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## Fenofibrate and pioglitazone improve endothelial function and reduce arterial stiffness in obese glucose tolerant men

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### Abstract

Obesity is a low grade inflammatory state associated with premature cardiovascular morbidity and mortality. Along with traditional risk factors the measurement of endothelial function, insulin resistance, inflammation and arterial stiffness may contribute to the assessment of cardiovascular risk.

We conducted a randomised placebo controlled trial to assess the effects of 12 weeks treatment with a PPAR $\alpha$  agonist (fenofibrate) and a PPAR $\gamma$  agonist (pioglitazone) on these parameters in obese glucose tolerant men. Arterial stiffness was measured using augmentation index and pulse wave velocity (PWV). E-selectin, VCAM-1 and ICAM-1 were used as markers of endothelial function.

Insulin sensitivity improved with pioglitazone treatment ( $p = 0.001$ ) and, in keeping with this, adiponectin increased by 85.2% ( $p < 0.001$ ). Pro-inflammatory cytokine levels (TNF $\alpha$ , IL-6 and IL-1 $\beta$ ) fell with both treatments ( $p < 0.01$  for TNF $\alpha$  and IL-1 $\beta$ ,  $p < 0.001$  for IL-6). VCAM-1 and ICAM-1 were reduced with both treatments ( $p < 0.001$  for VCAM-1,  $p < 0.05$  for ICAM-1) and E-selectin improved with pioglitazone treatment ( $p = 0.05$ ). Both treatments resulted in a fall in augmentation index. PWV fell by 17.4% with fenofibrate treatment ( $p < 0.001$ ) and 16.3% with pioglitazone treatment ( $p < 0.001$ ).

Pioglitazone and fenofibrate treatment of obese, glucose tolerant men reduces inflammation, improves markers of endothelial function and reduces arterial stiffness. These results suggest that treatment with PPAR agonists has potential to reduce the incidence of premature cardiovascular disease associated with obesity.

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**Keywords:** Obesity; PPARs; Inflammation; Arterial stiffness; Endothelial function

### 1. Introduction

Obesity is associated with premature cardiovascular morbidity and mortality. Although management of traditional risk factors is a key element in primary prevention it is suggested that up to 20% of cardiovascular events are not predicted by the information used in the Framingham coronary risk score [1]. Novel risk factors which may provide additional information to assess the probability of a vascular event include those

related to endothelial dysfunction and markers of inflammation [2].

The intact endothelium provides a physical barrier, balances thrombosis and fibrinolysis and regulates vascular tone through the secretion of vasoactive mediators which influence vascular smooth muscle contraction. Endothelial dysfunction appears to occur early in atherosclerosis and can be detected before there is clinical evidence of vascular disease [3]. The adhesion of leucocytes to the endothelium is important in the early development of atherosclerotic plaques and endothelial cell expression of adhesion molecules promotes recruitment of leucocytes at this site. Endothelial function may be assessed by quantifying plasma levels of endothelial

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products such as soluble forms of these adhesion molecules shed into the plasma from activated endothelial cells. Plasma levels of VCAM-1, ICAM-1 and E-selectin are increased in patients with cardiovascular disease [4]. Early changes in the endothelium affect the rate at which the pulse wave is propagated and also result in an altered waveform related to arterial reactivity ('stiffness').

Measurement of arterial stiffness may be used as a functional measure of the vasculature in order to estimate the risk of cardiovascular events. Arterial stiffness, which is predictive of vascular disease outcomes, can be measured by analysis of the arterial waveform to determine the pulse wave velocity (PWV) and augmentation index [5]. Increased augmentation index is associated with both all-cause and cardiovascular mortality in patients with end stage renal failure [6], while a PWV greater than 13 m/s has been demonstrated to be a strong predictor of cardiovascular mortality in hypertension [7]. Its assessment will therefore be useful in obese patients both to identify individuals at high risk of cardiovascular disease and indicate if intervention has been beneficial.

