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RESEARCH PAPER

Irbesartan-mediated reduction of renal and cardiac damage in insulin resistant JCR : LA-*cp* rats

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Background and purpose: Angiotensin II receptor antagonists (ARBs), originally developed for antihypertensive properties, have pleiotropic effects including direct vascular actions. We tested the hypothesis that the ARB irbesartan would be effective against micro- and macrovascular complications of the prediabetic metabolic syndrome using the obese, insulin-resistant JCR : LA-*cp* rat that exhibits micro- and macrovascular disease with ischaemic myocardial lesions and renal disease.
Experimental approach: Obese male rats were treated with irbesartan (30 mg·kg^{-1}·day^{-1}, incorporated into chow) from 12 to 25 weeks of age.
Key results: Irbesartan treatment caused no change in food intake or body weight. Fasting glycaemic control of the JCR : LA-*cp* rats was marginally improved, at the expense of increased plasma insulin levels (~50%). Fasting plasma triglycerides were marginally reduced (~25%), while cholesterol concentrations were unchanged. Elevated concentrations of adiponectin, monocyte chemotactic protein-1 and plasminogen activator inhibitor-1 were reduced along with severity of glomerular sclerosis. Macrovascular dysfunction (aortic hypercontractile response to noradrenergic stimulus and reduced endothelium-dependent relaxation) was improved and frequency of ischaemic myocardial lesions reduced (62%).
Conclusions and implications: Irbesartan reduces markers of inflammation and prothombotic status, improves macrovascular function and reduces glomerular sclerosis and myocardial lesions in a model of the metabolic syndrome. Unlike pharmaceutical agents targeted on metabolic dysfunction, irbesartan reduced end-stage disease without major reduction of plasma lipids or insulin. The protective effects appear to be secondary to unknown intracellular mechanisms, probably involving signal transduction pathways. Understanding these would offer novel pharmaceutical approaches to protection against cardiovascular disease.
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Keywords: metabolic syndrome; vascular function; glomerular sclerosis; PAI-1; MCP-1; adiponectin

Abbreviations: Ang II, angiotensin II; ARB, angiotensin II receptor antagonist; H&E, haematoxylin and eosin; HDL, high density lipoprotein; L-NAME, N^G-nitro-L-arginine methyl ester; LDL, low density lipoprotein; MCP-1, monocyte chemoattractant protein-1; NO, nitric oxide; ObR, leptin receptor; PAI-1, plasminogen activator inhibitor-1; PE, phenylephrine; TG, triglyceride; VLDL, very low density lipoprotein; VSMC, vascular smooth muscle cell

Introduction

The metabolic syndrome characterized by abdominal obesity, insulin resistance and consequent hyperinsulinaemia and dyslipidaemia has become recognized as a major public health problem in prosperous societies worldwide (Després *et al.*, 1996; Yusuf *et al.*, 2001). Prediabetic insulin resistance and associated metabolic abnormalities have driven a developing epidemic of type 2 diabetes, atherosclerosis and cardiovascular disease (Butler *et al.*, 2008). There is growing evidence that hyperinsulinaemia is a major determinant of vasculopathy, atherosclerosis and ischaemic cardiovascular disease associated with the metabolic syndrome (Després *et al.*, 1996; O'Brien and Russell, 1997). There is also a major contribution from apolipoprotein B containing lipid particles (Proctor *et al.*, 2004). In addition to the macrovascular disease, evident in coronary, cerebral and other large arteries, the metabolic syndrome is associated with microvascular complications that underlie a major portion of glomerular sclerosis and renal failure (Lorenzo *et al.*, 2008). The fundamental role of the metabolic syndrome in cardiovascular disease and mortality has led to a focus on therapeutic intervention to reduce obesity, related insulin resistance and

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hyperinsulinaemia. Changes in diet, food intake and physical activity have proven relatively ineffective in the human population and may well be confounded by environmental, behavioural and genetic factors (Hegele *et al.*, 2003). Thus, there have been significant efforts to develop effective pharmaceutical treatments, largely based on the use of animal models that mimic the metabolic and pathophysiological aspects of the metabolic syndrome (Proctor and Russell, 2006).

The rennin-angiotensin system plays a major role in blood pressure regulation and hypertension. Angiotensin converting enzyme (ACE) inhibitors reduce production of angiotensin II (Ang II) and have proven to be very useful antihypertensive agents. They have pleiotropic effects and we have reported that both captopril and ramipril are cardioprotective in an animal model of the metabolic syndrome and cardiovascular disease (Russell *et al.*, 1998a; 2004). Angiotensin II receptor antagonists (ARBs) are more recently developed agents that inhibit the rennin-angiotensin pathway by antagonizing the binding of Ang II to the AT_1 receptor (Alexander *et al.*, 2008). ARBs have been viewed as useful adjuncts to the treatment of cardiovascular disease because of their antihypertensive effects. However, there are recent indications that the effects of ARBs may be broader and include direct beneficial effects on diabetic vasculopathy (Karalliedde *et al.*, 2008; Negro, 2008).

The JCR : LA-*cp* rat is a unique strain that has been well established as an animal model of the metabolic syndrome, particularly in the study of the underlying mechanisms of associated cardiovascular disease (Proctor and Russell, 2006). Rats of this strain that are homozygous for the autosomal recessive *cp* gene (*cp/cp*) spontaneously develop the pathophysiological characteristics associated with the metabolic syndrome in humans (O'Brien *et al.*, 1999; Russell *et al.*, 1999). These include advanced intimal (atherosclerotic) lesions, myocardial ischaemic lesions and microvascular renal dysfunction (Russell and Proctor, 2008), but the animals are not hypertensive (Russell and Amy, 1986). Vascular dysfunction, at the macrovascular level, includes increased contractility and reduced endothelium-mediated vascular relaxation, and at the microvascular level is evident in albuminuria and glomerular sclerosis. Importantly, from an experimental point of view, we have shown that heterozygous (*cp*/+), or homozygous normal (+/+) rats are lean and metabolically normal and not distinguishable, providing a benchmark of normality.

