ELSEVIER

European Journal of Pharmacology 416 (2001) 133–139

ejp

www.elsevier.nl/locate/ejphar

Troglitazone corrects metabolic changes but not vascular dysfunction in dietary-obese rats

Ebrahim K. Naderali [a,*], Lucy C. Pickavance [a], John P.H. Wilding [a], Patrick J. Doyle [b], Gareth Williams [a]

[a] *Diabetes and Endocrinology Research Unit, Department of Medicine, University of Liverpool, UCD, Daulby Street, Liverpool L69 3GA, UK*
[b] *Novo-Nordisk A/S, Brudelysvej 28, 2880 Bagsvaerd, Denmark*

Received 26 October 2000; received in revised form 13 February 2001; accepted 20 February 2001

Abstract

Insulin resistance has been attributed to the defect in vascular function associated with obesity, type 2 diabetes and dyslipidaemia. We have investigated vascular effects of chronic (3-week) administration of troglitazone on female Wistar rats with moderate dietary obesity. Compared with lean controls, untreated obese rats had significantly higher body weights, fat pad masses, plasma triglycerides, free fatty acids and leptin levels (for all $P < 0.01$). These metabolic changes were corrected by troglitazone treatment. In mesenteric arteries, responses to noradrenaline or KCl were similar in all groups. However, in noradrenaline-preconstricted arteries, vasorelaxations to acetylcholine and insulin were significantly (50–60% less than in lean, $P < 0.001$) attenuated in both untreated and troglitazone-treated obese rats. Relaxations to sodium nitroprusside showed similar but lesser impairment in both untreated and troglitazone-treated obese animals. Our data show that although troglitazone markedly improved obesity-induced metabolic changes, it failed to correct vascular dysfunction associated with obesity in female Wistar rats.

Keywords: Obesity; Troglitazone; Endothelium; Vascular dysfunction

1. Introduction

The clustering of insulin resistance, abnormal glucose tolerance, abdominal obesity, dyslipidaemia and arterial hypertension known as the metabolic syndrome (syndrome X) (Reaven, 1988) is associated with substantially increased risk of cardiovascular disease, related to both hypertension and atherogenesis. Insulin resistance has been suggested to be the primary abnormality in syndrome X, but the mechanisms linking this with cardiovascular disorders are not fully understood, although direct effects of insulin on vascular smooth muscle proliferation (Capron et al., 1986), indirect effects via activation of the sympathetic nervous system (Anderson et al., 1991) and resistance to vasodilator effects of insulin (Laakso et al., 1992) have all been implicated.

The endothelium plays an important role in the regulation of vascular tone (Moncada et al., 1976; Abrams, 1997) by producing various vasoactive mediators which include nitric oxide (NO) and endothelin, both of which act on the underlying vascular smooth muscle to modulate arterial contractility (Mombouli and Vanhoutte, 1999). Endothelial dysfunction has been identified in several conditions that are associated with abnormal vascular reactivity and/or atherogenesis (Pinkney et al., 1997; Shimokawa, 1999). In particular, impaired NO-mediated vasorelaxation has been demonstrated in obesity, type 2 diabetes and hypertension (Creager et al., 1990; McVeigh et al., 1992; Steinberg et al., 1996; Watts et al., 1996) with the most striking abnormality being attenuation of acetylcholine-induced vasodilatation. Impaired endothelium-mediated vasorelaxation, which could contribute to hypertension, has been found in subjects at high risk of atherogenesis before detectable atheromatous lesions develop (Shimokawa,

* Corresponding author. Tel.: +44-151-706-4087; fax: +44-151-706-5797.
E-mail address: naderali@liverpool.ac.uk (E.K. Naderali).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)00866-4

1999; Boulanger, 1999). This is therefore regarded as a robust marker and predictor of atheroma formation and ultimately of coronary and peripheral arterial disease.

Abnormal vascular responses have previously been described in various animal models of obesity and insulin resistance, notably the fatty (*fa/fa*) Zucke (Walker et al., 1997), Zucker Diabetic Fatty (ZDF) and *cp/cp* rats (Russell et al., 1998). These animal models are all characterised by a primary defect in the leptin receptor, resulting in hyperphagia, reduced energy expenditure and ultimately obesity and severe insulin resistance. Reported vascular defects in these models include marked attenuation of vasorelaxation to acetylcholine and insulin, and their presence in these models has been interpreted as further evidence that insulin resistance causes the endothelial vascular dysfunction of syndrome X (Walker et al., 1997; Russell et al., 1998; O'Brien et al., 1998).

