Effects of rosiglitazone and pioglitazone on lipoprotein metabolism in patients with type 2 diabetes

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Abstract

Aims Previous studies have suggested that plasma lipids are affected differently by the PPAR-γ agonists pioglitazone and rosiglitazone. The aim of this study was to perform a quantitative lipoprotein turnover study to determine the effects of PPAR-γ agonists on lipoprotein metabolism.

Methods 24 subjects with type 2 diabetes treated with diet and/or metformin were randomised in a double blind study to receive 30mg pioglitazone, 8mg rosiglitazone or placebo once daily for 3 months. Before and after treatment absolute secretion rate (ASR) and fractional catabolic rate (FCR) of VLDL, IDL and LDL apolipoprotein B100 were measured with a 10 hour infusion of 1-^{13}C leucine.

Results There was a significant decrease in HbA1c and non-esterified fatty acids with pioglitazone ($p = 0.01$; $p = 0.02$) and rosiglitazone ($p = 0.04$; $p = 0.003$) respectively, but no change in plasma triglyceride or HDL cholesterol. Following rosiglitazone there was a significant reduction in VLDL apo B ASR ($p = 0.01$) compared to baseline, a decrease in VLDL triglyceride/apoB ($p = 0.01$), an increase in LDL2 cholesterol ($p = 0.02$) and a decrease in LDL3 cholesterol ($p = 0.02$). There was a decrease in VLDL triglyceride/apoB ($p = 0.04$) in the pioglitazone group. There was no significant difference in change in VLDL ASR or FCR between the 3 groups.

Conclusions In patients with type 2 diabetes and normal lipids treatment with rosiglitazone or pioglitazone had no significant effect on lipoprotein metabolism compared to placebo. Treatment with rosiglitazone resulted in a decrease in small dense LDL.

Keywords intermediate density lipoprotein, low density lipoprotein, NEFA, PPAR-γ agonist, type 2 diabetes, very low density lipoprotein

Abbreviations α-ketoisocaproate: α-KIC Apolipopotein B100: apo B Absolute secretion rate: ASR Fractional catabolic rate: FCR Gas chromotography mass spectrometry: GCMS Gas chromatography combustion isotope ratio mass spectrometry: GC-C-IRMS High density lipoprotein: HDL, Homeostatic model assessment: HOMA Intermediate density lipoprotein: IDL Lipoprotein lipase: LPL Low density lipoprotein: LDL

MTBSTFA: N-methyl-N-(tertbutyldimethylsilyl)-trifluoroacetamide Non esterified fatty acid: NEFA Peroxisome proliferator activated receptor: PPAR Very low density lipoprotein: VLDL

Introduction

The peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists pioglitazone and rosiglitazone were introduced in Europe in 2000 and the USA in 1999 for the treatment of type 2 diabetes [1]. As well as reducing blood glucose and HbA1c there is also evidence that these PPAR-γ agonists affect plasma lipid levels [2]. Since dyslipidaemia in diabetes contributes to the increased risk of macrovascular disease the effect of these drugs on dyslipidaemia is important. Interest in this has increased recently since the publication of a meta-analysis suggesting increased cardiovascular risk with rosiglitazone. The authors postulated that the adverse effect of rosiglitazone on plasma lipids may be a possible mechanism behind this increased risk [3].

Large double-blinded, placebo controlled studies of rosiglitazone show that it increases total, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol with no effect on triglyceride levels [4] [5] [6]. Similar studies with pioglitazone have found an increase in HDL cholesterol, a less consistent increase in LDL and total cholesterol and a reduction in triglyceride [7] [8] [9]. Systematic reviews [10] [11] have confirmed these differences. In the only head to head study 802 subjects with type 2 diabetes were randomised to pioglitazone 45mg or rosiglitazone 8mg for 24 weeks [12]. In the pioglitazone group there was a 12% reduction in triglyceride after 24 weeks treatment. In contrast in the rosiglitazone group there was an increase in triglyceride levels. HDL cholesterol and LDL cholesterol increased in both groups but the increase in HDL-cholesterol was greater in the pioglitazone group and the increase in LDL-cholesterol was greater in the rosiglitazone group. There was no difference in the effect on HbA1c or cardiovascular parameters.