Inflammation is central to the pathophysiology of atherosclerosis and is also thought to have a role in inducing insulin resistance. Adipose tissue is a source of inflammatory cytokines and in particular TNF $\alpha$  and IL-6; the levels of inflammatory markers may relate to adipose tissue mass [8] and potentially contribute to both the increased risk of cardiovascular disease and insulin resistance observed in obesity. By contrast increased levels of adiponectin, another cytokine derived from adipose tissue, are associated with insulin sensitivity; there is a tendency for lower levels of adiponectin in obesity [9]. The measurement of inflammatory markers, endothelial-derived adhesion molecules and arterial stiffness provides an assessment of cardiovascular risk which can be used to indicate response to treatment.

Obesity is associated with the metabolic syndrome and therefore dyslipidaemia and insulin resistance. PPAR $\alpha$  ligands are established agents in the management of hypertriglyceridaemia but may have additional effects on the arterial wall which could improve cardiovascular outcome [10]. PPAR $\gamma$  ligands were developed to improve insulin sensitivity however they demonstrate additional properties which suggest that they also could reduce cardiovascular risk in the metabolic syndrome [10]. This study was performed to assess the impact of PPAR $\alpha$  and PPAR $\gamma$  agonists on novel cardiovascular risk factors associated with obesity.

## 2. Methods

### 2.1. Randomised placebo controlled trial

A double blind randomised placebo controlled trial was conducted to examine the effect of a PPAR $\alpha$  agonist

and a PPAR $\gamma$  agonist on insulin resistance, inflammation, endothelial function and arterial stiffness in obese, glucose tolerant men. Obese men (BMI > 30 kg/m<sup>2</sup>) were randomised to 12 weeks treatment with either fenofibrate 160 mg once per day (as Supralip<sup>®</sup>, Fournier), pioglitazone hydrochloride (ACTOS<sup>®</sup>, Takeda) 30 mg once per day or placebo for 12 weeks. A group of lean, glucose tolerant men were recruited for comparison of baseline parameters (BMI < 25 kg/m<sup>2</sup>).

Inclusion criteria were as follows: non-smoker, no cardiovascular disease, no anti-hypertensive or lipid lowering therapy, blood pressure < 160/90 mmHg, fasting triglycerides < 6 mmol/l, total cholesterol < 7.5 mmol/l, fasting plasma glucose < 6.9 mmol/l and/or 2 h glucose < 7.8 mmol/l during OGTT (to exclude diabetes and impaired glucose tolerance). All patients gave written informed consent and the study protocol was approved by the Ethical Committee of Queens University, Belfast.

*Pre-treatment visit.* Patients attended after an overnight fast and a 75 g oral glucose tolerance test (OGTT) was performed to determine glucose tolerance and insulin sensitivity [11]. Blood was drawn and aliquoted for lipid profile, serum adiponectin, VCAM-1, ICAM-1, E-selectin, TNF $\alpha$ , IL-6 and IL-1 $\beta$ . Blood pressure was measured at the right brachial artery using the OMRON HEM-705CP automated sphygmomanometer. Arterial stiffness was measured by analysis of the arterial waveform to determine the augmentation index and pulse wave velocity (PWV) using the SphygmoCor<sup>TM</sup> pulse wave analysis system (AtCor Medical Pty. Ltd.). The software (SCOR-2000, version 6.3) generates a central waveform from the peripheral waveform using a validated transfer factor [5]. PWV in this study was measured by recording arterial pressure waveforms at the radial and carotid arteries sequentially.

*Treatment phase.* Obese subjects were randomised to treatment with placebo, pioglitazone or fenofibrate for 12 weeks.

*Final visit.* The procedures undertaken in pre-treatment visit were repeated for post-treatment assessment.

### 2.2. Laboratory methods

Plasma glucose, total cholesterol, HDL cholesterol and triglycerides were analysed on a VITROS 950 analyser (Ortho-Clinical Diagnostics) which uses slide based dry chemistry. LDL cholesterol was calculated using the Friedewald equation. Serum insulin was measured on an Abbott IMx analyser using a microparticle enzyme immunoassay technique (MEIA).

