*, 2002; Brand *et al.*, 2003; Chakrabarti *et al.*, 2003; Skrumsager *et al.*, 2003; Ye *et al.*, 2003; Saad *et al.*, 2004). Age-matched vehicle-treated lean (+/+) rats (LEAN) served as nondiabetic controls. Importantly, all lean ZDF rats used in these experiments were genotyped and found to be homozygous for the wild-type *fa* allele and thus did not have a defect in the leptin receptor.

In the diet-induced obesity (DIO) study, 40 6–7-week-old lean (+/+) male ZDF rats (148 ± 2 g; Genetic Models, Inc., Indianapolis, IN, U.S.A.) were housed in groups of four. Eight controls (CHOW) were fed pelleted chow and 32 rats were fed HPD for 10 weeks. The HPD consisted of 33% powdered chow, 33% condensed milk (Nestlé U.K. Ltd, York, U.K.) and 7% sucrose (Tate & Lyle, London, U.K.) by weight, with remainder water, as described previously (Wilding *et al.*, 1992). After 6 weeks on the HPD, rats underwent intervention treatment for 4 weeks with either vehicle (CON), RAGA, BF or PIO at the doses mentioned above.

Animals followed a 12-h light–dark cycle. Body weight and food intake were measured daily. U.K. Home Office regulations (Animal Scientific Procedures Act, 1986) were adhered to throughout.

Hyperglycaemic clamp

After the 28-day dosing period, and after a 12-h fast, all rats were subjected to a hyperglycaemic clamp. Rats were deeply

anaesthetised using a combination of Hypnorm[™] and Valium[®] (fentanyl/fluanisone (Janssen Pharmaceutical Ltd, Oxford, U.K.) and diazepam (Roche, Welwyn Garden City, Herts, U.K.); both 0.8 ml kg⁻¹, i.m.), followed by additional doses of Hypnorm[™] (~0.1 ml kg⁻¹, i.m.) every 30–40 min. Following catheterisation of the right jugular vein, a 500- μ l sample of blood was taken for later measurement of pre-clamp insulin concentrations by RIA (Pharmacia/Upjohn Diagnostics U.K., Lewes, Sussex, U.K.) and glucose by diagnostic kit (Roche Diagnostics, Milton Keynes, U.K.). Baseline tail-vein blood glucose concentration was measured with an electrochemical meter and electrodes (Precision Q.I.D.[™] Sensor and Precision Plus electrodes, Medisense[®], Abingdon, Oxon, U.K.).

A priming infusion of 20% D-glucose in water (w v⁻¹) was then begun at a rate of 100 mg kg min⁻¹, and was gradually reduced to 70, 57, 46 and 35 mg kg min⁻¹ every 2 min for the first 11 min of infusion, based on the method of Vettor *et al.* (1998). The glucose infusion rate (GIR) was then adjusted according to blood glucose readings taken every 10 min during a further 70-min maintenance period to clamp the glucose concentration at 24 mM. Tail-vein blood (~100 μ l) was collected at these time points for later measurement of plasma insulin levels. These values (μ U ml⁻¹) were plotted against time (min) and the area under the curve (AUC) calculated for the early- ($t=0$ –11 min) and late-phase ($t=20$ –90 min) insulin-secretory responses, as described previously (Giaccari *et al.*, 1995). Pre-clamp indices of insulin resistance and insulin secretion were calculated using homeostasis model assessment (HOMA) formulae (Matthews *et al.*, 1985): HOMA-IR = fasting insulin (μ U ml⁻¹) \times fasting glucose (mM)/22.5; HOMA- β = 20 \times fasting insulin (μ U ml⁻¹)/(fasting glucose (mM)-3.5).

Metabolic data and fat mass

At the end of the clamp, rats were killed by cardiac exsanguination. Post-clamp plasma concentrations of FFAs and TGs (Boehringer Mannheim, Milton Keynes, Bucks and Sigma-Aldrich Co., Ltd, Poole, Dorset, U.K.) were measured by diagnostic kits, and those of insulin and leptin by RIA (Biogenesis, Poole, Dorset, U.K.).

Body fat content was estimated by weighing dissected subcutaneous (SC, from below the sternum), perirenal and epididymal fat pads and expressing these as a percentage of final body weight.

Pancreatic insulin concentration

The pancreas was removed and snap-frozen in liquid nitrogen for later measurement of insulin content. Proteins were extracted from ~50-mg pieces of frozen pancreas by boiling in 0.5 ml of 0.1 M acetic acid for 5 min, cooling to room temperature and then sonicating for 30 s until well-homogenised. Protein was then pelleted by centrifugation at 14,000 r.p.m. for 5 min. A volume of 250 μ l of the supernatant was then neutralised with 215 μ l of 1 M NaOH, and insulin concentration then measured by RIA in the same way as for plasma concentration. The concentration of pancreatic insulin was then expressed per gram of tissue. As an estimate of insulin-secretory capacity, the ratio of pancreatic insulin content (nmol g⁻¹) to fasting plasma glucose (mM) was calculated.