The different effects of rosiglitazone and pioglitazone on lipid levels have yet to be explained. Lipoprotein turnover studies provide a methodology for investigating the mechanism behind changes in lipid concentrations. Several studies have investigated the effects of PPAR-γ agonists on lipoprotein turnover. The first, [13] randomised 24 subjects with type 2 diabetes to 8 weeks treatment with placebo, 200mg troglitazone or 600mg troglitazone. Very low density lipoprotein (VLDL) secretion was assessed using

1-^{13}C leucine infusion. A reduction in VLDL apolipoprotein (apo) B ASR was found with 600mg troglitazone which just failed to reach statistical significance. There was no other significant effect on lipoprotein turnover or lipid variables. In the second study [14] 8 subjects received 30-45mg pioglitazone for 12-14 weeks. Following treatment there was a reduction in VLDL triglyceride and an increase in the FCR of VLDL triglyceride but no change in VLDL apo B ASR, VLDL triglyceride ASR or LDL ASR or FCR. In the third study there was no change in triglyceride apoB production and clearance rates in 17 men without diabetes following 6 weeks of rosiglitazone treatment [15]. The most recent study randomised 12 patients with type 2 diabetes to pioglitazone or rosiglitazone for 20 weeks and found a reduction in hepatic de novo lipogenesis with pioglitazone but not rosiglitazone [16].

To determine the mechanism for the reported differences of pioglitazone and rosiglitazone on lipid levels, VLDL, intermediate density lipoprotein (IDL) and LDL apo B turnover were measured with stable isotopes in a placebo controlled double blind study in patients with type 2 diabetes.

Subjects and Methods

24 subjects with type 2 diabetes for more than 6 months were recruited from the diabetes clinic of the Royal Surrey County Hospital in Guildford and 6 local primary care diabetes clinics. Subjects were on diet alone or metformin and no lipid lowering medication. Informed consent was obtained and the study was approved by the South West Surrey Ethics committee. Subjects were randomised into 3 groups, one group received 8mg rosiglitazone, one group received 30mg pioglitazone and the third group received placebo tablets. Subjects and investigators were blinded to treatment group.

Before and after 3 months of treatment each subject had an identical metabolic study to measure lipoprotein turnover. After an overnight fast they were admitted to the clinical investigation unit and an intravenous cannula was inserted into each antecubital fossa. A bolus of 1-^{13}C leucine (1mg/kg) (Cambridge Isotopes, Andover MA, USA) was administered followed by a constant infusion of 1-^{13}C leucine (1mg/kg/hour) for 10 hours. Subjects were allowed water only throughout the study. Blood samples were taken at baseline and at 30, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, 540 and 600 minutes to measure VLDL, IDL and LDL apo B enrichment. At baseline and at 180, 360, 540 and 600 minute samples were taken to measure the concentration of VLDL, IDL and LDL-cholesterol, triglyceride and apo B. Enrichment of α-ketoisocaproate (α-KIC) was measured at baseline, 180, 360, 530 and 600 minutes and was used to provide a measure of intracellular ^{13}C leucine enrichment. In addition at baseline samples were taken for the measurement of LDL subfractions (LDL2 and LDL3), non esterified fatty acids (NEFAs), glucose, insulin and adiponectin.

Samples for VLDL, IDL and LDL were separated by sequential density ultracentrifugation in a Beckman Coulter Optima LE80-K ultracentrifuge (High Wycombe, UK) with a type 50.4 Ti fixed angle rotor as previously described [17]. LDL2 and LDL3 subfractions were separated from a baseline LDL fraction by adjusting the density to 1.044g/l then ultracentrifugation for 20h and removal of the top 1ml (LDL2).