The obese, disease-prone phenotype of the JCR : LA-*cp* rat is due to the *cp* mutation that results in a stop codon in the extracellular domain of the leptin receptor (ObR) (Wu-Peng *et al.*, 1997), leading to absence of the ObR in membrane-bound or soluble form. Absence of the leptin feedback signal leads to significant hyperphagia, obesity and rapid development of insulin resistance between the age of 4 and 7 weeks. There is an accompanying progressive development of a very low density lipoprotein (VLDL) hypertriglyceridaemia with delayed clearance of postprandial chylomicrons (Vance and Russell, 1990; Vine *et al.*, 2007). Adult *cp/cp* rats have essentially no insulin-mediated glucose uptake, but maintain euglycaemia at the expense of extreme hyperinsulinaemia, fasting and postprandial (Russell *et al.*, 1999). High insulin levels appear, in themselves, to have pathological effects and be a major contributor to the atherosclerosis and vascular dysfunction (Richardson *et al.*, 1998).

The hypothesis underlying this study was that irbesartan has pleitropic effects in the presence of the metabolic syndrome, beyond reduction of food intake, obesity, hyperlipidaemia or insulin resistance, and confers protection against end-stage vascular disease. Consequently, the focus of the study was on end-points of insulin/glucose metabolism and control, macrovascular function, glomerular damage and dysfunction and end-stage ischaemic lesions of the heart. The results indicate that irbesartan has beneficial effects on the vascular disease, in the absence of a significant reduction in food intake or on the principal parameters of the prediabetic metabolic syndrome.

Methods

Animals

Male JCR : LA-*cp* rats, *cp/cp* (obese) and +/? (lean; a 2:1 mix of *cp*/+ and +/+), were bred and maintained in our established rat colony (Russell *et al.*, 1995). The group of lean phenotype, +/?, animals were included throughout the study to provide a benchmark of normal physiology and metabolism. Rats were housed in an isolated high-efficiency particulate air filtered caging system (Techniplast S.p.a., Buguggiate, Italy) individually and placed on a reversed light cycle 1 week prior to the start of the experimental protocol to facilitate metabolic studies during the active (dark) phase of their diurnal cycle. All food was Lab Diet 5001 (PMI Nutrition International Inc., Brentwood, MO, USA). Rats were weighed and food intake determined twice per week throughout the experimental period. All care and treatment of the rats was in accordance with the Guidelines of the Canadian Council on Animal Care and was subject to prior review and approval by the Health Sciences Animal Policy Committee of the University of Alberta.

Experimental procedures

Rats were allowed to age within the breeding unit, without intervention, until 11 weeks of age, when they were transferred to the experimental facility and the Techniplast™ caging. After a 1-week acclimatization period, they were randomly assigned to treatment and control groups. At 24 weeks of age, a standardized meal tolerance test was performed, including collection of fasting blood. At 25 weeks of age, the rats were killed under isofluorane anaesthesia. Fed state blood was taken from the left ventricle for biochemical assays, together with the right kidney and the heart for histology, the thoracic aorta for assessment of vascular function and urine was collected from the bladder.

Meal tolerance test

The meal tolerance test was performed following a standardized protocol (Russell *et al.*, 1999). Rats were subjected to a mock procedure 1 week previously. The rats were deprived of food for 16 h over the light (inactive) period and the test was conducted in the early part of the dark period. Conscious

unrestrained rats were subjected to three blood samplings during each session. Animals were placed on a heated table to ensure vasodilatation of the tail, and 0.5 mL of blood was taken from the tip of the tail (0 min). Rats were then replaced in their cages and given a 5 g food pellet (the test meal). Timing began when 50% of the test meal had been consumed, and samples of blood were taken at 30 and 60 min for the analysis of glucose and insulin. All rats ate the full test meal within 15 min of presentation.

Drugs and chemicals

Irbesartan was provided by Sanofi-Synthelabo Recherche (Ruel-Malmaison, France). The agent was incorporated into powdered rat chow at a concentration, based on body weight and food consumption of the rats, to maintain a dose of 30 $mg \cdot kg^{-1} \cdot day^{-1}$, as recommended by Sanofi-Synthelabo Recherche. The chow was pelleted by extrusion through a die and air dried as previously described (Russell *et al*., 2000). Reagents and chemicals were obtained from Sigma Chemical (Oakville, ON, Canada).

Analytical methods

Plasma glucose was determined using a glucose oxidase assay procedure (Diagnostic Chemicals Ltd., Charlottetown, PEI, Canada) and insulin assayed by rat ELISA (Mercodia AB, Uppsala, Sweden). Colorimetric assays obtained from Wako Pure Chemicals USA, Inc. (Richmond, VA, USA) were used to determine: plasma triglyceride (L-Type TG-H), total cholesterol (Cholesterol E) and low density lipoprotein (LDL) cholesterol (L-Type LDL-C). High density lipoprotein (HDL) cholesterol was measured using direct HDL assay (Diagnostic Chemicals). Adiponectin was measured by radioimmunoassay (#MADP-60HK) by enzyme-linked immunosorbent assay using LINCOplex™ plates (Linco Research, St Charles, MO, USA). Monocyte chemoattractant protein-1 (MCP-1) and plasminogen activator inhibitor-1 (PAI-1) activity were measured by immunoassays (R&D Systems, Minneapolis MN, USA and Diapharma Group Inc., West Chester, OH, USA respectively). Urine albumin and creatinine measurements were performed on a Beckman Coulter LX20i analyser using immuno turbidimetric and Jaffé methods respectively.

Vascular function studies

The vascular function of aortic rings, with intact endothelium, was assessed using established methods (Russell *et al*., 2001). Briefly, the thoracic aorta was excised, trimmed of adhering fat and connective tissue, and cut into 3 mm-long transverse rings. Rings were mounted on stainless steel hooks under 1.5 g resting tension in 10 mL organ baths and bathed at 37°C in Krebs solution (containing in mM: NaCl 116, KCl 5.4, $CaCl_2$ 1.2, $MgCl_2$ 2, Na_2PO_4 1.2, glucose 10 and $NaHCO_3$ 19) and gassed with 95% O_2 and 5% CO_2. Tension was recorded isometrically with Grass FTO3C transducers (Grass Medical Instruments, Quincy, MA, USA) and displayed on a Digi-Med tissue force analyser (Model 210, Micro-Med, Louisville, KY, USA), linked to an IBM-compatible computer that acquired data digitally using DMSI 210/4 (Micro-Med) software.