Thiazolidinediones, such as troglitazone, are effective agents in the management of type 2 diabetes mellitus. They bind to the nuclear peroxisome proliferator-activated receptor-γ (PPAR-γ), and consequent improvements in insulin resistance and glucose metabolism are principally attributed to decreased free fatty acid concentrations (Lefebvre et al., 1997; Murakami et al., 1998). They improve metabolic abnormalities in animal models of insulin resistance and type 2 diabetes (Fujiwara et al., 1988; Shimabukuro et al., 1998; Pickavance et al., 1999) and in humans with type 2 diabetes (Suter et al., 1992; Nolan et al., 1994; Kumar et al., 1996). Recent studies have suggested that thiazolidinediones may also have independent beneficial effects on the vasculature. They have been reported to lower blood pressure in hypertensive fatty Zucker rats (Yoshioka et al., 1993), obese diabetic rats (Yoshimoto et al., 1997) and diet-induced hypertensive rats (Buchanan et al., 1995). The mechanisms by which the blood pressure falls is not fully understood but may include induction of vasorelaxation (Buchanan et al., 1995; Walker et al., 1998; Kawasaki et al., 1998) and a reduction in peripheral vascular resistance (Ghazzi et al., 1997). Furthermore, recent work has identified PPAR-γ receptors in vascular cells (Lijima et al., 1998; Kato et al., 1999); thiazolidinediones could affect vascular tone by influencing the production or release of vasoactive endothelial products such as nitric oxide (NO). Other possibilities include direct interaction with calcium channels (Walker et al., 1998; Nakamura et al., 1998) to inhibit contraction; improving endothelial function (Ghazzi et al., 1997), thus potentiating vasorelaxation or by simply improving metabolic changes in patients with type 2 diabetes (Okuno et al., 1997), thereby potentiating vasodilatation.

This study was designed to evaluate vascular effects of chronic administration of troglitazone in dietary obese female Wistar rats. We studied resistance arteries because these vessels represent endothelial function throughout the vasculature and are believed to be involved in determining the increase in peripheral resistance that leads to the development of hypertension (Christensen and Mulvany, 1993).

2. Materials and methods

2.1. Animals

Adult female Wistar rats ($n = 15$) were randomly divided into a control group maintained on ordinary chow diet ($n = 5$, 176 ± 3 g) and a test group ($n = 10$, 171 ± 5 g), which was fed a highly palatable diet for 11 weeks.

All animals had free access to water and housed in groups of 2 or 3 under controlled environmental conditions (19–22°C; 30–40% humidity) and a 12 h light/dark cycle (lights on at 07:00 h). Lean controls were fed a standard laboratory pelleted diet (CRM Biosure, Cambridge, UK), while test groups had free access to a highly palatable, high-energy diet consisting of 33% (by weight) ground pellet diet, 33% Nestle condensed milk, 7% sucrose and 27% water. After 8 weeks, half of the test group was given troglitazone (300 mg^{-1} kg^{-1} day^{-1}) and the remainder were given vehicle (1% carboxymethyl cellulose at 1 ml/kg body weight; Sigma, Pool, Dorset, UK) was given by gavage daily for 3 weeks, before being killed. We have used female animals because troglitazone has greater bioavailibity in female rats than their male counterparts (Kawai et al., 1997).

The rats were killed by CO_2 inhalation, and the gonadal and perirenal fat masses and the gastrocnemius muscle dissected and weighed. Blood was removed by cardiac puncture into cold heparinized tubes. The plasma was immediately separated by centrifugation before being frozen at −40°C for later measurements of blood analytes (glucose, insulin, leptin, free fatty acids and triglycerides). Plasma glucose concentration was determined using a glucose oxidase method, and free fatty acids and triglyceride concentrations were measured using commercial diagnostic kits (Boehringer-Mannheim, Milton Keynes, Bucks and Sigma Diagnostics, Poole, Dorset, UK). Insulin and leptin concentrations were measured by radioimmunoassay (RIA) kits (Pharmacia/Upjohn Diagnostics, Lewes, Sussex and Linco Research, Biogenesis, Poole, Dorset, UK, respectively).