Adiponectin was analysed using Quantikine<sup>®</sup> human adiponectin immunoassay kit (R&D systems). TNF $\alpha$ , IL-6 and IL-1 $\beta$  were measured in serum using Quantikine<sup>®</sup> high sensitivity human TNF $\alpha$ , human IL-6 and human IL-1 $\beta$  immunoassay kits (R&D systems). VCAM-1, ICAM-1 and E-selectin were measured in serum using Parameter<sup>®</sup>

Table 1  
Age, BMI, blood pressure and lipid profiles of patients in each group at baseline and after treatment in the obese groups

Group (number of subjects)	Control [9]	Placebo [15]	Pioglitazone [16]	Fenofibrate [16]
Age (year)	50.3 ± 9.4	48.1 ± 8.3	47.9 ± 8.5	46 ± 7.6
BMI (kg/m <sup>2</sup> )				
Pre	23.4 ± 1.3***	35.4 ± 6.4	34.1 ± 3.6	32.1 ± 2.2
Post	–	35.0 ± 6.8	33.9 ± 3.8	32.0 ± 2.5
% change	–	–1.3	–0.6	–0.3
Waist circumference (cm)				
Pre	86.3 ± 7.4***	116.5 ± 17.4	111.4 ± 9.0	108.5 ± 4.1
Post	–	116.0 ± 17.3	109.4 ± 9.2	107.3 ± 6.8
% change	–	–0.4	–1.8	–1.1
Hip circumference (cm)				
Pre	88.2 ± 7.1***	112.5 ± 14.8	107.8 ± 7.2	105.7 ± 3.4
Post	–	112.6 ± 15.6	105.9 ± 7.9	104.9 ± 5.5
% change	–	0.1	–1.8	–0.8
Systolic BP (mmHg)				
Pre	121.6 ± 11.6*	133.5 ± 8.9	131.4 ± 8.3	130.3 ± 7.0
Post	–	126.6 ± 11.1	128.3 ± 3	124.3 ± 8.0
% change	–	–5.2	–2.4	–4.7
Diastolic BP (mmHg)				
Pre	79.9 ± 6.4	84.5 ± 5.9	84.6 ± 5.3	84.6 ± 5.7
Post	–	80.1 ± 6.2	82.2 ± 4.2	79.8 ± 6.6
% change	–	–5.2	–2.8	–5.7
Cholesterol				
Pre	4.82 ± 0.75	5.29 ± 0.89	5.27 ± 0.65	5.34 ± 0.68
Post	–	5.18 ± 1.08	5.26 ± 0.79	4.94 ± 0.84*
% change	–	–2.1	–1.9	–7.5
HDL cholesterol				
Pre	1.46 ± 0.35**	1.10 ± 0.23	1.28 ± 0.25	1.13 ± 0.26
Post	–	1.20 ± 0.27	1.34 ± 0.18	1.24 ± 0.27
% change	–	9.1	4.7	9.7
LDL cholesterol				
Pre	2.87 ± 0.63	3.07 ± 0.65	3.32 ± 0.63	3.30 ± 0.76
Post	–	2.95 ± 0.88	3.21 ± 0.63	3.07 ± 0.77
% change	–	–3.9	–3.3	–7.0
Triglycerides				
Pre	1.11 ± 0.28*	2.45 ± 1.47	1.88 ± 0.84	2.12 ± 1.09
Post	–	2.27 ± 1.3	1.72 ± 0.54	1.39 ± 0.80*
% change	–	–18	–8.5	–34.4

Results are expressed as mean ± S.D. Waist and hip circumference values were transformed to natural logarithms for statistical analysis. Lean vs. obese groups at baseline compared using ANOVA: \* $p < 0.05$ , \*\* $p = 0.01$ , and \*\*\* $p < 0.001$ . Obese groups post-treatment compared using univariate analysis of covariance.

human sVCAM-1, sICAM-1 and sE-selectin immunoassay kits (R&D systems).

### 2.3. Statistics

Statistical advice was obtained from the Northern Ireland Clinical Research Support Centre, Research and Development Office (NI). Statistical comparisons were performed using SPSS version 12.0. Lean and obese groups were compared at baseline by ANOVA. The obese groups were compared after treatment using univariate analysis of covariance with pre-treatment values as a covariant; this analyses the degree of change observed for significance. Non-parametric data were log transformed to natural logarithms to allow parametric testing.