Statistical analyses

Data are expressed as mean \pm s.e.m. Differences were considered statistically significant at $P < 0.05$ unless otherwise stated. Parameters were compared between diabetic (ZDF-CON) and LEAN controls (prevention and intervention studies) or between DIO-CON and CHOW (DIO study) by unpaired (two-sample) *t*-tests or Mann-Whitney test for non-normally distributed data. Significance of trend across obese groups was tested using Cuzick's test. The drug effect on each of the parameters was measured by comparing values in each of the treated ZDF or DIO groups with those in their obese control groups by one-way ANOVA followed by Dunnett's test for multiple comparisons with a control. Using the trapezoidal rule, insulin secretion ($\mu\text{U ml min}^{-1}$) was calculated as area under the curve (AUC) of plasma insulin concentration ($\mu\text{U ml}^{-1}$) during the 90-min hyperglycaemic clamp, and the incremental change from baseline was calculated. Steady-state (S-S) GIR was taken as the mean rate over the final 30 min of the clamp. All statistical analyses were carried out using StatsDirect statistical software (v. 2.3.8, © 1990–2001 StatsDirect Ltd).

Results

Prevention study in ZDF rats: effects on energy intake, body weight and body composition

After the prevention treatment period, CON rats were heavier than their LEAN counterparts ($+19\%$; 344 ± 6 vs 289 ± 3 g; $P < 0.0001$), with increased fat mass. RAGA and PIO, but not BF, increased food intake, doubled food efficiency and enhanced weight gain in these animals (Table 1).

Prevention effects on metabolic parameters

CON rats were insulin-resistant (HOMA-IR: $+763\%$; $P < 0.05$), had reduced β -cell function (HOMA- β : -68% ; $P < 0.01$), and about half the pancreatic insulin content of LEAN rats, although this latter value was not significant. RAGA and PIO significantly improved insulin sensitivity (both by 72% vs CON; $P < 0.01$), and there were trends toward improved β -cell function and increased pancreatic insulin content, both absolute and relative to plasma glucose, across treatments ($P = 0.0001$, 0.059 and 0.03 , respectively). Indeed, RAGA increased HOMA- β several-fold ($+607\%$; $P < 0.01$).

These rats also showed increased FFA and TG levels, which were reduced by all treatments (TGs only in the case of BF, although reduction in FFAs approached significance ($P = 0.08$)). Circulating leptin concentrations were approximately doubled by RAGA and PIO treatments (Table 2).

Desired S-S blood glucose concentration during the clamp could not be attained in the CON- and BF-treated groups because these animals were already hyperglycaemic beyond 24 mM (Figure 1a). Consequently, GIR was negligible. GIR in PIO- and RAGA-treated groups was normalised (Figure 1b), although there was no change in insulin-secretory response. The PIO-treated group showed enhanced insulin release throughout the clamp, but this was not significant (Figure 1c).

Intervention study in ZDF rats: effects on energy intake, body weight and body composition

Despite almost identical body weight to lean controls (-0.02% ; 345 ± 6 vs 348 ± 3 g; $P > 0.05$), CON rats had greater fat pad masses. Interestingly, whereas only PIO enhanced food intake, both PIO and RAGA induced weight gain to match that of LEAN rats. This is consistent with increased food efficiency of more than six-fold (both $P < 0.0001$; Table 1).

Table 1 Effects of PPAR agonists on cumulative energy intake, body weight and body composition in obese and lean ZDF rats