2.2. Assessment of vascular function

Eight third-order mesenteric arteries (180–220 μm diameter, 2 mm lengths) were carefully dissected from each animal. Each artery was freed of fat and connective tissue and mounted on two 40-μm diameter stainless-steel wires in an automated myograph (Cambustion, Cambridge, UK), based on the principle of the Mulvany myograph which measures isometric tension generated in response to various stimuli. The vessels (in duplicate) were incubated in a 5-ml organ bath containing physiological salt solution

(PSS; composition [in mM]: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.026 and glucose 5.5) gassed with 95% O_2 and 5% CO_2 at 37°C.

After 30 min of equilibration, the length-tension characteristics for each vessel were determined and set to its normalised diameter, as previously described (Naderali et al., 2000). The computer calculated the target tension that each vessel should develop in response to a maximal stimulus. Arteries were allowed a further 30 min to equilibrate before being depolarized twice with high-potassium physiological salt solution (KPSS, 125 mM) in which NaCl in normal PSS was replaced by an equimolar concentration of KCl. Cumulative concentration–response curves to either KCl (10–125 mM) or noradrenaline (0.5–6 μM) were then carried out. Any vessel failing to reach its predetermined target tension in response to vasoconstriction with KCl (125 mM) was discarded.

2.3. Assessment of defect in vascular relaxation

To characterize defects in NO and endothelial function, we measured relaxation induced in noradrenaline-preconstricted arteries following exposure to endothelium-dependent (acetylcholine and insulin) and endothelium-independent vasodilator (sodium-nitroprusside). Arteries were contracted with a supramaximal concentration of noradrenaline (8 μM). When contraction reached a plateau after 2 min, concentration–response curves were carried out to either acetylcholine, sodium nitroprusside (for all both, 10 nM–100 μM) or insulin (50–2500 mU/l), in random order.

2.4. Reagents

Noradrenaline, acetylcholine and sodium nitroprusside were all obtained from Sigma (Poole, Dorset, UK) and human insulin (Humulin-S; 100 U/ml) from Eli Lilly (Indianapolis, IN, USA). The working concentrations of insulin preservative (glycerine and *m*-cresol) following dilution of standard 100 U/ml insulin solution have no detectable vasoactivity in this system (Walker et al., 1997).

2.5. Data interpretation and statistical analyses

Vasoconstriction in response to KCl and noradrenaline was expressed as absolute force generated. Relaxation in response to acetylcholine, sodium nitroprusside or insulin was calculated as the percentage reduction from the maximal tension generated in response to the supramaximal concentration of noradrenaline (8 μM).

Data are expressed as mean ± S.E.M. Statistical significance was tested using repeated-measures analysis of variance (ANOVA) or the Mann–Whitney test, as appropriate, using the statistical package, Arcus Pro-stat (Version 3.23; Iain Buchan, Liverpool, UK). Results were considered statistically significant at the $P < 0.05$ level.

2.6. Homeostasis model assessment

Homeostasis Model Assessment, which employs measures of fasting plasma concentrations of glucose and insulin, was used to assess insulin resistance as described by Matthews et al., (1985).

Table 1
Physiological and metabolic characteristics of chow-fed and highly palatable cafeteria diet rats. Data are mean ± S.E.M.

	Chow-fed	Obese untreated	Obese + troglitazone
Body weight (g)			
· Initial	176 ± 3	172 ± 7	–
· Before dosing (g)	241 ± 10	278 ± 26[a]	273 ± 24[b]
· Final	250 ± 6	309 ± 17[a]	274 ± 7[b]
· Gained	74 ± 4	140 ± 19[a]	103 ± 14[b,c]
Gonadal fat-pad mass (g)	0.76 ± 0.05	1.72 ± 0.32[a]	0.77 ± 0.22[c]
Perirenal fat-pad mass (g)	2.20 ± 0.08	4.50 ± 0.75[a]	2.06 ± 0.44[c]
Gastrocnemius muscle mass (g)	1.60 ± 0.06	1.77 ± 0.07	1.63 ± 0.03
Fat/lean ratio	1.85 ± 0.07	3.47 ± 0.47[a]	1.71 ± 0.37[c]
Heart weight (g)	0.97 ± 0.08	1.01 ± 0.03	1.28 ± 0.02[b,c]
Plasma glucose (mM)	7.96 ± 0.35	7.71 ± 0.80	7.33 ± 0.29[b,c]
Plasma insulin (μU/ml)	12.69 ± 2.39	13.92 ± 1.73	9.45 ± 0.54[b,c]
Homeostasis Model Assessment Index	4.41 ± 0.65	4.87 ± 1.03	3.08 ± 0.19[b,c]
Plasma leptin (ng/ml)	5.97 ± 0.60	10.98 ± 0.30[a]	5.74 ± 1.07[c]
Plasma triglycerides (mM)	0.23 ± 0.06	0.55 ± 0.13[a]	0.18 ± 0.02[b,c]
Plasma free fatty acids (mM)	0.33 ± 0.05	0.40 ± 0.03[a]	0.17 ± 0.04[b,c]

[a] Obese untreated vs. chow-fed.
[b] Obese + troglitazone vs. chow-fed.
[c] Obese + troglitazone vs. obese untreated.