The bottom fraction was then adjusted to density 1.063g/l followed by ultracentrifugation for 20h and removal of the top 1ml (LDL3).

VLDL, IDL and LDL apo B was precipitated by isopropanol [18], and hydrolysed using hydrochloric acid. VLDL and IDL samples were derivatised with N-methyl-N-(tertbutyldimethylsilyl)-trifluoroacetamide (MTBSTFA) VLDL and IDL apo B enrichment was measured using gas chromatography mass spectrometry (GCMS), (Agilent 5973 N MSD; Agilent Technologies, Bracknell, U.K.) in the electron impact mode with selected ion monitoring of $[M\text{-butyl}]^+$ ions at mass to charge ratio (m/z) of 302 and 303.

Since the isotopic enrichment of LDL apo B is much lower than VLDL and IDL this was determined using a N-acetyl n-propyl ester derivative on a gas chromatography combustion isotope ratio mass spectrometer (GC-C-IRMS), specifically a Sira Series 2 IRMS (VG Instruments, Hellingly, UK) coupled to an Orchid Gas Chromatograph Interface Module (Europa Scientific, Crewe, UK). Data were analysed using the manufacturer's software (Orchid Post Processor, Version 2.3c. Europa Scientific). Isotope abundance was expressed relative to pulse peaks of reference CO_2 gas. The enrichment of α-KIC, was measured as the quinoxalinol-*tert*-butyldimethylsilyl derivative. GCMS analysis used electron impact ionisation with selected ion monitoring of the ions at m/z 259 and 260.

Apo B concentration was determined using an in-house sandwich enzyme-linked immunoassay method combining a polyclonal antibody against apo B (the Binding Site Ltd, Birmingham, UK) as capturing antibody and a monoclonal antibody raised specifically against apo B100 as second antibody (Ottawa Heart Institue, Ottawa, Canada). Triglyceride, cholesterol, VLDL, IDL, LDL apo B, triglyceride, cholesterol, HDL-cholesterol and NEFA concentrations were all determined using commercially available kits. Insulin was measured using an in-house double antibody radioimmunoassay (RIA). Glucose and HbA1c were measured on an ADVIA 1650 Chemistry system (Bayer Healthcare, Leverusen, Germany). Plasma adiponectin was

measured by RIA using a commercially available kit from Linco Research Inc. (St. Louis, MO, USA).

Data analysis

Homeostatic model assessment insulin resistance index (HOMA2 IR) was calculated using the HOMA computer model (HOMA2, version 2.2) [19].

VLDL, IDL and LDL fractional secretion rate (FSR) was analysed using Simulation Analysis and Modelling (SAAM) II software, (SAAM, Seattle, WA, USA). This uses a multicompartmental model and incorporates a forcing function corresponding to precursor (α-KIC) enrichment and a delay function accounting for the amount of time required for synthesis and secretion of VLDL apo B. At steady state, shown by constant concentration of VLDL, IDL and LDL apo B, the proportion of apo B being removed from the circulation per unit time (fractional catabolic rate; FCR) is equal to the FSR. ASR (mg/kg/day) is the product of FSR and the apo B pool size divided by body weight (kg). Apo B pool size (mg) is the product of apo B concentration and plasma volume. Plasma volume was calculated using the method of Pearson [20].

Power calculation

The primary endpoint was the change in VLDL secretion rate from baseline for each treatment versus the change from baseline for placebo. We estimated that a study with 8 patients in each group would have a power of 80% to detect a difference of 45% in the production rate of VLDL at the 5% level of significance. For this calculation it was assumed that the standard deviation in production rate of VLDL apo B metabolism would be 30% (ref). Since an exercise programme which improves insulin sensitivity has been shown to reduce VLDL production rate by 49% [21] , a similar order of magnitude was expected for the glitazones.