## 3. Results

### 3.1. Body mass index

As patients were recruited on the basis of body mass index the lean group had BMIs which would be categorised as normal. The obese groups had BMIs of greater than 30.0 kg/m<sup>2</sup> before treatment and among the obese groups there was no significant weight change over the study period (Table 1).

### 3.2. Blood pressure

The normal weight group had lower systolic pressure ( $p < 0.05$ ) but similar diastolic pressure compared with base-

line levels in the obese groups. Blood pressure was not significantly altered by treatment.

### 3.3. Lipid profile

In keeping with dyslipidaemia associated with the metabolic syndrome, HDL-cholesterol was higher ( $p=0.01$ ) and triglycerides lower ( $p<0.05$ ) in the normal weight individuals relative to the obese groups (Table 1). After fenofibrate treatment total cholesterol fell by 7.5% ( $p<0.05$ ) and triglycerides improved by 34.4% ( $p<0.05$ ). There was no significant change in lipid profile with either placebo or pioglitazone.

### 3.4. Oral glucose tolerance

Obese patients had higher plasma glucose at 30 and 60 min than the lean group during the OGTT but fasting levels were similar (Fig. 1). Plasma glucose was less than 7.8 mmol/l at 120 min after 75 g of oral glucose indicating that, despite obesity, the patients who entered the treatment phase of the study had normal glucose tolerance.

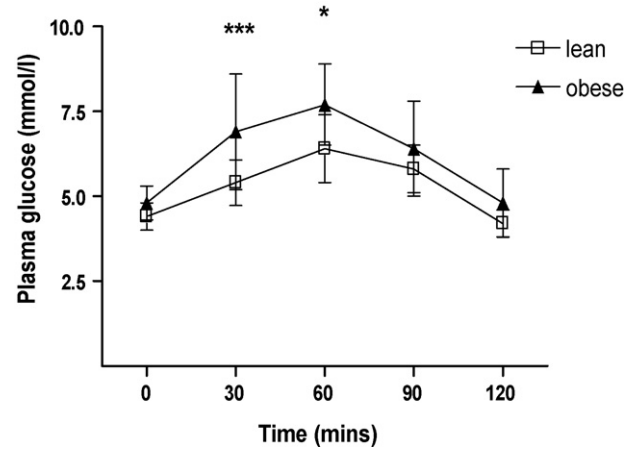


Fig. 1. Graph of mean plasma glucose at 30 min intervals during 75 g OGTT in lean and obese subjects. Error bars indicate S.D. Number of subjects: lean,  $n=9$ ; obese,  $n=47$ . Lean vs. obese groups at each time point compared using ANOVA: \* $p<0.05$  and \*\*\* $p<0.001$ .

Table 2

Fasting insulin and integrated insulin response to oral glucose in all groups at baseline and after treatment in obese subjects

Groups (number of subjects)	Control [9]	Placebo [15]	Pioglitazone [16]	Fenofibrate [16]
Fasting insulin (mU/ml)				
Pre	7.1 ± 2.3**	22.6 ± 11.1	16.9 ± 7.2	17.3 ± 7.8
Post	–	20.5 ± 10.8	10.2 ± 4.4*	12.8 ± 5.0*
% change	–	–9.3	–39.6	–26.0
Insulin AUC (mU/ml 180 min)				
Pre	113.5 ± 53.3***	298.7 ± 1696	246.9 ± 1878	227.9 ± 1015
Post	–	297.9 ± 208.3	158.3 ± 83.2####	216.7 ± 92.2
% change	–	–0.3	–35.9	–4.9

Integrated insulin response was calculated using area under the curve (AUC) of serum insulin at 30 min intervals up to 180 min during 75 g OGTT. Fasting insulin and insulin AUC values were transformed to natural logarithms for statistical analysis. Results are expressed as mean ± S.D. Lean vs. obese groups at baseline compared using ANOVA: \*\* $p<0.01$  and \*\*\* $p<0.001$ . Obese groups post-treatment compared using univariate analysis of covariance: \* $p<0.05$  and #### $p=0.001$ .