3. Results

3.1. Body weight and metabolic data

Rats given the highly palatable diet progressively gained more weight than the chow-fed controls and were 15% heavier immediately before the 3-week treatment period. During treatment, the rate of weight gain was attenuated in troglitazone-treated rats, but these remained significantly obese when compared with chow-fed controls (Table 1).

3.2. Effects of dietary obesity

The gonadal and perirenal fat pad masses weighed 110% more ($P < 0.001$) in untreated obese than the chow-fed controls, while gastrocnemius muscle weight was comparable (Table 1). As a result, untreated obese rats had a significantly ($P < 0.001$) higher fat/lean ratio than the chow-fed groups (Table 1). There were no significant changes in heart weight between untreated obese and chow-fed rats.

Terminal plasma free fatty acid ($P < 0.05$) and triglyceride ($P < 0.001$), but not insulin, glucose and leptin levels and Homeostasis Model Assessment index, were significantly higher in dietary-obese than in control animals (Table 1).

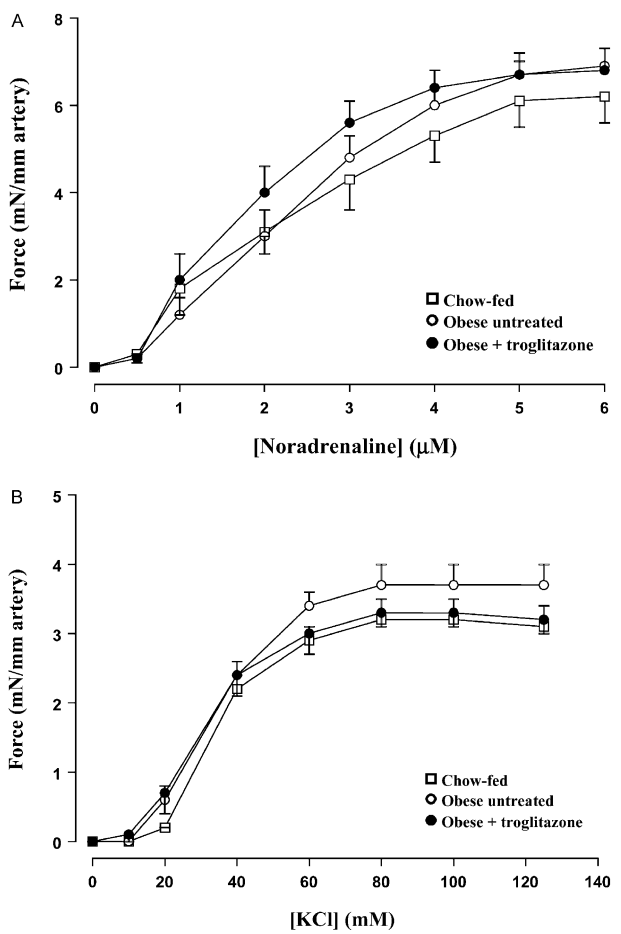

Fig. 1. Effects of (A) KCl (10–125 mM) and (B) noradrenaline (0.5–6 μM) on arteries from untreated, troglitazone-treated dietary-obese and lean control animals. Data represent mean ± S.E.M. The concentration–response curves for the all three groups did not differ significantly.