Statistical analysis

Data is presented as mean ± SEM except for non-parametrically distributed variables (triglyceride, ASR and FCR) which is presented as median (interquartile range).

Comparison between variables at baseline was made using one way ANOVA or Kruskal-Wallis for non-parametrically distributed variables. Post-hoc analysis was performed using the Bonferroni post-hoc test. Within each group, a comparison of variables before and after treatment was performed using Student's t-test or Wilcoxon rank test for non-parametrically distributed variables. Change in variables after treatment was compared across groups using ANOVA or Kruskal-Wallis as above. All statistical analysis was performed using SPSS. P values of less then 0.05 were regarded as significant.

Results

Subjects' baseline characteristics are summarised in table 1. Following 3 months of treatment there was a significant increase in weight in the pioglitazone group ($p = 0.04$). HbA1c decreased following treatment in the pioglitazone (-0.71% ± 0.20, $p = 0.01$) and rosiglitazone group (-0.48% ± 0.19, $p = 0.04$). There was a decrease in the IR component of HOMA2 in the rosiglitazone group (-0.34 ± 0.15, $p = 0.06$) but not in the pioglitazone group (-0.04, $p = 0.83$).

Lipid concentrations

Plasma lipid concentrations before and after treatment are summarised in table 2, 3 and 4. Prior to treatment plasma triglyceride, LDL cholesterol and HDL cholesterol were near normal. Only 6 subjects had a plasma triglyceride greater than 2.0mmol/l; 4 subjects had a LDL cholesterol greater than 3.0 mmol/l and 7 subjects had an HDL cholesterol below 1mmol/l.

There was a significant decrease in NEFAs with pioglitazone and rosiglitazone treatment ($p= 0.02$; $p = 0.003$ respectively) but no other change in lipid variables although there was a trend for an increase in HDL cholesterol in the rosiglitazone group ($p = 0.07$), table 2. In the pioglitazone and the rosiglitazone groups there was a significant decrease in VLDL triglyceride/apo B, ($p = 0.04$, $p = 0.01$ respectively). There was no significant change in VLDL or IDL apo B, cholesterol, triglyceride or cholesterol/apo B in any of the groups. Following treatment there was a significant increase in LDL cholesterol in the placebo group and a significant increase in LDL cholesterol/apo B in the rosiglitazone group ($p = 0.008$), with a trend for a decrease in the pioglitazone group ($p = 0.07$) (table 3). When LDL was subfractionated there was a significant increase in LDL 2 cholesterol and apo B ($p = 0.02$; $p = 0.02$) and a decrease in LDL 3 cholesterol and apo B ($p = 0.02$; $p = 0.06$) following rosiglitazone, table 4.

Lipoprotein metabolism data

The effects of treatment on lipoprotein metabolism are summarised in table 5. There was a significant decrease in VLDL apo B ASR in the rosiglitazone group following

treatment. There was no change in VLDL apo B ASR or FCR in the other 2 groups following treatment. There was no significant difference in the change in VLDL ASR or FCR between groups. There was no significant change in ASR or FCR of IDL and LDL apo B in any group following treatment.

Adiponectin

There was a significant increase in adiponectin in the pioglitazone and the rosiglitazone group following treatment (pioglitazone from 5.46µg/ml ± 1.07 to 10.34µg/ml ± 1.71, p = 0.001, and rosiglitazone from 7.33µg/ml ± 1.16 to 15.93µg/ml ± 3.40, p = 0.002).