The contractile response of endothelium-intact rings of aortae to phenylephrine (PE) was assessed through concentration–response curves for PE (1 nM to 300 mM). The basal nitric oxide (NO)-mediated relaxation of aortic rings (precontracted with PE to 80% of maximal contraction) was assessed by determining the relaxant response to different concentrations of the endothelial NO-releasing agent ACh and the nitrate donor sodium nitro prusside (SNP). Direct assessment of NO-mediated effects was also determined through addition of N^G-nitro-L-arginine methyl ester (L-NAME), at 10^{-4} M, in order to inhibit nitric oxide synthase (NOS).

Histology

Kidneys were cut through the hilum on the long axis, fixed in formalin, subjected to conventional processing and sectioning, followed by haematoxylin and eosin (H&E) staining. The extent of glomerular sclerosis was determined using a similar process to that of Schäfer *et al*. (2004) and guided by the interpretation of Ferrario and Rastaldi (2006). Four fields of view of the right kidney of each rat were recorded at ×2 magnification on a digital camera system. Histological images were visualized using Photoshop (V7.0, Adobe Systems Inc., San Jose, CA, USA), examined blind, and all glomeruli in each field (minimum of 40 per kidney) rated as normal or sclerotic (Russell and Proctor, 2007). Results are expressed as % of glomeruli that exhibited sclerosis.

Hearts were cut transversely into four blocks, fixed in formalin, subjected to conventional processing, embedded in a single paraffin block and sectioned, followed by H&E staining. The heart sections were examined blind by an experienced observer and the number of ischaemic lesions identified in each of the sections summed for each heart. The lesions were categorized by four stages as previously described (Russell *et al*., 1995).

Statistical analysis

Results are expressed as mean ± SEM and were analysed using SigmaStat (Jandel Scientific, San Rafael, CA, USA) and plotted using SigmaPlot (Systat Software, Inc. San Jose, CA, USA) and Prism (Graphpad, San Diego, CA, USA). Results were compared using one-way analysis of variance (ANOVA) followed by multiple comparison tests. Concentration–response curves were analysed using the program ALLFIT (DeLean *et al*., 1978), which fits the complete data set to the logistic equation and permits independent testing of differences between individual parameters. A value of $P < 0.05$ was taken as being statistically significant.

Results

Food intake and body weight

Figure 1 shows food intake and body weights of *cp/cp* rats, control and irbesartan-treated, over the period from 12 to 24 weeks of age with data from +/? control rats shown for reference. Irbesartan did not alter food intake or body weight of *cp/cp* rats.

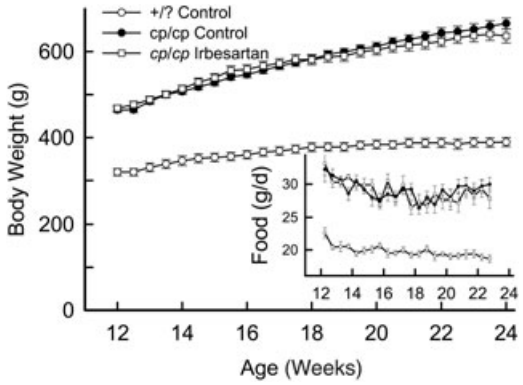

Figure 1 Food intake and body weight of JCR : LA-cp rats during treatment from 12 to 24 weeks of age. Values are mean ± SEM, 10 rats per group. There were no significant differences between control and irbesartan-treated *cp/cp* rats ($P > 0.05$).

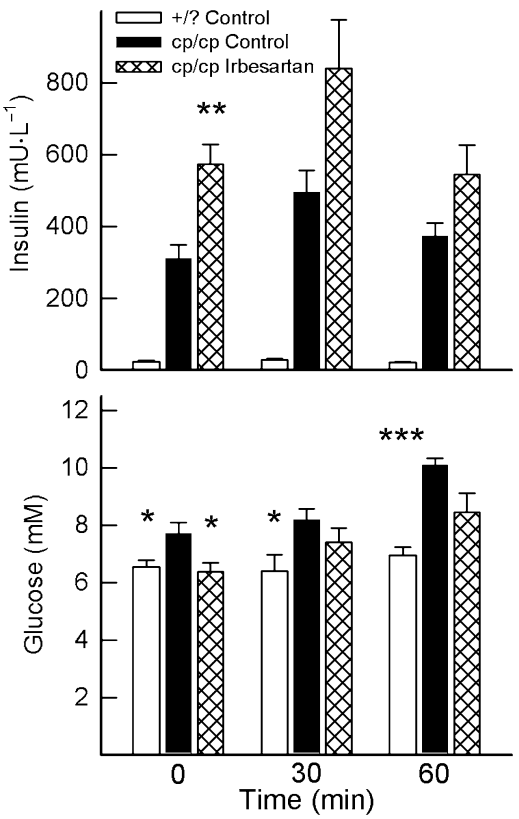

Figure 2 Plasma insulin (upper panel) and glucose (lower panel) concentrations in the meal tolerance test. Values are mean ± SEM, 10 rats per group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus *cp/cp* control.

Insulin and glucose metabolism

Fasting (0 min) and postprandial (30 and 60 min during the meal tolerance test) plasma insulin and glucose concentrations are shown in Figure 2. The +/? control rats had modestly lower fasting glucose concentrations than the *cp/cp* controls in the presence of markedly lower plasma insulin levels. Irbesartan-treated *cp/cp* rats had significantly higher fasting insulin levels than the *cp/cp* controls ($P < 0.01$), accompanied by lower glucose concentrations ($P < 0.05$ vs. *cp/cp* control). At the 30- and 60-min postprandial time points, +/? rats continued to have very low insulin levels and significantly lower glucose concentrations ($P < 0.05$ and $P < 0.001$). The apparently lower glucose concentrations and higher insulin levels of the irbesartan-treated *cp/cp* rats through the 30 and 60 min time points were not significant compared with *cp/cp* controls ($P > 0.05$).

Plasma lipids

Fasting plasma lipid concentrations of the rats are shown in Figure 3. As previously reported, HDL and total cholesterol levels were significantly lower in the +/? control rats than in *cp/cp* controls (Vance and Russell, 1990). Irbesartan treatment had no effect on cholesterol lipoprotein fractions of *cp/cp* rats. However, plasma TG levels of irbesartan-treated rats were modestly decreased compared with *cp/cp* controls ($P < 0.05$).