Table 3

Serum levels of E-selectin, ICAM-1 and VCAM-1 at baseline in all groups and after treatment in the obese groups

Groups (number of subjects)	Control [9]	Placebo [15]	Pioglitazone [16]	Fenofibrate [16]
E-selectin (ng/ml)				
Pre	43.1 ± 13.1	57.6 ± 20.6	62.5 ± 23.3	53.2 ± 20.8
Post	–	59.5 ± 27.5	51.6 ± 20.2~	50.9 ± 20.9
% change	–	3.3	–17.4	–4.3
ICAM-1 (ng/ml)				
Pre	266.3 ± 31.2	285.8 ± 57.1	276.9 ± 64.6	293.5 ± 87.5
Post	–	289.5 ± 64.2	257.3 ± 65.1*	257.9 ± 36.7*
% change	–	1.3	–7.1	–12.1
VCAM-1 (ng/ml)				
Pre	567.7 ± 134.7	632.8 ± 222.8	682.2 ± 246.5	676.6 ± 297.4
Post	–	648.4 ± 220.7	583.4 ± 212.4**	574.9 ± 229.3**
% change	–	2.5	–14.5	–15.0

Results are expressed as mean ± S.D. Lean vs. obese groups at baseline compared using ANOVA. Obese groups post-treatment compared using univariate analysis of covariance: ~ $p=0.05$ , \* $p<0.05$ , and \*\* $p<0.01$ .

Table 4  
Mean plasma levels of cytokines at baseline in all groups and after treatment in obese groups

Groups (number of subjects)	Control [9]	Placebo [15]	Pioglitazone [16]	Fenofibrate [16]
Plasma TNF $\alpha$ (pg/ml)				
Pre	1.27 $\pm$ 0.23*	2.09 $\pm$ 1.38	1.63 $\pm$ 0.49	2.00 $\pm$ 1.07
Post	–	2.00 $\pm$ 1.22	1.43 $\pm$ 0.42 <sup>##</sup>	1.48 $\pm$ 0.4 <sup>##</sup>
% change	–	–4.3	–12.3	–26.0
Plasma IL-6 (pg/ml)				
Pre	1.55 $\pm$ 0.35	1.61 $\pm$ 0.65	1.90 $\pm$ 0.95	1.70 $\pm$ 0.65
Post	–	1.89 $\pm$ 0.74	1.29 $\pm$ 0.43 <sup>###</sup>	1.33 $\pm$ 0.41 <sup>###</sup>
% change	–	17.4	–32.1	–21.8
Plasma IL-1 $\beta$ (pg/ml)				
Pre	0.09 $\pm$ 0.07	0.24 $\pm$ 0.16	0.30 $\pm$ 0.28	0.40 $\pm$ 0.30
Post	–	0.23 $\pm$ 0.16	0.13 $\pm$ 0.24 <sup>##</sup>	0.18 $\pm$ 0.31 <sup>##</sup>
% change	–	–4.2	–56.7	–55.0
Adiponectin ( $\mu$ g/ml)				
Pre	6.5 $\pm$ 3.2	4.9 $\pm$ 2.0	6.1 $\pm$ 2.3	5.2 $\pm$ 1.8
Post	–	5.0 $\pm$ 2.0	11.3 $\pm$ 4.6 <sup>###</sup>	5.0 $\pm$ 1.7
% change	–	2.0	85.2	–3.8

Results are expressed as mean  $\pm$  S.D. Values for TNF $\alpha$  and IL-6 were transformed to natural logarithms for statistical analysis. For TNF $\alpha$ , IL-6 and adiponectin: lean vs. obese groups were compared at baseline using ANOVA: <sup>##</sup> $p < 0.01$ . Obese groups post-treatment compared using univariate analysis of covariance: \* $p < 0.01$ , <sup>##</sup> $p < 0.01$  and <sup>###</sup> $p < 0.001$ . IL-1 $\beta$ : lean vs. obese groups at baseline compared using one-way ANOVA by ranks. Obese groups post-treatment analysed by calculating the change (before–after) and comparing these using a Kruskal–Wallis one-way analysis of variance by ranks: <sup>##</sup> $p < 0.01$ .