Table 1

Baseline characteristics and changes following treatment

	Placebo		**Pioglitazone**		**Rosiglitazone**	
Sex (M/F)	7/1		7/1		4/4	
Age (years)	60.75 ± 3.45		61.00 ± 3.93		66.50 ± 2.51	
Time since diagnosis (years)	2.88 ± 0.40		4.00 ± 0.80		4.38 ± 1.03	
Number on metformin	5		5		6	
	Baseline	3 months	Baseline	3 months	Baseline	3 months
Weight (kg)	103.91 ± 5.61	104.38 ± 5.76	96.39 ± 3.62	98.33 ± 3.96[a]	91.95 ± 6.52	92.63 ± 6.44
BMI (kg/m^2)	31.96 ± 1.56	32.10 ± 1.64	30.81 ± 1.26	31.45 ± 1.45[b]	29.99 ± 1.50	30.13 ± 1.45
Fasting glucose (mmol/L)	8.21 ± 0.36	8.55 ± 0.47	8.15 ± 0.30	8.15 ± 0.31	8.11 ± 0.75	6.83 ± 0.50
HbA1c (%)	6.60 ± 0.14	6.79 ± 0.24	7.53 ± 0.21[c]	6.81 ± 0.18[d]	6.94 ± 0.30	6.46± 0.19[e]
HOMA2 IR	1.5 ± 0.30	1.84 ± 0.37	1.75 ± 0.30	1.71 ± 0.45	1.10 ± 0.18	0.76 ± 0.13[f]

[a] p = 0.04 compared to baseline, [b] p = 0.04 compared to baseline, [c] p = 0.03 compared to placebo, [d] p = 0.01 compared to baseline, [e] p = 0.04 compared to baseline, [f] p = 0.06 compared to baseline

Table 2

Lipid concentrations at baseline and after treatment

	Placebo	**Pioglitazone**	**Rosiglitazone**
Total cholesterol (mmol/L)			
Baseline	4.52 ± 0.19	5.14 ± 0.35	5.08 ± 0.29
After treatment	4.77 ± 0.27	5.49 ± 0.41	5.32 ± 0.25
LDL cholesterol (mmol/L)			
Baseline	2.06 ± 0.10	2.82 ± 0.33	2.42 ± 0.22
After treatment	2.50 ± 0.19[a]	2.42 ± 0.27	2.58 ± 0.21
Triglyceride (mmol/L)			
Baseline	2.15 (1.26, 2.44)	1.61 (1.42, 1.93)	1.38 (1.21, 1.68)
After treatment	2.22 (1.76, 2.37)	2.12 (1.34, 2.55)	1.41 (1.17, 1.59)
HDL cholesterol (mmol/L)			
Baseline	1.09 ± 0.06	1.23 ± 0.16	1.30 ± 0.08
After treatment	1.15 ± 0.10	1.28 ± 0.17	1.45 ± 0.12[b]
NEFA (mmol/L)			
Baseline	0.64 ± 0.06	0.66 ± 0.08	0.68 ± 0.09
After treatment	0.72 ± 0.08	0.48 ± 0.04[c]	0.49 ± 0.10[d]

[a] $p = 0.03$ compared to baseline, [b] $p = 0.07$ compared to baseline, [c] $p = 0.02$ compared to baseline, [d] $p = 0.003$ compared to baseline