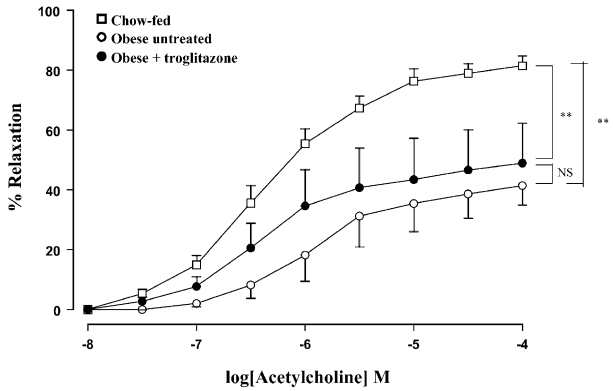

Fig. 2. Relaxation curves for acetylcholine on arteries from lean control and dietary-obese rats. Arteries were precontracted with 8 μM noradrenaline. When contraction reached a plateau after 2 min, concentration–response curves to acetylcholine was carried out. Data represent mean ± S.E.M. The concentration–response curves for the chow-fed and dietary-obese (both untreated and troglitazone-treated) groups differ significantly (by ANOVA, $P < 0.001$), however, there was no significant difference between ACh-induced vasorelaxation of arteries from untreated and troglitazone-treated dietary-obese rats.

3.3. Effects of troglitazone in dietary obesity

Troglitazone reversed all the metabolic changes induced by the palatable diet. In troglitazone-animals gonadal and perirenal fat pad masses, fat/lean ratio, and leptin levels were similar to those of chow-fed animals and were significantly ($P < 0.01$) lower than untreated obese rats. In fact, troglitazone-treated animals had significantly ($P < 0.05$)

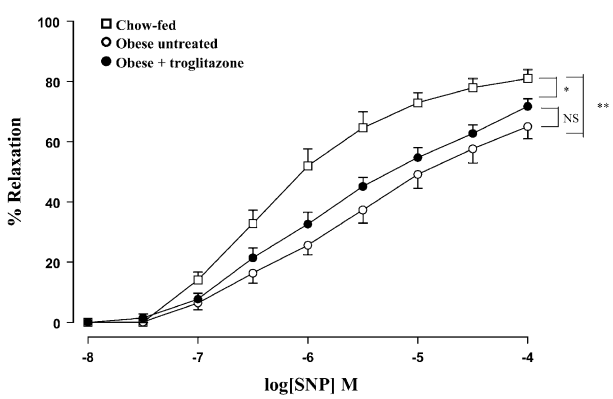

Fig. 3. Relaxation curves for sodium nitroprusside on arteries from lean control and dietary-obese rats. Arteries were precontracted with 8 μM noradrenaline. When contraction reached a plateau after 2 min, concentration–response curves to sodium nitroprusside was carried out. Data represent mean ± S.E.M. The concentration–response curves for the chow-fed and dietary-obese (both untreated and troglitazone-treated) groups differ significantly (by ANOVA, $P < 0.001$). There was no significant difference between SNP-induced vasorelaxation of arteries from untreated and troglitazone-treated dietary-obese rats.

lower plasma glucose, insulin, triglyceride, free fatty acids levels and Homeostasis Model Assessment index than not only untreated obese group, but also when compared with chow-fed animals (Table 1). Troglitazone-treated animals had significantly ($P < 0.05$) higher heart weight than either untreated dietary-obese or chow-fed animals.

3.4. Vascular responses

There were no significant differences in vessel diameter between the untreated dietary-obese, troglitazone-treated dietary-obese and chow-fed animals (230 ± 10, 228 ± 5 and 227 ± 7 μm), respectively.

3.4.1. Contractile responses

The contractile responses of vessels from chow-fed, untreated dietary-obese and troglitazone-treated dietary-obese animals to increasing concentrations of KCl (10–125 mM) and noradrenaline (0.5–6 μM) displayed the characteristic sigmoid relationship with no significant differences between the three groups either overall (by ANOVA) or at any single concentration (Fig. 1).

3.4.2. Endothelium-dependent relaxation

Arteries from chow-fed rats that were pre-contracted with noradrenaline (8 μM) demonstrated progressive relaxation to cumulative additions of acetylcholine (10 nM–100 μM), achieving a maximum of $83 \pm 5\%$ relaxation at an acetylcholine concentration of 100 μM (Fig. 2). Arteries from untreated obese or troglitazone-treated obese rats that were similarly exposed to acetylcholine displayed marked impairment of the relaxation responses, with a significant 50% reduction ($P < 0.001$) in maximum relaxation and a significant rightward shift compared with vessels from chow-fed rats (Fig. 2).