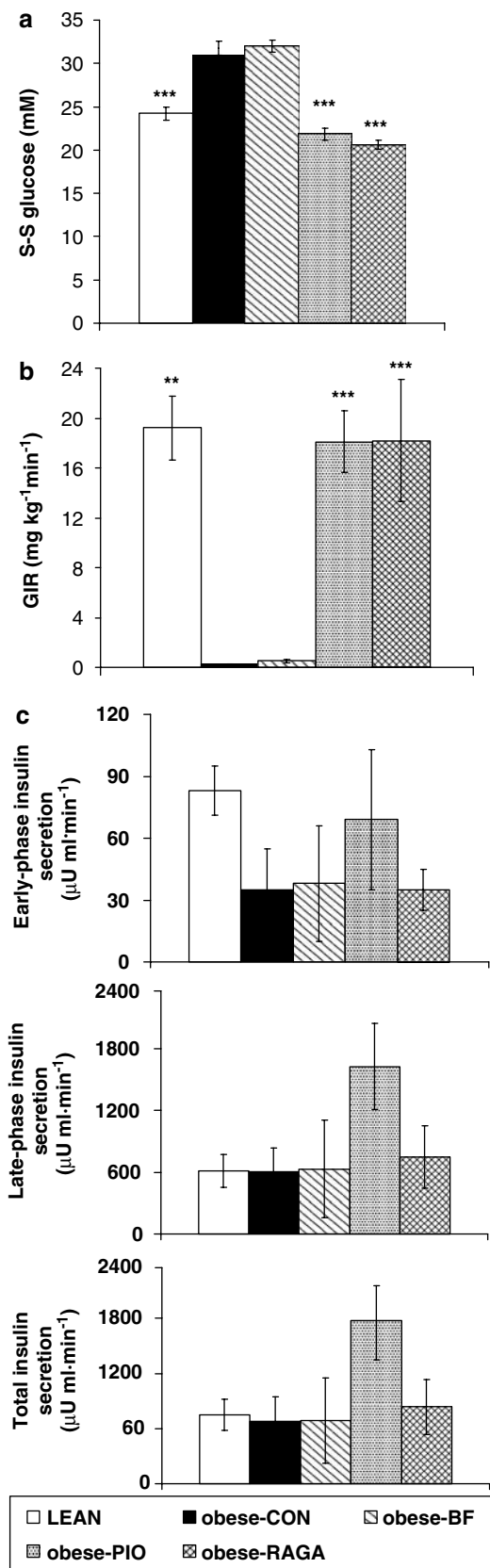
Treatment	Energy intake (kJ)	Food efficiency (g MJ ⁻¹)	Body weight gain (g)	% epididymal fat	% perirenal fat	% SC fat
<i>Prevention study in ZDF</i>						
CON	14,579 \pm 421	127 \pm 9	8.0 \pm 0.8	0.79 \pm 0.03	0.98 \pm 0.05	0.093 \pm 0.008
BF	15,037 \pm 655	122 \pm 10	7.7 \pm 1.2	0.75 \pm 0.02	0.93 \pm 0.05	0.111 \pm 0.014
PIO	17,583 \pm 262***	245 \pm 4***	13.4 \pm 0.4***	1.08 \pm 0.03***	1.30 \pm 0.08**	0.106 \pm 0.020
RAGA	17,863 \pm 250***	247 \pm 5***	13.2 \pm 0.4***	1.04 \pm 0.02***	1.26 \pm 0.06**	0.118 \pm 0.017
LEAN	9604 \pm 111**	113 \pm 6	10.9 \pm 0.7*	0.29 \pm 0.01***	0.27 \pm 0.02**	0.048 \pm 0.006**
<i>Intervention study in ZDF</i>						
CON	18,356 \pm 418	17 \pm 4	0.24 \pm 0.22	0.80 \pm 0.03	1.07 \pm 0.05	0.101 \pm 0.009
BF	19,232 \pm 286	22 \pm 4	0.05 \pm 0.23	0.80 \pm 0.03	1.05 \pm 0.03	0.096 \pm 0.010
PIO	20,071 \pm 457**	130 \pm 13***	6.19 \pm 0.86***	1.13 \pm 0.04***	1.34 \pm 0.02***	0.126 \pm 0.014
RAGA	19,777 \pm 738	132 \pm 22***	6.20 \pm 1.32***	1.10 \pm 0.02***	1.40 \pm 0.04***	0.142 \pm 0.015*
LEAN	10,127 \pm 179**	64 \pm 3***	5.38 \pm 0.17***	0.29 \pm 0.01***	0.34 \pm 0.03***	0.054 \pm 0.003**
<i>Intervention study in DIO</i>						
CON	9760 \pm 80	2.3 \pm 0.3	23 \pm 3	0.58 \pm 0.02	0.70 \pm 0.03	0.086 \pm 0.009
BF	10,573 \pm 230**	2.1 \pm 0.2	22 \pm 2	0.59 \pm 0.03	0.69 \pm 0.04	0.076 \pm 0.016
PIO	11,171 \pm 163***	4.4 \pm 0.2***	50 \pm 2***	0.78 \pm 0.04***	0.74 \pm 0.04	0.081 \pm 0.017
RAGA	11,146 \pm 232***	4.2 \pm 0.2***	46 \pm 2***	0.78 \pm 0.01***	0.86 \pm 0.02**	0.105 \pm 0.023
CHOW	8859 \pm 120***	1.5 \pm 0.3	14 \pm 3*	0.35 \pm 0.01***	0.39 \pm 0.02***	0.058 \pm 0.004*

Animals were treated for 28 days with ragaglitazar (RAGA, 1.5 mg kg⁻¹), pioglitazone (PIO, 30 mg kg⁻¹) and bezafibrate (BF, 100 mg kg⁻¹). Values are expressed as mean \pm s.e.m. ($n = 8$); * $P < 0.05$; ** $P < 0.01$; *** $P \leq 0.0001$ as compared to obese control (CON). Food efficiency = Δ body weight/ Σ energy intake. Fat pad mass expressed as weight of a single pad as % of terminal body weight.