Table 3

VLDL, IDL and LDL lipid concentrations before and after treatment

	Placebo	Pioglitazone	Rosiglitazone
VLDL apo B (mg/L)			
Baseline	46.26 ± 10.84	38.15 ± 5.84	31.88 ± 4.26
After treatment	49.36 ± 10.86	44.20 ± 8.40	34.55 ± 5.67
VLDL cholesterol (mmol/L)			
Baseline	0.47 ± 0.10	0.51 ± 0.12	0.28 ± 0.04
After treatment	0.53 ± 0.10	0.54 ± 0.12	0.27 ± 0.04
VLDL triglyceride (mmol/L)			
Baseline	1.44 (0.85, 1.87)	1.41 (0.92, 1.72)	0.85 (0.78, 0.98)
After treatment	1.55 (1.17, 1.78)	1.17 (1.05, 1.58)	0.83 (0.69, 0.86)
VLDL triglyceride/apo B			
Baseline	30.64 ± 6.19	31.04 ± 3.91	25.51 ± 2.70
After treatment	34.56 ± 9.07	25.29 ± 3.71[a]	20.64 ± 2.47[b]
IDL apo B (mg/L)			
Baseline	27.27 ± 4.04	35.05 ± 7.74	28.64 ± 4.45
After treatment	24.63 ± 2.69	31.89 ± 7.24	35.04 ± 5.35
IDL cholesterol (mmol/L)			
Baseline	0.089 ± 0.011	0.150 ± 0.034	0.096 ± 0.015
After treatment	0.095 ± 0.011	0.145 ± 0.036	0.109 ± 0.014
IDL triglyceride (mmol/L)			
Baseline	0.07 ± 0.009	0.10 ± 0.019	0.08 ± 0.011
After treatment	0.08 ± 0.009	0.10 ± 0.025	0.08 ± 0.011
IDL triglyceride/apo B			
Baseline	2.42 ± 0.33	2.71 ± 0.22	2.64 ± 0.24
After treatment	2.86 ± 0.30	2.81 ± 0.16	2.68 ± 0.34
LDL apo B (mg/L)			
Baseline	586.67 ± 28.01	715.42 ± 84.49	651.39 ± 73.73
After treatment	618.61 ± 50.96	667.08 ± 54.78	640.00 ± 56.41
LDL cholesterol/apoB			
Baseline	1.37±0.06	1.54±0.08	1.46±0.06
After treatment	1.57±0.07	1.40±0.08[c]	1.56±0.05[d]
LDL triglyceride (mmol/L)			
Baseline	0.33 ± 0.05	0.44 ± 0.07	0.37 ± 0.06
After treatment	0.31 ± 0.07	0.38 ± 0.05	0.36 ± 0.06

[a] p = 0.04 compared to baseline, [b] p = 0.01 compared to baseline, [c] p = 0.07 compared to baseline, [d] p = 0.008 compared to baseline

Table 4

LDL subfractions before and after treatment

	Placebo	Pioglitazone	Rosiglitazone
LDL2 cholesterol (mmol/L)			
Baseline	0.78 ± 0.08	1.08 ± 0.15	1.02 ± 0.14
After treatment	0.80 ± 0.10	1.21 ± 0.07	1.39 ± 0.20[a]
LDL3 cholesterol (mmol/L)			
Baseline	1.28 ± 0.16	1.25 ± 0.15	1.33 ± 0.12
After treatment	1.41 ± 0.18	1.53 ± 0.23[b]	0.96 ± 0.14[c]
LDL2 apo B (mmol/L)			
Baseline	0.21 ±0.02	0.26 ± 0.03	0.25 ± 0.03
After treatment	0.21 ± 0.02	0.29 ± 0.03	0.34 ± 0.05[d]
LDL3 apo B (mmol/L)			
Baseline	0.37 ± 0.05	0.37 ± 0.04	0.38 ± 0.05
After treatment	0.41 ± 0.41	0.46 ± 0.07	0.30 ± 0.05[e]

[a] p = 0.02 compared to baseline, [b] p = 0.05 compared to baseline, [c] p = 0.02 compared to baseline, p = 0.03 compared to placebo, p = 0.004 compared to pioglitazone, [d] p = 0.02 compared to baseline, [e] p = 0.06 compared to baseline, p = 0.03 compared to pioglitazone