### 3.5. Insulin resistance

Two surrogate markers of insulin resistance were used: fasting insulin and the integrated insulin response to a 75 g oral glucose tolerance test (measured by the area under the curve of the serum insulin levels up to 3 h [11]). Both these parameters were much lower in the normal weight group (both  $p < 0.001$ ) compared to the obese subjects (Table 2). Fasting insulin improved by 39.6% in the pioglitazone treated group and by 26% with fenofibrate treatment (both  $p < 0.05$  when compared with placebo). The integrated insulin response was significantly reduced only by pioglitazone treatment where it fell by 35.9% ( $p = 0.001$ ).

### 3.6. Soluble adhesion molecules

ICAM-1, VCAM-1 and E-selectin were found to vary widely among individuals with a coefficient of variation of approximately 20% for ICAM-1 and 35% for VCAM-1 and E-selectin (Table 3). Both pioglitazone treatment and fenofibrate resulted in significant falls in ICAM-1 ( $p < 0.05$ ) and VCAM-1 ( $p < 0.01$ ) (Table 3). Absolute E-selectin levels were similar following fenofibrate and pioglitazone treatment but this represented a non-significant fall of 4.3% with fenofibrate and significant fall of 17.4% with pioglitazone (Table 3). These results indicate an improvement in endothelial function with both pioglitazone and fenofibrate. In the group receiving pioglitazone treatment the change in VCAM-1 correlated with the improvement in insulin sensitivity measured by the integrated insulin response ( $r = 0.47$ ,  $p = 0.029$ ) (Table 3).

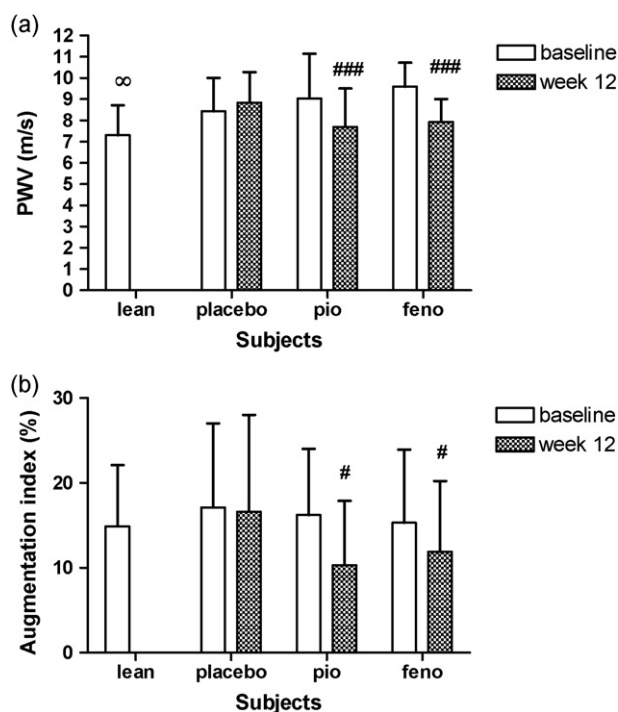


Fig. 2. Mean PWV and augmentation index in all groups at baseline and obese groups after treatment: (a) PWV and (b) augmentation index; pio, pioglitazone; feno, fenofibrate. Numbers in study: lean,  $n = 9$ ; placebo,  $n = 15$ ; pio,  $n = 16$ ; feno,  $n = 16$ . Error bars indicate S.D. Lean vs. obese groups at baseline compared using ANOVA:  $\infty p = 0.012$ . Obese groups post-treatment compared using univariate analysis of covariance: <sup>#</sup> $p < 0.05$ ; <sup>###</sup> $p < 0.001$ .

### 3.7. Cytokines/adipokines

Baseline TNF $\alpha$  was significantly lower in the normal weight group; all three pro-inflammatory cytokines measured fell with both active treatments in the obese subjects (Table 4). Adiponectin showed a tendency (not significant) to be lower in obese groups at baseline and rose significantly (by 85.2%,  $p < 0.001$ ) in the group receiving pioglitazone (Table 4); fenofibrate and placebo treatment did not cause any change in adiponectin levels.