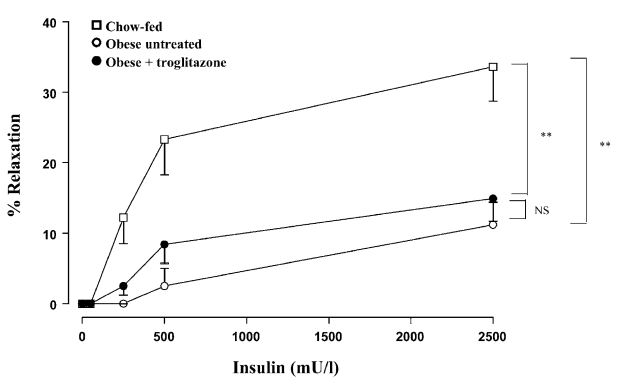

Fig. 4. Relaxation curves for insulin on arteries from lean control and dietary-obese rats. Arteries were precontracted with 8 μM noradrenaline. When contraction reached a plateau after 2 min, concentration–response curves to insulin was carried out. Data represent mean ± S.E.M. The concentration–response for the chow-fed and dietary-obese (both untreated and troglitazone-treated) groups differ significantly (by ANOVA, $P < 0.001$). There was no significant difference between insulin-induced vasorelaxation of arteries from untreated and troglitazone-treated dietary-obese rats.

Sodium nitroprusside had similar effects to those of acetylcholine. Maximum relaxation of arteries from chow-fed rats was $81 \pm 3\%$ at 100 μM sodium nitroprusside (Fig. 3), and the relaxation responses of arteries from dietary-obese rats (both troglitazone-treated and untreated animals) again displayed a significant ($P < 0.001$) right- and downward shift with up to 25% reduction in maximum relaxation (Fig. 3).

3.4.3. Insulin responses

Insulin induced a concentration-dependent relaxation of vessels from chow-fed rats. There was a significant (60%, $P < 0.001$) loss of insulin-induced vasorelaxation on arteries from dietary-obese (both troglitazone-treated and untreated) rats compared with their chow-fed counterparts (Fig. 4).

4. Discussion

In this study, the highly palatable diet induced obesity with striking increases in fat pad masses, plasma free fatty acids, triglycerides, and leptin levels. These metabolic changes resemble those seen in human obesity (Suter et al., 1992; Carey et al., 1997; Tack et al., 1998). These diet-induced metabolic abnormalities were corrected by troglitazone, although the modest effect on the attenuation of weight gain with troglitazone seen in this study might be attributed to additional effect of high volume of the administered compound. Such observations have also been reported in genetically obese animals such as Zucker rats (Murakami et al., 1998; Suter et al., 1992; Kumar et al., 1996; Tack et al., 1998; Cominacini et al., 1998). However, it is notable that these dietary obese animals were not insulin-resistant, at least with respect to glucose metabolism, as their fasting glucose and insulin and Homeostasis Model Assessment index were similar to chow-fed controls, although troglitazone did result in improved insulin sensitivity. This is consistent with our previous observations in this model after a similar period on the diet in male rats (Pickavance et al., 1999), and with a euglycaemic clamp study (unpublished observations).

Insulin resistance is associated with a decreased vasodilator response to insulin (Laakso et al., 1990, 1992; Feldman and Bierbrier, 1993). Because vasodilator effects of insulin is NO-dependent (Walker et al., 1997; Steinberg et al., 1994), thus this impairment may reflect endothelial dysfunction. Therefore, troglitazone, an insulin-sensitiser, might be expected to improve insulin-dependent and/or endothelium-dependent vascular function if insulin resistance is present. Indeed, animal studies have shown improvement of skin blood flow in diabetic rat (Fujiwara et al., 1988), attenuation of induced contraction in porcine coronary (Kawasaki et al., 1998) and rat tail (Song et al., 1997) arteries.

In agreement with our previous findings (Naderali et al., 1999), dietary-induced obesity had no significant effects on vascular contractility, as vasoconstriction induced by KCl and noradrenaline was similar in magnitude and pattern in both chow-fed and dietary-obese groups. Moreover, 3-week treatment of dietary-obese animals with troglitazone did not alter vascular contractility, supporting previous observations made in lean and obese Zucker rats (Walker et al., 1997) and in patients with type 2 diabetes (Lind et al., 1995), suggesting that insulin does not have a role in regulating vascular contractility. In contrast, acetylcholine- and insulin-induced vasorelaxation was attenuated in arteries from dietary-obese animals, indicating impairment of endothelial function. Attenuation of insulin-induced, as well as acetylcholine-induced, vasorelaxation seen in our study indicate that endothelial dysfunction induced by dietary-obesity is not corrected by chronic administration of troglitazone. Moreover, in agreement with some animal and human studies (Creager et al., 1990; Watts et al., 1996; Williams et al., 1996), sodium nitroprusside-induced vasorelaxation was also reduced in dietary-obese animals compared to chow-fed controls, indicating impairment of NO-generation by vascular smooth muscle cells. This finding suggests that troglitazone had also no effect on smooth muscle-dependent NO generation.