Table 5

VLDL, IDL and LDL kinetic data

	Placebo	**Pioglitazone**	**Rosiglitazone**
VLDL FCR (pools/day)			
Baseline	4.88 (3.91, 6.80)	4.15 (3.55, 5.83)	6.74 (5.80, 7.98)
After treatment	4.18 (3.51, 5.50)	3.97 (3.63, 5.07)	5.75 (4.77, 6.53)
VLDL ASR (mg/kg/day)			
Baseline	6.58 (5.06, 9.66)	5.68 (4.75, 6.54)	7.24 (6.28, 8.04)
After treatment	6.24 (4.85, 8.60)	6.04 (5.03, 7.05)	5.83 (5.06, 6.84)[a]
IDL FCR (pools/day)			
Baseline	4.80 (2.37, 5.90)	4.67 (2.76, 5.56)	7.63 (6.14, 8.12)
After treatment	5.12 (3.60, 7.24)	4.13 (3.59, 5.66)	5.57 (5.02, 6.52)
IDL ASR (mg/kg/day)			
Baseline	4.48 (2.55, 5.68)	3.60 (3.28, 6.23)	6.45 (5.51, 7.81)
After treatment	3.76 (2.92, 5.72)	4.15 (3.24, 5.52)	6.22 (3.72, 8.74)
LDL FCR (pools/day)			
Baseline	0.51 (0.43, 0.60)	0.41 (0.31, 0.54)	0.45 (0.40, 0.62)
After treatment	0.53 (0.34, 0.60)	0.44 (0.29, 0.56)	0.48 (0.43, 0.72)
LDL ASR (mg/kg/day)			
Baseline	10.33 (8.92, 10.71)	9.05 (7.41, 10.09)	10.77 (8.34, 13.22)
After treatment	9.78 (9.00, 10.72)	9.60 (7.58, 11.04)	12.14 (8.81, 14.29)

[a] $p = 0.01$ compared to baseline

Discussion

This study compared the effect of placebo, pioglitazone and rosiglitazone on lipids and lipoprotein kinetics in patients with type 2 diabetes with near normal plasma lipid levels. There was a modest improvement in HbA1c and a decrease in NEFAs with pioglitazone and rosiglitazone. Although there was no significant change in plasma triglycerides there was a decrease in VLDL triglyceride/apo B suggesting a decrease in large VLDL particles (VLDL1) with both treatments. LDL cholesterol also did not change but there was an increase in large buoyant LDL cholesterol (LDL2) and a decrease in small dense LDL (LDL3) in the rosiglitazone group. There was no significant difference in lipoprotein metabolism after treatment between the 3 groups. The only significant finding was a reduction in VLDL apo B ASR in the rosiglitazone group, however there was no difference between treatment and placebo in this or any other measurement.

The findings are surprising since previous studies have shown that the PPAR-γ agonists affect plasma lipids and that pioglitazone has a more favourable effect than rosiglitazone. A number of mechanisms may account for this. Subjects had well controlled plasma lipids and diabetes at baseline. The most marked changes in lipids seen in studies of the thiazolidinediones tend to be in subjects with dyslipidaemia at baseline. Indeed it has been suggested that the reason that pioglitazone is thought to have a more beneficial effect on lipids is that subjects in the original pioglitazone studies had more dyslipidaemia at baseline [11]. Mean LDL cholesterol at baseline was greater than 3mmol/l in the 3 largest studies of rosiglitazone and pioglitazone [5] [6] [7] [8] [9] [22] and triglyceride was greater than 2.5mmol/L in 4 of these studies [5,7-9]. Since the publication of trials such as the Heart Protection Study [23] the lipid concentrations in the present study are more reflective of current targets and clinical practice.

In previous studies of PPAR-γ agonists changes in lipid concentrations have been relatively small. The increase in HDL cholesterol with pioglitazone and rosiglitazone has been reported to be between 10 and 15%, the increase in LDL and total cholesterol with rosiglitazone is of a similar magnitude and the reduction in triglyceride with pioglitazone varies from 5 to 24% but is usually about 10%. In a head to head study [12] the change in triglyceride was -12% and +15%, the change in total cholesterol was +5.7% and +15.9% and the change in HDL cholesterol was +14.9% and +7.8% with pioglitazone and

rosiglitazone respectively. In a recent study in younger men without diabetes (mean age 48 years) with BMI ranging from 20.0 to 41.6 kg/m^2 and triglyceride levels between 0.61 and 4.0mmol/l rosiglitazone was shown to increase triglycerides by 32 % [15]. In contrast a number of studies have shown rosiglitazone to have no significant effect on plasma triglycerides as in the current study [4], [5].