Table 2 Effects of PPAR agonists on metabolic parameters in obese and lean ZDF rats

Treatment	Insulin ($\mu\text{U ml}^{-1}$)	Pre-clamp			Pancreatic insulin (nmol g^{-1})	Post-clamp			Leptin (ng ml^{-1})
		Glucose (mM)	HOMA-IR	HOMA- β (%)		Pancreatic insulin/plasma glucose	FFA (mM)	TG (mM)	
<i>Prevention study in ZDF</i>									
CON	16.2 \pm 1.8	22.8 \pm 1.5	16.4 \pm 2.3	17.8 \pm 2.9	2.8 \pm 0.8	0.10 \pm 0.03	1.23 \pm 0.10	3.97 \pm 0.77	6.5 \pm 0.6
BF	15.0 \pm 4.4	23.0 \pm 1.6	16.2 \pm 5.0	15.0 \pm 4.2	3.9 \pm 0.9	0.13 \pm 0.04	0.96 \pm 0.12	1.63 \pm 0.47**	6.6 \pm 0.6
PIO	13.3 \pm 0.9	7.4 \pm 0.7***	4.5 \pm 0.6**	71.6 \pm 12.2	5.8 \pm 2.1	0.60 \pm 0.21**	0.57 \pm 0.04***	0.91 \pm 0.10***	12.2 \pm 1.1***
RAGA	15.9 \pm 2.7	7.2 \pm 0.6***	4.7 \pm 0.7**	125.9 \pm 40.2**	5.2 \pm 0.9	0.56 \pm 0.15**	0.60 \pm 0.06***	1.11 \pm 0.09***	12.5 \pm 1.2***
LEAN	7.3 \pm 0.9**	6.3 \pm 0.5**	1.9 \pm 0.2*	56.1 \pm 12.1**	4.5 \pm 0.8	0.77 \pm 0.17**	0.70 \pm 0.09**	0.80 \pm 0.13**	2.7 \pm 0.3***
<i>Intervention study in ZDF</i>									
CON	15.3 \pm 2.3	23.9 \pm 1.6	16.6 \pm 3.3	13.0 \pm 1.3	1.2 \pm 0.3	0.06 \pm 0.02	1.17 \pm 0.10	3.38 \pm 0.45	5.8 \pm 0.2
BF	14.8 \pm 1.9	19.9 \pm 1.8	13.3 \pm 2.6	19.8 \pm 3.3	1.1 \pm 0.5	0.04 \pm 0.01	1.01 \pm 0.10	2.90 \pm 0.69	5.2 \pm 0.2
PIO	18.3 \pm 2.9	11.0 \pm 1.2***	9.2 \pm 2.2	55.9 \pm 10.2**	2.2 \pm 0.4	0.21 \pm 0.05*	0.61 \pm 0.07***	1.15 \pm 0.15**	8.0 \pm 0.4*
RAGA	15.4 \pm 1.4	10.4 \pm 1.5***	6.8 \pm 0.8*	60.4 \pm 12.9**	2.9 \pm 1.0	0.21 \pm 0.06	0.62 \pm 0.03***	1.59 \pm 0.23*	9.7 \pm 1.1**
LEAN	15.2 \pm 0.4	6.7 \pm 0.5**	4.6 \pm 0.4**	105.6 \pm 15.7**	2.4 \pm 0.4	0.36 \pm 0.08**	0.51 \pm 0.10**	0.92 \pm 0.19**	3.1 \pm 0.4***
<i>Intervention study in DIO</i>									
CON	9.8 \pm 1.5	6.3 \pm 0.3	2.8 \pm 0.5	74.7 \pm 12.5	5.8 \pm 2.4	0.55 \pm 0.12	1.97 \pm 0.10	1.90 \pm 0.15	6.8 \pm 0.7
BF	6.4 \pm 2.0	7.8 \pm 1.1	2.5 \pm 1.2	36.9 \pm 7.6	7.3 \pm 2.2	0.93 \pm 0.30	1.21 \pm 0.12***	1.09 \pm 0.14***	5.2 \pm 1.0
PIO	6.8 \pm 1.1	4.7 \pm 0.9	1.3 \pm 0.3	37.8 \pm 4.7	5.9 \pm 1.2	1.69 \pm 0.55	0.70 \pm 0.04***	0.78 \pm 0.04***	7.4 \pm 0.6
RAGA	8.4 \pm 1.2	5.4 \pm 1.1	2.0 \pm 0.4	104.5 \pm 39.6	4.6 \pm 1.0	0.79 \pm 0.26	0.90 \pm 0.07***	0.93 \pm 0.09***	7.2 \pm 0.6
CHOW	5.1 \pm 0.3**	6.4 \pm 0.5	1.4 \pm 0.1*	43.6 \pm 9.7	1.8 \pm 0.4*	0.28 \pm 0.09	1.22 \pm 0.04**	1.00 \pm 0.011**	3.2 \pm 0.4**