Cytokines and thrombosis

As shown in Figure 4, MCP-1 levels were significantly elevated in the *cp/cp* rats compared with +/? rats (59%, $P < 0.01$) and were significantly reduced by irbesartan treatment (30%, $P < 0.05$). The markedly elevated plasma levels of PAI-1 (fourfold, $P < 0.01$) were also reduced by irbesartan (46%, $P < 0.01$). In addition, elevated plasma levels of the adipose tissue-related cytokine adiponectin (2.8-fold, $P < 0.001$) were lowered significantly (30%, $P < 0.01$) by irbesartan.

Vascular function

The contractile response of aortic rings to the adrenoceptor agonist PE and the relaxant response to ACh are shown in Figure 5 and Table 1. Aortae from *cp/cp* rats have enhanced PE-mediated contractility compared with +/? rats. Hypercontractility was reduced in aortae from irbesartan-treated rats, in both the absence and presence of the inhibitor of NO synthesis, L-NAME ($P < 0.001$ and $P < 0.05$ respectively). Irbesartan had no effect on the EC_{50} for PE (Table 1). ACh-mediated relaxation of PE precontracted aortic rings of *cp/cp* rats is significantly impaired, with reduced maximal relaxation compared with +/? control rats. Aortic rings from irbesartan-treated rats had increased maximal ACh-mediated relaxation, and increased sensitivity to ACh, indicated by a 65% decrease in EC_{50} ($P < 0.001$). In addition, the EC_{50} for SNP-mediated relaxation was significantly reduced in aortae from irbesartan-treated rats.

Renal function and glomerular sclerosis

Urinary albumin excretion was markedly elevated in the *cp/cp* rats at 24 weeks of age ($P < 0.001$), as shown in Figure 6, and was accompanied by increased severity of glomerular sclerosis ($P < 0.05$). Treatment of *cp/cp* rats with irbesartan did not significantly reduce the albumin/creatinine ratio. However, the fraction of glomeruli that were sclerotic was significantly reduced in the irbesartan-treated rats compared with controls ($P < 0.05$).

Ischaemic myocardial lesions

As shown in Figure 7, there were relatively few Stage 1 lesions (areas of early ischaemia or necrosis) or Stage 3 lesions (foci of

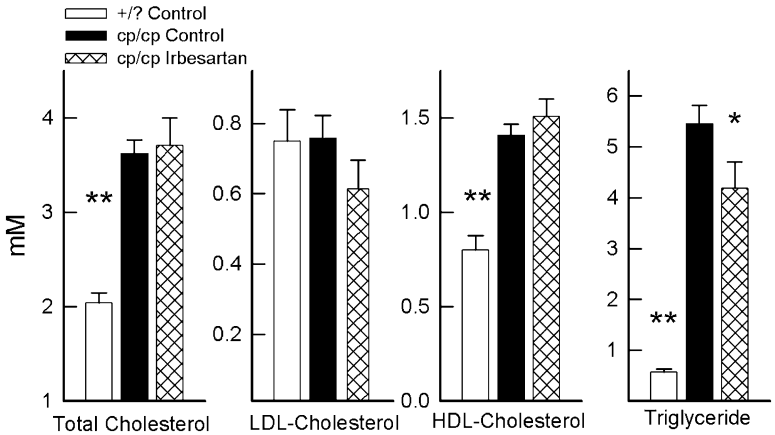

Figure 3 Fasting plasma lipid concentrations in irbesartan-treated JCR : LA-cp rats. Values are mean ± SEM, 10 rats per group. **P* < 0.05; ***P* < 0.0001 versus *cp/cp* control. HDL, high density lipoprotein; LDL, low density lipoprotein.

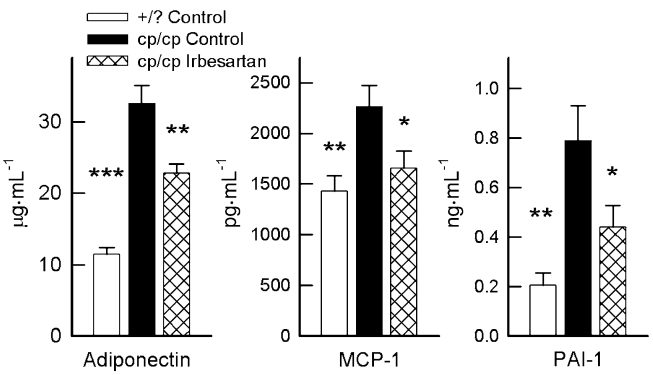

Figure 4 Concentrations of adiponectin, MCP-1 and PAI-1 in plasma of irbesartan-treated *cp/cp* rats. Values are mean ± SEM, 7–10 animals per group for adiponectin and PAI-1 and 4–6 animals for MCP-1. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 versus *cp/cp* control. MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1.

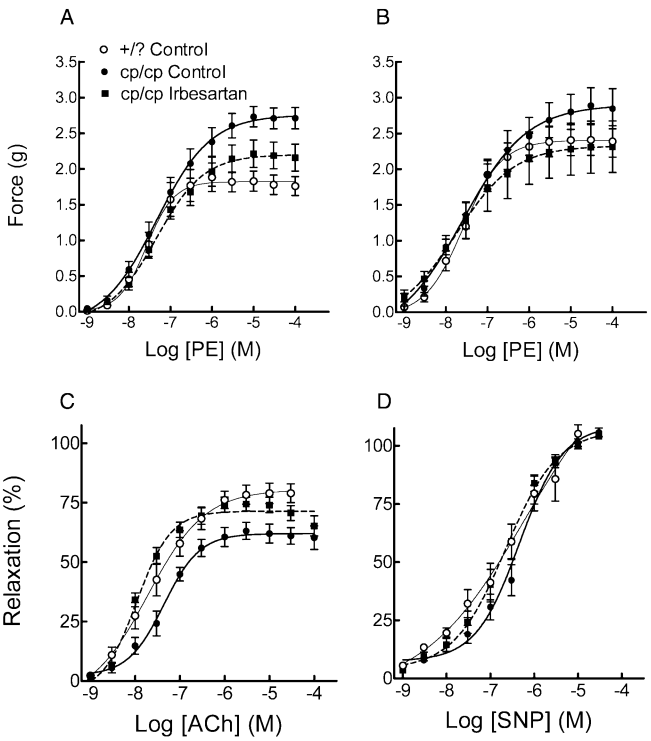

Figure 5 Effect of irbesartan treatment on aortic vascular function in *cp/cp* rats. (A) PE-mediated contractile response in the absence of L-NAME. (B) PE-mediated contractile response in the presence of L-NAME, 10^{-4} M. (C) ACh-mediated concentration–relaxant response of PE pre-contracted aortic rings. (D) SNP-mediated relaxant response of PE pre-contracted aortic rings. Values are mean ± SEM, 10 rats per group. The concentration–response curves were calculated using the logistic equation and the ALLFIT program, with values of the parameters shown in Table 1, together with statistical significance of differences. L-NAME, N^G-nitro-L-arginine methyl ester; PE, phenylephrine; SNP, sodium nitro prusside.