Despite the lack of change in lipid levels in the current study there were changes in VLDL and LDL particle sizes. In both treatment groups there was a significant decrease in VLDL/apo B suggesting a decrease in large VLDL (VLDL1). A recent study has shown that the increase in VLDL triglyceride in diabetes is due to an increase in VLDL1 production rate and that VLDL2 production rate is unaffected by diabetes [24]. This suggests that VLDL1 and VLDL2 are independently regulated. It has been suggested that the increased VLDL1 in type 2 diabetes results in an increase in small dense LDL and a reduction in HDL, increasing the risk of atherosclerosis [25]. However a decrease in small dense LDL (LDL3), an increase in large buoyant LDL (LDL2) and an increase in HDL cholesterol was found in the rosiglitazone treated group but not in the pioglitazone group. This effect of rosiglitazone to shift LDL phenotype from dense to large buoyant subfractions has been reported in animal [26] and human studies [27] and may explain the tendency of rosiglitazone to increase LDL cholesterol.

Despite the similar changes in VLDL triglyceride/apo B with rosiglitazone and pioglitazone there was no change in VLDL apo B ASR compared to placebo although there was a significant reduction compared to baseline in the rosiglitazone group. There was a similar decrease in NEFAs in both groups. There is evidence that increased delivery of plasma NEFAs to the liver increases hepatic triglyceride synthesis and the assembly of VLDL and that this is fundamental in the development of diabetic dyslipidaemia [28]. Since VLDL apo B ASR was not decreased compared to placebo in either treatment factors other than NEFA flux must be important in controlling VLDL apo B ASR.

A study performed in a hamster model of insulin resistance [29] showed that rosiglitazone reduced VLDL apo B secretion rate with no effect on VLDL triglyceride clearance rate with an associated reduction in expression of microsomal transfer protein.

Similarly troglitazone (600mg per day) has been shown to decrease VLDL apo B ASR with no effect on VLDL apo B FCR [13] whereas a recent study found no change in the ASR or FCR of triglyceride rich apoB in men without diabetes treated with rosiglitazone when compared to placebo [15].

The lack of effect of pioglitazone on VLDL apo B kinetics is in keeping with previous studies. Treatment with 30-45mg of pioglitazone for 12-14 weeks in patients with type 2 diabetes was shown to have no effect on VLDL apo B kinetics [14]. The patients in this study had a much greater triglyceride than the current study (triglyceride was > 2.0mmol/l in 6 out of 8 patients) and pioglitazone reduced plasma triglyceride and VLDL triglyceride with no change in VLDL apo B suggesting a decrease in particle size as in the current study. There was an increased FCR of VLDL triglyceride, but there was no change in VLDL triglyceride ASR. Fibrates have also been shown to increase the FCR of VLDL [30], explained by hepatic activation of PPAR-α increasing expression of lipoprotein lipase and decreasing hepatic apo CIII synthesis. Pioglitazone may thus have PPAR-α like action. The lack of this effect in the current study may be due to the lower triglyceride concentrations.

This study demonstrates that in people with diabetes and well controlled lipids that although the addition of rosiglitazone and pioglitazone had no effect of plasma triglycerides or LDL cholesterol there was a decrease in VLDL triglyceride/apo B ratio suggesting a shift from large VLDL1 to small VLDL2. In addition rosiglitazone increased large buoyant LDL and decreased small dense LDL, an effect not seen with pioglitazone. This may explain the reported effects of rosiglitazone to increase LDL cholesterol, an effect which has been postulated to be the cause of the increased cardiovascular risk seen with this drug.

Acknowledgements: The study was supported by an unrestricted educational grant from Takeda to D. Russell-Jones. Takeda were not involved in any aspect of study inception, design or analysis and the authors also gratefully acknowledge Cedar Charity for funding the salary of A. Brackenridge.

Declaration of competing interests: Nil else to declare.

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