Animals were treated for 28 days with ragaglitazar (RAGA, 1.5 mg kg⁻¹), pioglitazone (PIO, 30 mg kg⁻¹) and bezafibrate (BF, 100 mg kg⁻¹). Values are expressed as mean \pm s.e.m. ($n=8$); * $P<0.05$; ** $P<0.01$; *** $P\leq 0.0001$ as compared to obese control (CON). HOMA-IR = insulin resistance index = glucose (mM) \times insulin ($\mu\text{U ml}^{-1}$)/22.5; HOMA- β = insulin secretion index = $20 \times$ insulin ($\mu\text{U ml}^{-1}$)/(glucose (mM)-3.5). Pancreatic insulin/plasma glucose ratio = (nmol g⁻¹)/mM (fasting plasma glucose).



Intervention effects on metabolic parameters

CON rats showed marked insulin resistance (HOMA-IR: +261%; $P < 0.01$) and reduced β -cell function (HOMA- β : -88%; $P < 0.01$), with a corresponding (nonsignificant) decrease in pancreatic insulin content compared to LEAN. RAGA and PIO improved insulin sensitivity (HOMA-IR: -59% (RAGA) and -44% (PIO); $P < 0.05$ and $P = 0.09$, respectively) and β -cell function, as shown by HOMA- β (>3-fold; both $P < 0.01$) and pancreatic insulin/plasma glucose ratio (both +250%; $P = 0.052$ and 0.045 , respectively), and there was a significant trend toward improvement in pancreatic insulin content (+142% (RAGA) and +83% (PIO); $P = 0.03$). Plasma FFA and TG levels were also elevated in CON rats but significantly reduced by RAGA and PIO. BF had no effect on lipids. Both RAGA and PIO increased circulating leptin (Table 2).

As in the prevention study, because of pre-existing hyperglycaemia, S-S blood glucose concentration could not be attained, and glucose was not infused in CON- and BF-treated groups (Figure 2a, b). S-S GIR in RAGA- and PIO-treated groups, although greater than that required in CON- and BF-treated groups, was not significantly so (Figure 2b), but nevertheless was sufficient in PIO-treated animals to significantly increase late-phase and total insulin secretion (Figure 2c).

Intervention study in DIO rats: effects on energy intake, body weight and body composition

After 10 weeks on the HPD, CON rats were 12% heavier than CHOW rats (410 ± 4 vs 365 ± 6 g; $P < 0.0001$). During the 4-week treatment period, energy intake had remained higher in these animals than in CON rats (+10%; $P < 0.0001$), and this was associated with greater body weight gain (final body weight: 426 ± 6 vs 380 ± 8 g; $P < 0.01$). PIO-, BF- and RAGA-treated animals showed a further increase in food intake (+11–14%; all $P < 0.01$) that was associated with greater weight gain in PIO and RAGA groups. Consistent with this, food efficiency nearly doubled and percentage body fat content, greater in CON than LEAN rats, was further increased by PIO and RAGA (Table 1).