chronic inflammatory cells without cell dropout) in the hearts of any of the groups of rats. There were no significant differences between the groups. Stage 2 lesions (areas with strong inflammatory cell infiltration and cell lysis) were infrequent in the hearts of +/? control rats and frequent in the hearts of *cp/cp* control rats ($P < 0.001$). Irbesartan treatment of *cp/cp* rats reduced the frequency of Stage 2 lesions to the range seen in the hearts of +/? rats ($P < 0.01$). Stage 4 lesions were relatively infrequent in these 24-week-old hearts reflecting the cumulative nature of these mature scarred lesions and the relatively young age of the rats. No Stage 4 lesions were seen in irbesartan-treated hearts, but the low frequency of these lesions in these younger rats makes this observation non-significant ($P > 0.05$).

Discussion

The absence of any effect of irbesartan on food intake or body weight is consistent with it being a specific antagonist of a peptide hormone receptor (i.e. an ARB). The reduction in fasting glucose concentrations may be interpreted as reflecting an improvement in glucose control. This is consistent with the physiological adaptation of the untreated *cp/cp* rat that maintains euglycaemia in the presence of the developing extreme insulin resistance through a compensating hyperinsulinaemia (Russell *et al.*, 1999). However, the present results do not indicate a major increase in insulin sensitivity, which

Table 1 Vascular function of JCR : LA-*cp* rats: effects of irbesartan

	+/? rats	*cp/cp rats*	
	Control	*Control*	*Irbesartan*
PE contractility			
Maximum (g)	1.82 ± 0.07***	2.77 ± 0.10	2.18 ± 0.09***
EC_{50} (M X10^{-8})	2.62 ± 0.57**	6.02 ± 1.35	5.46 ± 1.35
PE contractility – L-NAME			
Maximum (g)	2.40 ± 0.08	2.85 ± 0.12	2.31 ± 0.15*
EC_{50} (M X10^{-8})	2.71 ± 0.06	3.70 ± 1.02	2.16 ± 0.10
ACh-relaxation			
Maximum (%)	79.4 ± 2.6***	62.9 ± 1.7	71.8 ± 1.4***
EC_{50} (M X10^{-8})	2.61 ± 0.52	3.58 ± 0.62	1.26 ± 0.15***
SNP-relaxation			
Maximum (%)	130 ± 18	115 ± 5	109 ± 4.2
EC_{50} (M X10^{-7})	4.6 ± 3.7	4.26 ± 0.97	2.05 ± 0.42*

Data are expressed as mean ± SEM; 10 rats per group; each rat 24 weeks of age.
*P < 0.05; **P < 0.01; ***P < 0.001 versus values for *cp/cp* control rats.
L-NAME, N^G-nitro-L-arginine methyl ester; PE, phenylephrine; SNP, sodium nitro prusside.

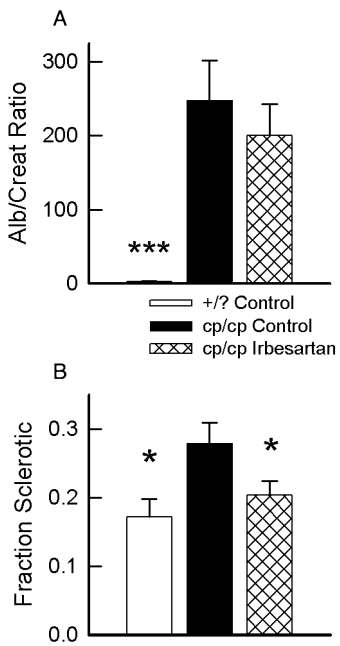

Figure 6 Urinary albumin/creatinine ratio, as an index of renal vascular function (A), and fractional glomerular sclerosis (B) of control and irbesartan-treated rats. Values are mean ± SEM, 10 rats per group. *P < 0.05; ***P < 0.001 versus *cp/cp* control.

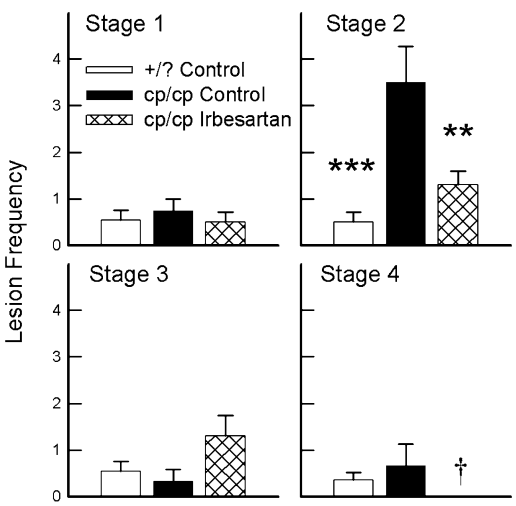

Figure 7 Frequency of ischaemic myocardial lesions in irbesartan-treated and control rats. Lesion stages as described in the Methods. Values are mean ± SEM, 10 rats in each group. **P < 0.01; ***P < 0.005 versus *cp/cp* control. No Stage 4 lesions were detected in the hearts of irbesartan-treated *cp/cp* rats (†), but the difference was not statistically significant (P > 0.05).

has been seen in this model with other agents (Russell *et al.*, 2000). Given that hyperinsulinaemia plays a major role in the development of atherosclerosis and myocardial damage in the *cp/cp* rat, the substantial increases in plasma insulin seen in the irbesartan-treated rat cannot be viewed as beneficial.

The modest 20% reduction in fasting plasma TG concentrations caused by irbesartan treatment of *cp/cp* rats must reflect a reduction in VLDL, but the mechanism while clearly a pleiotropic effect is not clear. Consistent with the primary cholesterol bearing lipoprotein fraction in the rat being HDL, there was no effect on cholesterol levels. TG-rich particles and remnants play an important role in atherogenesis (Proctor *et al.*, 2004) and the reduction in TG may play a role in vascular protection seen with irbesartan treatment of the *cp/cp* rat, but we feel this is too small to account for all of the beneficial effects seen.