In conclusion, these data indicate that the vascular defects in endothelium and vascular smooth muscle that are present in dietary-obesity are not corrected by troglitazone. This is in spite of troglitazone's ability to correct the obesity-induced metabolic changes, and improve insulin sensitivity, even though insulin resistance was not apparent in untreated dietary obese animals. One explanation for this is that the vascular defect in dietary-obesity is not directly due to insulin resistance, suggesting that other mechanisms are involved; alternatively, after 8 weeks of feeding animals with a highly palatable diet, damage to vascular and endothelial function may have become established to the extent that 3 weeks of troglitazone treatment was insufficient to reverse the abnormalities present. Moreover, we cannot exclude possible detrimental effects of fatty acid composition of diet itself. Although troglitazone reduced plasma free fatty acids, this might not have affected all fatty acids.

Acknowledgements

This study was supported by Novo-Nordisk A/S, Denmark.

References

Abrams, J., 1997. Role of endothelial dysfunction in coronary artery disease. Am. J. Cardiol. 79, 2–9.

Anderson, E.A., Balon, T.W., Hoffman, R.P., Sinkey, S.A., 1991. Marked hyperinulinaemia produces both sympathic nueral activation and vasodilataion in normal humans. J. Clin. Invest. 87, 2246–2252.

Boulanger, C.M., 1999. Secondary endothelial dysfunction: hypertension and heart failure. J. Mol. Cell. Cardiol. 31, 39–49.

Buchanan, T.A., Meehan, W.P., Jeng, Y.Y., Yang, D., Chan, T.M., Nadler, J.L., Scott, S., Rude, R.K., Hsueh, W.A., 1995. Blood pressure lowering by pioglitazone. Evidence for a direct vascular effect. J. Clin. Invest. 96, 354–360.

Capron, L., Jarnet, J., Kazandjian, S., Housset, E., 1986. Growth-promoting effects of diabetes and insulin on arteries. An in vivo study of the rat aorta. Diabetes 35, 973–978.

Carey, V.J., Walters, E.E., Colditz, G.A., Solomon, C.G., Willett, W.C., Rosner, B.A., Speizer, E.E., Manson, J.E., 1997. Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The nurses' health study. Am. J. Epidemiol. 145, 614–619.

Christensen, K.L., Mulvany, M.J., 1993. Mesenteric arcade arteries contribute substantially to vascular resistance in conscious rats. J. Vasc. Res. 30, 73–79.

Cominacini, L., Garbin, U., Fratta-Pasini, A., Campagnola, M., Davoli, A., Foot, E., Sighieri, G., Sirroin, A.M., Casciov, L., Feerannini, E., 1998. Troglitazone reduces LDL oxidation and lowers plasma E-selectin concentration in NIDDM patients. Diabetes 47, 130–133.

Creager, M.A., Cooke, J.P., Mendelsohn, M.E., Gallagher, S.J., Coleman, S.M., Loscalzo, J., Dzau, V.J., 1990. Impaired vasodilatation of forearm resistance vessels in hypercholesterolemic humans. J. Clin. Invest. 86, 228–234.

Feldman, R.D., Bierbrier, G.S., 1993. Insulin-mediated vasodilation: impairment with increased blood pressure and body mass. Lancet 342, 707–709.

Fujiwara, T., Yoshioka, S., Yoshioka, T., Ushiyama, I., Horikoshi, H., 1988. Characterization of new oral antidiabetic agent CS-045. Studies in KK and ob/ob mice and Zucker fatty rats. Diabetes 37, 1549–1555.

Ghazzi, M.N., Perez, J.E., Antonucci, T.K., Driscoll, J.H., Huang, S.M., Faja, B.W., Whitcomb, R.W., 1997. Cardiac and glycemic benefits of troglitazone treatment in NIDDM. The troglitazone study group. Diabetes 46, 433–439.

Kato, K., Satoh, H., Endo, Y., Yamada, D., Midorikawa, S., Sato, W., Mizuno, K., Fujita, T., Tsukamoto, K., Watanabe, T., 1999. Thiazolidinediones down-regulate plasminogen activator inhibitor type 1 expression in human vascular endothelial cells: a possible role for PPARgamma in endothelial function. Biochem. Biophys. Res. Commun. 258, 431–435.