### 3.8. Arterial stiffness

Arterial stiffness as measured by PWV was lower in lean individuals ( $p = 0.012$ ). Augmentation index is recognised to be variable among individuals; this was reflected in the large standard deviation found (Fig. 2(b)). Both augmentation index (Fig. 2(b)) and PWV (Fig. 2(a)) were reduced significantly after each treatment compared with placebo ( $p < 0.05$  for augmentation index and  $p < 0.001$  for PWV). Blood pressure, which influences arterial stiffness, did not change significantly in any of the three groups suggesting that changes in arterial stiffness measured in this study are due to factors other than this.

## 4. Discussion

As the prevalence of obesity increases strategies to effectively tackle both obesity and its consequences are therefore necessary. Subjects in this study were recruited in the early phase of the syndrome(s) associated with obesity; that is, they were clinically well, had normal glucose tolerance and were without symptomatic cardiovascular disease. However, they were insulin resistant, a state that precedes the onset of type 2 diabetes and is an independent risk factor for the development of cardiovascular disease [12]. Treatment of obesity with diet and exercise leading to weight reduction would be an ideal strategy but this approach yields suboptimal results since, for most affected individuals, these positive life-style changes prove difficult to maintain. The present study demonstrates the potential role of PPAR agonists in reducing the vascular risk factors associated with obesity.

Inflammation appears to play an important role in the development of insulin resistance and endothelial dysfunction in obese individuals. The inflammatory adipokines TNF $\alpha$  and IL-6 are implicated in the pathogenesis of insulin resistance. In contrast to TNF $\alpha$  and IL-6, adiponectin reduces plasma glucose, increases fatty acid oxidation in skeletal muscle and augments insulin induced inhibition of hepatic glucose output [13]. The production of TNF $\alpha$  and IL-6 increase [14] while adiponectin is reduced [9] in obesity. Similar to the current study Tsigos et al. reported that plasma TNF $\alpha$  positively correlates with abdominal obesity [15]; however, there is no consensus about the contribution of fat depots to circulating levels of TNF $\alpha$  [14]. The

increase in pro-inflammatory cytokines in adipose tissue is closely linked to the increase in adipose tissue resident macrophages [16]. The thiazolidinediones (TZDs) are known to have anti-inflammatory properties; one of the TZDs, rosiglitazone, has been shown to reduce the number of macrophages in white adipose tissue in mice [16], and hence to reduced macrophage-derived inflammatory cytokines. TZDs also promote adipocyte differentiation and it is suggested that the smaller adipocytes which result from this are more insulin sensitive than large adipocytes [17]. In the present study pioglitazone treatment caused a marked increase in plasma adiponectin levels as well as a reduction in TNF $\alpha$  and IL-6. Fenofibrate treatment has also been shown to reduce inflammation. For example, fibrates inhibit AP-1 and NF $\kappa$ B mediated transcription of IL-6 in VSMC [18]. These effects may be widespread and mediate the direct anti-inflammatory effects of fenofibrate. Fibrates may potentially improve insulin sensitivity through their effects on lipids and inflammatory markers [10]. However, although fenofibrate treatment did result in a fall in total cholesterol, triglycerides and circulating pro-inflammatory cytokines it did not improve adiponectin levels or insulin sensitivity in this study. It is possible that the differences in adiponectin response may explain in part why pioglitazone had a more marked effect on insulin sensitivity than fenofibrate.

The improvement in circulating cytokines and adipokines during treatment with PPAR $\alpha$  and PPAR $\gamma$  agonists was paralleled by reduced levels of circulating adhesion molecules. E-selectin, important for initiating the capture and adhesion of leucocytes, is produced only by the endothelium whereas ICAM-1 and VCAM-1 have a wider tissue distribution that may limit the degree to which elevated plasma levels can be attributed to endothelial dysfunction [4]. The elevated levels of pro-inflammatory cytokines in obesity may promote the expression of all adhesion molecules and contribute to the development of atherosclerosis. NF $\kappa$ B may be a final common pathway in regulating expression of cell adhesion molecules with inflammation, dyslipidaemia, oxidative stress and advanced glycation endproducts influencing it. In health nitric oxide (NO) inhibits NF $\kappa$ B activation [19] and when there is impairment of NO production, as is found in endothelial dysfunction preceding atherosclerosis [3], this inhibitory effect is lost. Fenofibrate has been found to increase the half-life of eNOS by stabilizing its mRNA and this may be a mechanism through which fibrates improve endothelial function [20]. There may also be direct effects of PPAR agonists on adhesion molecule production since endothelial cells possess PPAR receptors and reduced adhesion molecule expression has been shown in vitro [21].