Intervention effects on metabolic parameters in DIO rats

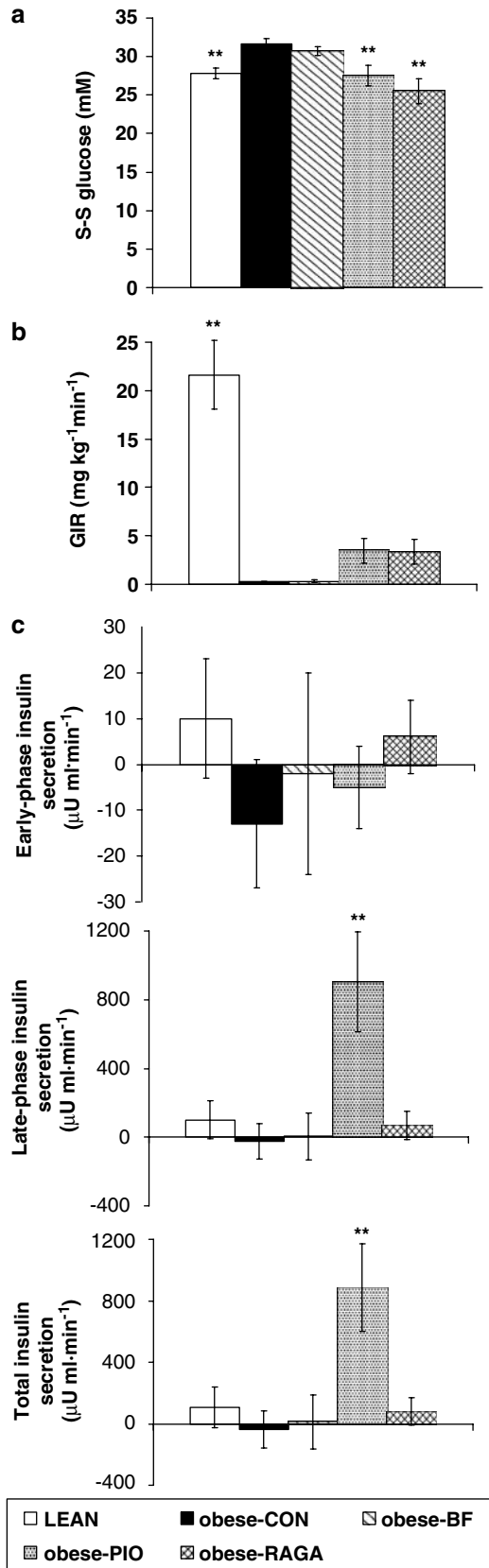
CON rats were more insulin-resistant than CHOW rats (HOMA-IR: +100%; $P < 0.05$). This was not significantly affected by any treatment, although HOMA-IR was numerically lower in all treated groups and similar to that in CHOW rats (one-way ANOVA: $P > 0.05$). β -Cell function was not significantly different between the groups (HOMA- β or pancreatic insulin/plasma glucose ratio), although pancreatic

Figure 1 Effects of PPAR agonist prevention therapy in ZDF (*fa/fa*) rats. (a) S-S blood glucose concentration, (b) GIR and (c) incremental change from baseline in insulin secretion during the hyperglycaemic clamp. Animals were treated once daily for 28 days with RAGA (1.5 mg kg^{-1}), PIO (30 mg kg^{-1}) and BF (100 mg kg^{-1}), then clamped at 24 mM glucose, as described in Methods. Vehicle-treated obese (CON) and lean (LEAN) rats were used as controls. Values are expressed as mean \pm s.e.m. ($n = 8$); ** $P < 0.01$; *** $P < 0.0001$ as compared to CON (ANOVA).

insulin content was three-fold higher in CON than CHOW rats ($+222\%$; $P<0.05$). This was not altered by treatment, however. All treatments reduced FFA and TG concentrations,

with PIO and RAGA having a greater effect than BF. Plasma leptin concentration was higher in CON than CHOW rats, but was not altered by any treatment (Table 2).

Blood glucose concentration of approximately 22 mM was attained in all groups over the final 30 min of the clamp (all $P>0.05$; Figure 3a). There were no significant differences between S-S GIRs required to maintain hyperglycaemia in each group (all $P>0.05$; Figure 3b). However, as observed in the studies of diabetic ZDF rats, insulin secretion in the PIO group was enhanced throughout the clamp, but only significantly in the early phase ($P<0.05$ vs CON), consistent with the observed increase, albeit nonsignificant, in the GIR of these animals. CHOW rats, however, showed significant increases above baseline compared to CON rats in both the late phase and total insulin secretion (Figure 3c).



Discussion

In these studies, we have shown that the PPAR γ agonist, PIO and the PPAR α/γ agonist, RAGA, are effective at improving insulin action in the obese, diabetic ZDF rat. These benefits were greater earlier in the course of diabetes, but only nonsignificant trends were seen in the insulin-resistant DIO model. Indices showing improved β -cell function (HOMA- β and pancreatic insulin content/plasma glucose ratio) were generally consistent, agreeing with *in vitro* observations suggesting that PPAR γ and dual PPAR α/γ agonists, including RAGA, prevent β -cell death and alter expression of markers of β -cell survival such as insulin and GLUT2 in diabetic rodent models (Murakami *et al.*, 1999; Finegood *et al.*, 2001; Sturis *et al.*, 2002; Brand *et al.*, 2003; Pickavance *et al.*, 2003; Yajima *et al.*, 2003).