Adiponectin (also known as Acrp30 or GBP28) is a cytokine, exclusively secreted by adipocytes, and there is an unresolved debate over the role of adiponectin and whether it is involved in cardiovascular disease (Empana, 2008). However, there is growing evidence that insulin resistance and hyperinsulinaemia stimulate adiponectin secretion (Fernández-Veledo *et al.*, 2008) and our data show markedly elevated plasma adiponectin levels in the *cp/cp* rat. Plasma concentrations in the *cp/cp* rat are approximately sevenfold higher than those reported for *fa/fa* Zucker diabetic fatty rats, which are type 2 diabetic, as opposed to being insulin-resistant, and have essentially normal insulin levels (Szöcs *et al.*, 2008). While the mechanistic relationships remain obscure, it is clear that

adiponectin, at least in the *cp/cp* rat, is implicated in the metabolic status and susceptibility to vascular disease, possibly acting as an inflammatory cytokine. The reduction of adiponectin levels observed in irbesartan treated *cp/cp* rats is, thus, consistent with the associated reduction in micro- and macrovascular disease. At some level, it appears that irbesartan reduces the pro-inflammatory state of the *cp/cp* rat, through a pathway not directly related to obesity and insulin metabolism. It remains unclear whether the role of adiponectin is causal or merely as a marker.

Monocyte chemoattractant protein-1 is the principal chemotactic factor for the migration of monocytes/macrophages and mediation of chronic inflammation (Dragomir and Simionescu, 2006). Our data show higher MCP-1 levels in the *cp/cp* rat in comparison with +/? rats, consistent with the effect of hyperinsulinaemia on MCP-1 secretion (Fernández-Veledo *et al*., 2008). Greater expression of MCP-1 in the *cp/cp* rat is also consistent with our earlier observations of widespread activation and endothelial adherence of macrophages in atherosclerosis-prone adult *cp/cp* rats (Russell *et al*., 1995; 1998a; Schepers *et al*., 2006). In addition, Takebayashi *et al*. (2006) have shown a potentially important association between MCP-1 and urinary albumin excretion in non-obese type 2 diabetic patients, analogous to our observations in the *cp/cp* rat. Our results show a reduced incidence of glomerular sclerosis with irbesartan treatment, without any change in urinary albumin loss. This is an apparently contradictatory finding as glomerular sclerosis is viewed as the end-stage of microvascular damage, first evident in capillary dysfunction. The reduction in glomerular damage in the irbesartan-treated rats may, thus, be mediated through effects on a process separate from that leading to glomerular capillary permeability and albuminuria.

Humans exhibiting the metabolic syndrome have approximately twofold higher plasma PAI-1 levels (Bahia *et al*., 2006), even in the absence of evidence of type 2 diabetes. The *cp/cp* rat has also been shown to have elevated circulating levels of PAI-1 (Schneider *et al*., 1998), consistent with the finding of both macro- and micro-thrombi in the arterial system (Russell *et al*., 1998b). The reduction in PAI-1 levels in irbesartan-treated *cp/cp* rats indicates a reduction in the prothrombotic status and should be beneficial in terms of reduced obstructive vascular disease. Reduced incidence of glomerular sclerosis and reduced frequency of Stage 2 lesions in the heart is consistent with this concept.

Irbesartan treatment had significant effects on the macrovascular dysfunction of the *cp/cp* rats. The reduction in the PE-stimulated contractile response was evident in the absence of the NOS inhibitor L-NAME, and present, although less marked in the presence of L-NAME. Together with the marked changes in the relaxant response to ACh on irbesartan treatment, and the smaller but still significant changes in SNP-stimulated relaxation, these results suggest irbesartan improves the status of the endothelium and, to a lesser degree, the vascular smooth muscle cells (VSMCs). The reduction in the relaxant EC_{50} for SNP, a direct NO donor, further indicates a change in the VSMC. In addition, the increased relaxation response to ACh and reduced EC_{50} indicate an improvement in endothelial cell function and NO release. Paradoxically, these changes occurred in the presence of an increase in hyperinsulinaemia, which is a critical mediator of vascular dysfunction and cardiovascular disease in prediabetic and type 2 diabetic states (Russell *et al*., 1995; O'Brien and Russell, 1997). The improvement in vascular function, together with reduced PAI-1 levels, may be expected to lead to a reduction in vasospastic and thrombotic events and ischaemic damage to organs, such as the heart.

Macrovascular disease in the *cp/cp* rat leads to atherosclerosis (Proctor and Russell, 2006), thrombotic disease and ischaemic lesions (Russell *et al*., 2005). The Stage 1 lesions, seen in hearts of *cp/cp* rats, are areas of necrosis and early ischaemic damage. They have a short lifespan and progress to Stage 2 or 3 lesions with infiltration of chronic inflammatory cells. The number of Stage 1 lesions present, at any point in time, is small and differences between groups difficult to demonstrate. Stage 2 lesions represent the longer active stage of resolution of lesions of significant size, and while infrequent in +/? rats, are frequent in the *cp/cp* rats. Irbresartan treatment reduced the frequency of these early end-stage lesions in *cp/cp* rats to the range seen in the lean normal +/? rats, indicating an effective reduction in the development of end-stage ischaemic lesions. Stage 3 lesions, as small foci of chronic inflammatory cell activity, are short lived, relatively infrequent and nonspecific. Stage 4 lesions reflect the persistent, or remnant scarred lesions that were of a sufficient size to be visible after resolution and contraction of the collagen structure, accumulating with aging of the rat. The small number of Stage 4 lesions present in the relatively young *cp/cp* rats in this study precluded statistical analysis.