The improvement in E-selectin, VCAM-1 and ICAM-1 in vivo with pioglitazone has not been previously reported. In endothelial cell culture troglitazone treatment resulted in a parallel fall in expression of adhesion molecules and NF $\kappa$ B [22]. This fall may be due to increased NO levels and PPAR $\gamma$  ligands increase NO release in vitro but do not increase eNOS expression or protein levels [23]. Pioglitazone

zone is an insulin sensitizer but the direct effect of insulin on adhesion molecules is uncertain. Adiponectin, which in the present study increased with pioglitazone treatment, has been shown to attenuate the TNF $\alpha$  stimulated increase in E-selectin, VCAM-1, and ICAM-1 on the cell surface of endothelial cells in culture [24]. There are, therefore, several mechanisms acting on the anti-inflammatory pathway which may produce the improvement in endothelial function with pioglitazone.

Fenofibrate, as a PPAR $\alpha$  agonist, has potent triglyceride lowering effects with a modest effect on cholesterol. The mechanism for the fall in VCAM-1 and ICAM-1 with this treatment may be due to its lipid lowering properties particularly the reduction of VLDL. Fenofibrate has also been shown to have anti-inflammatory effects and improve insulin sensitivity both of which may alter VCAM-1, ICAM-1 and E-selectin levels. Fenofibrate reduces NF $\kappa$ B binding to its recognition site on the VCAM-1 promoter thus down regulating NF $\kappa$ B activity and VCAM-1 expression [25].

The vascular stiffness associated with obesity may be a direct effect of insulin resistance, fatty acids or pro-inflammatory cytokines. An increased augmentation index has been demonstrated in patients with rheumatoid arthritis who did not have evidence of vasculitis, cardiovascular disease or known cardiac risk factors [26]. This would suggest that inflammation per se may increase arterial stiffness and contribute to the excess cardiovascular mortality in rheumatoid arthritis. Obesity is recognised to be an inflammatory state and treatments which reduce this such as PPAR agonists may be important in improving vascular outcomes in this group of patients. The improvements in arterial stiffness with fenofibrate demonstrated in this study are novel and may be the result of a reduction in free fatty acids and anti-inflammatory effects.

Like fenofibrate, pioglitazone also reduced inflammation and the fall in TNF $\alpha$  in particular may reduce arterial stiffness. TZDs have a marked influence on insulin sensitivity and insulin resistant states such as type 2 diabetes and impaired glucose tolerance which are associated with arterial stiffness [27]. Improving insulin sensitivity with pioglitazone may therefore be an important mechanism by which PPAR $\gamma$  agonists reduce arterial stiffness. Insulin promotes the release of nitric oxide [28] but insulin resistance, and therefore hyperinsulinaemia, is correlated with increased arterial stiffness and reduced responsiveness to exogenous insulin. This may reflect a difference in the response of the endothelium to insulin in insulin sensitive and insulin resistant states. Indeed a correlation has been shown between reduced insulin stimulated glucose uptake and reduced augmentation index in response to insulin infusion in non-diabetic men [29]. This suggests that in insulin resistant states the blood vessels are also less reactive to insulin stimulation.

The value of improving arterial stiffness is highlighted by a study which reports that augmentation index measured using the same technique as used in this project correlated with coronary atherosclerosis found at angiography [30].

In conclusion this investigation has demonstrated that a PPAR $\alpha$  agonist (fenofibrate) and a PPAR $\gamma$  agonist (pioglitazone) reduce inflammation, adhesion molecules and arterial stiffness in obese glucose tolerant men suggesting that there is evidence to explore the long-term use of these agents in prevention of coronary heart disease in these conditions.

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