Paradoxically, we have not been able to demonstrate improved insulin secretion with RAGA under hyperglycaemic clamp conditions, and only inconsistent improvement with PIO. This may be because insulin secretion is already maximal in these insulin-resistant animals. The apparent increase in insulin secretion seen with PIO but not RAGA intervention therapy, in the face of similar levels of hyperglycaemia and GIRs, could represent a superior effect of RAGA on insulin sensitivity, despite similar HOMA-IR values, or on non-insulin-mediated glucose disposal after a glucose load; that is, an effect which is independent of any change in insulin sensitivity. However, further studies would be required to investigate this phenomenon.

Despite their obesity and insulin resistance, DIO ZDF lean rats did not develop diabetes. Thus, a leptin receptor mutation appears to be necessary for the development of diabetes in this model. Diabetes developed at a younger age and lower body weight in the obese mutant than in the DIO model, suggesting

Figure 2 Effects of PPAR agonist intervention therapy in ZDF (*fa/fa*) rats. (a) S-S blood glucose concentration, (b) GIR and (c) incremental change from baseline in insulin secretion during the hyperglycaemic clamp. Animals were treated once daily for 28 days with RAGA (1.5 mg kg⁻¹), PIO (30 mg kg⁻¹) and BF (100 mg kg⁻¹), then clamped at 24 mM glucose, as described in Methods. Vehicle-treated obese (CON) and lean (LEAN) rats were used as controls. Values are expressed as mean \pm s.e.m. ($n=8$); ** $P<0.01$ as compared to CON (ANOVA).

that the islet defect is related directly to the leptin receptor mutation, rather than to the degree of obesity. This is in agreement with findings that overexpression of the normal

leptin receptor in the islets of pre-diabetic ZDF rats reverses the diabetic phenotype (Zhou *et al.*, 1998). Our findings seem at odds with those of Griffen *et al.* (2001), which show that a β -cell defect in insulin gene transcription is present in lean ZDF rats and leads to the development of diabetes in the face of insulin resistance. However, it is possible that the modest insulin resistance observed in our DIO rats was not severe or prolonged enough to allow expression of this phenotype.

Consistent with recent human data showing comparable glycaemic control and lipid profile between RAGA and PIO (Saad *et al.*, 2004), but in contrast to some other reported animal data (Brand *et al.*, 2003; Chakrabarti *et al.*, 2003; Larsen *et al.*, 2003), we have not found any greater effect of RAGA compared to PIO on circulating FFAs and TGs, despite an equivalent effect on insulin resistance and glucose-lowering. Moreover, because both PIO and RAGA caused weight gain, increasing fat mass and leptin concentrations, we can deduce that there was no neutralisation of weight gain by the α component of RAGA, which appears to contradict previous reports (Larsen *et al.*, 2003; Yajima *et al.*, 2003; Ye *et al.*, 2003). However, trends were present for weight gain in these studies, and the lack of an effect may simply represent shorter treatment duration or lack of statistical power. The weak retention by PIO of α activity (Sakamoto *et al.*, 2000) could also explain the lack of difference between PIO and RAGA. The rodent model (diet and strain) and fat depot examined may also explain why our results conflict with those of others. Whereas other workers have tested RAGA in wild-type Sprague–Dawley or Wistar rats fed a high-fat diet (Larsen *et al.*, 2003; Ye *et al.*, 2003), we have used a monogenic obesity-prone model. Certainly, in conjunction with findings that expression of PPAR α target genes in liver is altered by RAGA within these other paradigms (Brand *et al.*, 2003; Larsen *et al.*, 2003; Ye *et al.*, 2003), the above rationalisation suggests that it would be imprudent to conclude that RAGA has no PPAR α effect in the ZDF rat.

ZDF rats may be poor responders to PPAR α agonism in general due to reduced islet PPAR α expression (Zhou *et al.*, 1998). If this were also true of expression in the liver, the other tissue to which PPAR α is localised, it might explain why BF had no significant effect on insulin resistance, whereas others have shown α agonists to improve insulin sensitivity in rodent models of obesity such as Zucker (*fa/fa*) rats and high-fat-fed mice and rats (Guerre-Millo *et al.*, 2000; Ye *et al.*, 2001). BF may not have had significant effects on most parameters because it is only a moderately weak PPAR α agonist compared to fenofibrate in rodents, although potency in humans is approximately equivalent (Willson *et al.*, 2000). We know that the batch of drug used was effective insofar as weight gain was attenuated and FFA and TG levels, at least in DIO and