A variety of pharmaceutical interventions have been studied in the *cp/cp* rat. ACE inhibitors, endopeptidase inhibitors, insulin sensitizers and ethanol have all been shown to reduce hyperinsulinaemia, hypertriglyceridaemia, vascular dysfunction and myocardial lesions (Russell and Proctor, 2008). Recently, we have shown that these substances also reduce renal microvascular dysfunction and damage (Russell *et al*., 2004; Proctor *et al*., 2007). A common element to the beneficial effects of interventions that are protective against vascular disease has been a significant reduction of insulin resistance and hyperinsulinaemia. In contrast to other agents, irbesartan induced only a modest, and probably biologically unimportant, reduction in TG levels and actually increased insulin levels. This suggests that irbesartan is protective against cardiovascular disease through previously unrecognized pleiotrophic effects. The reduction in markers of inflammation and thrombotic susceptibility, improved macrovascular function and reduced renal and myocardial end-stage disease are consistent with irbesartan having a direct effect on the VSMC, possibly through intracellular signalling pathways. Recently, it has been demonstrated that inhibition of the rennin-angiotensin system combined with calcium channel antagonists reduces plasma levels of inflammatory markers, including MCP-1, and increases adiponectin levels (Ogawa *et al*., 2008). Similarly, Vieitez *et al*. (2008) have shown that irbesartan lowers the expression of cytokines related to inflammation in the kidney of the cytotoxic streptozotocin model of type 1 diabetes. These findings are consistent with our earlier finding that calcium channel antagonists have antiatherogenic and cardioprotective activity in the *cp/cp* rat (Russell *et al*., 1997). The available evidence suggests

not only that MCP-1 and adiponectin mediate effects through AMPK (Ogawa *et al*., 2008; Sharma *et al*., 2008), but also that AMPK metabolism is abnormal in the *cp/cp* rat (Atkinson *et al*., 2003) and that the underlying mechanisms are complex. Negro (2008) has extensively reviewed the role of the renin-angiotensisn system and endothelium in vascular dysfunction and their relationship with many of the factors identified as being involved in the vascular, myocardial and renal disease in the *cp/cp* rat (Russell and Proctor, 2006; 2007). He particularly emphasized the crucial role of Ang II in the endothelial dysfunction and NO deficiency seen in the *cp/cp* rat. Further, he suggested that irbesartan-mediated 'beneficial effects beyond blood pressure control' follow from 'blockade of the renin-angiotensin system'. These mechanisms potentially offer novel pharmaceutical approaches to protection against cardiovascular disease associated with the prediabetic metabolic syndrome.

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Conflict of interest

The authors state no conflict of interest.

References

Alexander SPH, Mathie A, Peters JA (2008). Guide to receptors and channels (GRAC), 3rd edn. *Br J Pharmacol* **153** (Suppl. 2): S1–S209.

Atkinson LL, Kozak R, Kelly SE, Onay Besikci A, Russell JC, Lopaschuk GD (2003). Potential mechanisms and consequences of cardiac triacylglycerol accumulation in insulin-resistant rats. *Am J Physiol Endocrinol* **284**: E923–E930.

Bahia L, Aguiar LG, Villela N, Bottino D, Godoy-Matos AF, Geloneze B *et al*. (2006). Relationship between adipokines, inflammation, and vascular reactivity in lean controls and obese subjects with metabolic syndrome. *Clinics* **61**: 433–440.

Butler J, Mooyaart MS, Dannemann N, Bamberg F, Shapiro DO, Ferencik M *et al*. (2008). Relation of the metabolic syndrome to quantity of coronary atherosclerotic plaque. *Am J Cardiol* **101**: 1127–1130.

DeLean A, Munson PJ, Rodbard D (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am J Physiol* **235**: E97–E102.

Després J-P, Lamarche B, Mauriège P, Cantin B, Dagenais GR, Moorjani S *et al*. (1996). Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med* **334**: 952–957.

Dragomir E, Simionescu M (2006). Monocyte chemoattractant protein-1 – a major contributor to the inflammatory process associated with diabetes. *Arch Physiol Biochem* **112**: 239–244.

Empana J-P. (2008). Adiponectin isoforms and cardiovascular disease: the epidemiological evidence has just begun. *Eur Heart J* **29**: 1221–1223.

Fernández-Veledo S, Nieto-Velasquez I, de Castro J, Ramos MP, Brüderlein S, Möller P, Lorenzo M (2008). Hyperinsulinemia induces insulin resistance on glucose and lipid metabolism in a human adipocyte cell line: paracrine interaction with myocytes. *J Clin Endocrin Metab* **93**: 2866–2876.

Ferrario F, Rastaldi MP (2006). Histopathological atlas of renal diseases: diabetic nephropathy. *J Nephrol* **19**: 1–5.

Hegele RA, Zinman B, Hanley AJ, Harris SB, Barrett PH, Cao H (2003). Genes, environment and Oji-Cree type 2 diabetes. *Clin Biochem* **36**: 163–170.

Karalliedde J, Smith A, DeAngelos L, Mirenda V, Kandra A, Botha J *et al*. (2008). Valsartan improves arterial stiffness in type 2 diabetes independently of blood pressure lowering. *Hypertension* **51**: 1–7.

Lorenzo C, Nath SD, Hanley AJ, Abboud HE, Haffner SM (2008). Relation of low glomerular filtration rate to metabolic disorders in individuals without diabetes and with normoalbuminuria. *Clin J Am Soc Nephrol* **3**: 783–789.

Negro R (2008). Endothelial effects of antihypertensive treatment: focus on irbesartan. *Vasc Health Risk Manag* **4**: 89–101.

O'Brien SF, Russell JC (1997). Insulin resistance and vascular wall function: lessons from animal models. *Endocrinol Metab* **4**: 155–162.

O'Brien SF, Russell JC, Davidge ST (1999). Vascular wall dysfunction in JCR : LA-cp rats: effects of age and insulin resistance. *Am J Physiol* **277**: C987–C993.

Ogawa S, Mori T, Nako K, Ito S (2008). Combination therapy with Renin-Angiotensin system inhibitors and the calcium channel blocker azelnidipine decreases plasma inflammatory markers and urinary oxidative stress markers in patients with diabetic nephropathy. *Hypertens Res* **31**: 1147–1155.

Proctor SD, Russell JC (2006). Small animal models of cardiovascular disease: tools for study of roles of the metabolic syndrome, dyslipidemias and atherosclerosis. *J Cardiovasc Pathol* **15**: 318–330.

Proctor SD, Vine DF, Mamo JC (2004). Arterial permeability and efflux of apolipoprotein B-containing lipoproteins assessed by in situ perfusion and three-dimensional quantitative confocal microscopy. *Arterioscler Thromb Vasc Biol* **24**: 2162–2167.

Proctor SD, Kelly SE, Stanhope KL, Havel PJ, Russell JC (2007). Synergistic effects of conjugated linoleic acid and chromium picolinate improve vascular function and renal pathophysiology in the insulin-resistant JCR : LA-cp rat. *Diab Obesity Metab* **9**: 87–95.