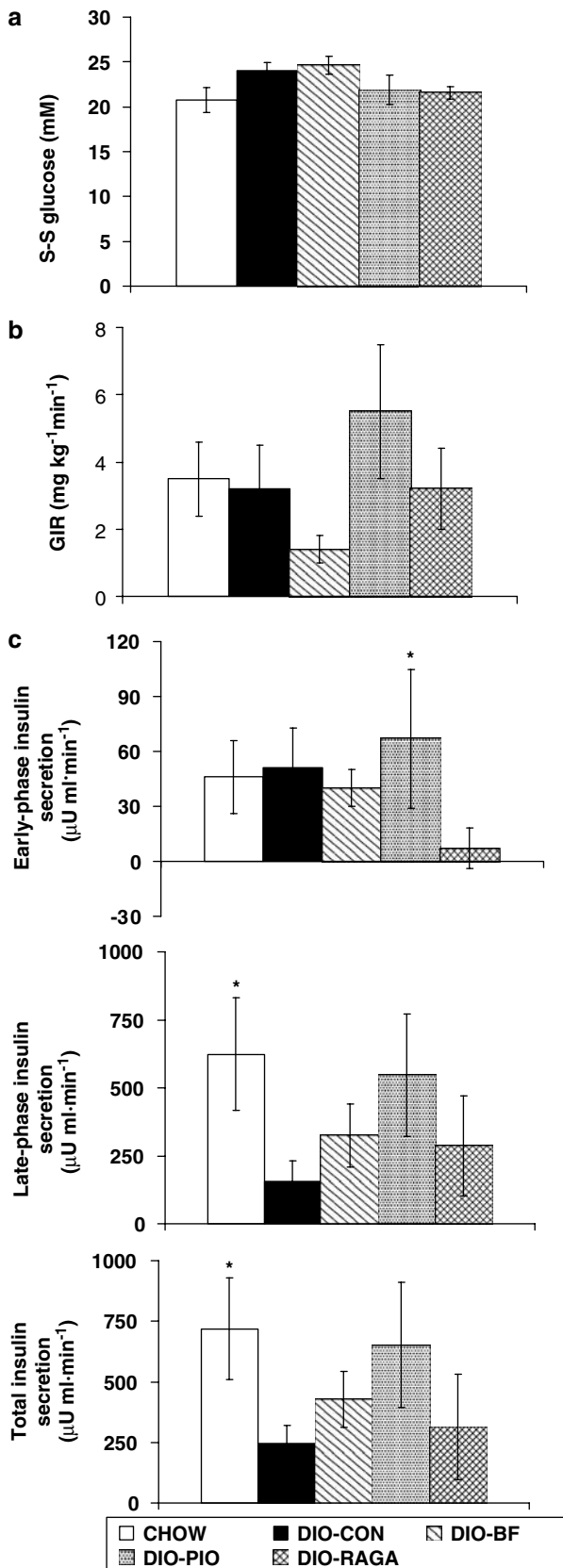


Figure 3 Effects of PPAR agonist intervention therapy in ZDF (+/+) DIO rats. (a) S-S blood glucose concentration, (b) GIR and (c) incremental change from baseline in insulin secretion during the hyperglycaemic clamp. Animals were treated once daily for 28 days with RAGA (1.5 mg kg⁻¹), PIO (30 mg kg⁻¹) and BF (100 mg kg⁻¹), then clamped at 24 mM glucose, as described in Methods. Vehicle-treated DIO (CON) and lean (CHOW) rats were used as controls. Values are expressed as mean \pm s.e.m. ($n = 8$); * $P < 0.05$ as compared to CON (ANOVA).

younger diabetic animals, were reduced. Although others have effectively reduced circulating insulin, FFA and TG concentrations and increased pancreatic insulin content with BF treatment in older, overtly diabetic ZDF rats, this may be due to their use of a different dosage and longer duration of treatment (Harmon *et al.*, 2001).

Conclusion

In summary, treatment with PPAR γ and α/γ agonists is more effective when applied early in the natural development of diabetes in the ZDF rat. At the doses used here, RAGA improves insulin sensitivity, β -cell function and metabolic profile equally with PIO and better than BF alone, although enhanced insulin secretion could not be demonstrated dynamically, perhaps because of limitations of hyperglycaemic clamp methodology in this model. Unchanged insulin-secre-

tory response in younger ZDF rats treated with RAGA, despite increased GIR, suggests that the main effect of this compound is through improved insulin sensitivity. Lack of effect on energy accumulation and other outcome variables normally suppressed by BF treatment further emphasise the point that in future the ZDF rat should be employed with caution as a model for studies on PPAR α compounds. In addition, we have shown that although the lean (+/+) ZDF rat develops obesity and insulin resistance on a HPD, it is not prone to diabetes like its obese (*fa/fa*) mutant counterpart.

We are very grateful to the Biomedical Services Unit staff at the University of Liverpool, in particular Miss Juliet McAdams, Miss Joanne Sanders and Miss Catherine Edwards, for their excellent care of the animals.

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(Received August 12, 2004
Revised September 27, 2004
Accepted October 5, 2004)