Richardson M, Schmidt AM, Graham SE, Achen B, DeReske M, Russell JC (1998). Vasculopathy and insulin resistance in the JCR : LA-cp rat. *Atherosclerosis* **138**: 135–146.

Russell JC, Amy RM (1986). Plasma lipids and other factors in the LA/N corpulent rat in the presence of chronic exercise and food restriction. *Can J Physiolo Pharmacol* **64**: 750–756.

Russell JC, Proctor SD (2006). Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia and atherosclerosis. *Cardiovasc Pathol* **15**: 318–330.

Russell JC, Proctor SD (2007). Increased insulin sensitivity and reduced micro and macro vascular disease induced by 2-deoxy-D-glucose during metabolic syndrome in obese JCR : LA-*cp* rats. *Br J Pharmacol* **151**: 216–225.

Russell JC, Proctor SD (2008). Macro and micro vascular disease in the insulin resistant JCR : LA-cp rat. In: Hansen B, Bray G (eds). *The Metabolic Syndrome Epidemiology, Clinical Treatment, and Underlying Mechanisms*. Humana Press: Totawa, NJ, pp. 137–166.

Russell JC, Amy RM, Graham SE, Dolphin PJ, Wood GO, Bar-Tana J (1995). Inhibition of atherosclerosis and myocardial lesions in the JCR : LA-cp rat by *β,β'*-tetramethyl hexadecanedioic acid (MEDICA 16). *Atheroscler Thromb Vasc Biol* **15**: 918–923.

Russell JC, Dolphin PJ, Graham SE, Amy RM (1997). Cardioprotective and hypolipidemic effects of nisoldipine in the JCR : LA-cp rat. *J Cardiovasc Pharmacol* **29**: 586–592.

Russell JC, Graham SE, Amy RM, Dolphin PJ (1998a). Inhibition of myocardial lesions in the JCR : LA-corpulent rat by captopril. *J Cardiovasc Pharmacol* **31**: 971–977.

Russell JC, Graham SE, Richardson M (1998b). Cardiovascular disease in the JCR : LA-cp rat. *Mol Cell Biochem* **188**: 113–126.

Russell JC, Graham SE, Dolphin PJ (1999). Glucose tolerance and insulin resistance in the JCR : LA-cp rat: effect of miglitol (Bay m1099). *Metabolism* **48**: 701–706.

Russell JC, Ravel D, Pégorier J-P, Delrat P, Jochemsen R, O'Brien SF *et al*. (2000). Beneficial insulin-sensitizing and vascular effects of S15261 in the insulin-resistant JCR : LA-cp rat. *J Pharmacol Exp Ther* **295**: 753–760.

Russell JC, McKendrick JD, Dubé GP, Dolphin PJ, Radomski MW (2001). Effects of LY117018 and the estrogen analogue, 17-ethinylestradiol, on vascular reactivity, platelet aggregation, and lipid metabolism in the insulin-resistant JCR : LA-cp rat: role of nitric oxide. *J Cardiovasc Pharmacol* **37**: 119–128.

Russell JC, Kelly SE, Schäfer S (2004). Vasopeptidase inhibition improves insulin sensitivity and endothelial function in the JCR : LA-cp rat. *J Cardiovasc Pharmaco* **44**: 258–265.

Russell JC, Kelly SE, Shäfer S (2005). Insulin-sensitizing and cardiovascular effects of the sodium-hydrogen exchange inhibitor, cariporide, in the JCR : LA-cp rat. *J Cardiovasc Pharmacol* **46**: 746–753.

Schäfer S, Steioff K, Linz W, Bleich M, Busch AE, Löhn M (2004). Chronic vasopeptidase inhibition normalizes diabetic endothelial dysfunction. *Eur J Pharmacol* **484**: 361–362.

Schepers A, Eefting D, Bonta PI, Grimbergen JM, de Vries MR, van Weel V *et al*. (2006). Anti-MCP-1 gene therapy inhibits vascular smooth muscle cells proliferation and attenuates vein graft thickening both in vitro and in vivo. *Arterioscler Thromb Vasc Biol* **26**: 2063–2069.

Schneider DJ, Absher PM, Neimane D, Russell JC, Sobel BE (1998). Fibrinolysis and atherogenesis in the JCR : LA-cp rat in relation to insulin and triglyceride concentrations in blood. *Diabetologia* **41**: 141–147.

Sharma K, RamachandraRao S, Qui G, Usui K, Zhu Y, Dunn SR *et al*. (2008). Adiponectin regulates albuminuria and podocyte function in mice. *J Clin Invest* **118**: 1645–1656.

Szöcs Z, Brunmair B, Stadlbaur K, Nowotny P, Bauer L, Luger A *et al*. (2008). Age-dependent development of metabolic derangement and effects of intervention with pioglitazone in Zucker diabetic fatty (ZDF) rats. *J Pharmacol Exp Ther* **326**: 323–329.

Takebayashi K, Matsumoto S, Aso Y, Inulai T (2006). Association between circulating monocyte chemoattractant protein-1 and urinary albumin excretion in nonobese Type 2 diabetic patients. *J Diab Complic* **20**: 98–104.

Vance JE, Russell JC (1990). Hypersecretion of VLDL, but not HDL, by hepatocytes from the JCR : LA-corpulent rat. *J Lipid Res* **31**: 1491–1501.

Vieitez P, Gómez O, Uceda ER, Vera ME, Molina-Hilgado E (2008). Systemic and local effects of angiotensin II blockade in experi, emtal diabetic nephropathy. *J Renin Angiotensin Aldosterone Syst* **9**: 96–102.

Vine D, Takechi R, Russell JC, Proctor SD (2007). Impaired postprandial apolipoprotein-B48 metabolism in the obese, insulin-resistant JCR : LA-cp rat: increased atherogenicity for the metabolic syndrome. *Atherosclerosis* **190**: 282–290.

Wu-Peng XS, Chua SC, Okada N, Liu SM, Nicolson M, Leibel RL (1997). Phenotype of the obese Koletsky (*f*) rat due to Tyr763Stop mutation in the extracellular domain of the leptin receptor (Lepr): evidence for deficient plasma-to-CSF transport of leptin in both the Zucker and Koletsky obese rat. *Diabetes* **46**: 513–518.

Yusuf S, Reddy S, Ôunpuu S, Anand S (2001). Global burden of cardiovascular diseases Part I: general considerations, the epidemiological transition, risk factors, and impact of urbanization. *Circulation* **104**: 2746–2753.