Kawai, K., Kawasaki-Tokui, Y., Odaka, T., Tsuruta, F., Kazui, M., Iwabuchi, H., Nakamura, T., Kinoshita, T., Ikeda, T., Yoshioka, T., Komai, T., Nakamura, K.-I., 1997. Disposition and metabolism of the new oral antidiabetic drug troglitazone in rats, mice and dogs. Drug Res. 47, 356–368.

Kawasaki, J., Hirano, K., Nishimura, J., Fujishima, M., Kanaide, H., 1998. Mechanisms of vasorelaxation induced by troglitazone, a novel antidiabetic drug, in the porcine coronary artery. Circulation 198, 2446–2452.

Kumar, S., Boulton, A.J., Beck-Nielsen, H., Berthezene, F., Muggeo, M., Persson, B., Spinas, G.A., Donoghue, S., Lettis, S., Stewart-Long, P., 1996. Troglitazone, an insulin action enhancer, improves metabolic control in NIDDM patients. Diabetologia 39, 701–709.

Laakso, M., Edelman, S.V., Brechtel, G., Baron, A.D., 1990. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. J. Clin. Invest. 85, 1844–1852.

Laakso, M., Edelman, S.V., Brechtel, G., Baron, A.D., 1992. Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. Diabetes 41, 1027–1032.

Lefebvre, A.M., Peinado-Onsurbe, J., Leitersdorf, I., Briggs, M.R., Paterniti, J.R., Fruchart, J.C., Fieret, C., Auwerx, J., Staels, B., 1997. Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. Arterioscler. Thromb., Vasc. Biol. 17, 1756–1764.

Lijima, K., Yoshizumi, M., Ako, Y., Eto, M., Kim, S., Hashmoto, M., Sugimoto, N., Liang, Y.Q., Sudoh, N., Taba, K., Ouchi, Y., 1998. Expression of peroxisome proliferator-activated receptor gamma (PPAR gamma) in rat aortic smooth muscle cells. Biochem. Biophys. Res. Commun. 247, 353–356.

Lind, L., Berne, C., Lithell, H., 1995. Prevalence of insulin resistance in essential hypertension. J. Hypertens. 12, 1457–1462.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., Turner, R.C., 1985. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412–419.

McVeigh, G.E., Brennan, G.M., Johnston, G.D., McDermott, B.J., McGarth, L.T., Henry, W.R., Andrews, J.W., Hayes, J.R., 1992. Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 35, 771–776.

Mombouli, J.-V., Vanhoutte, P.M., 1999. Endothelial dysfunction: from physiology to therapy. J. Mol. Cell. Cardiol. 31, 61–74.

Moncada, S., Gryglewski, R., Bunting, S., Vane, J.R., 1976. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 263, 663–665.

Murakami, K., Tobe, K., Ide, T., Mochizuki, T., Ohoshi, M., Akanuma, Y., Yazaki, Y., Kadowaki, T., 1998. A novel insulin sensitizer acts as a coligand for peroxisome proliferator-activated receptor-alpha (PPAR-alpha) and PPAR-gamma: effect of PPAR-alpha activation on abnormal lipid metabolism in liver of Zucker fatty rats. Diabetes 47, 1841–1847.

Naderali, E.K., Doyle, P., Jackson, M.J., Williams, G., 1999. Evidence of impaired endothelial function in dietary-induced obese rats. J. Physiol. 518, 83 pp., Abstract.

Naderali, E.K., Doyle, P., Williams, G., 2000. Resveratrol induces vasorelaxation of mesenteric and uterine arteries from female guinea-pigs. Clin. Sci. 98, 537–643.

Nakamura, Y., Ohya, Y., Onaka, U., Fujii, K., Abe, I., Fujishima, M., 1998. Inhibitory action of insulin-sensitising agents on calcium channels in smooth muscle cells from resistance arteries of guinea-pig. Br. J. Pharmacol. 123, 675–682.

Nolan, J.J., Ludvik, B., Beerdsen, P., Joyce, M., Olefsky, J., 1994. Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N. Engl. J. Med. 331, 1188–1193.

O'Brien, S.F., McKendrick, J.D., Radomski, M.W., Davidge, S.T., Russell, J.C., 1998. Vascular wall reactivity in conductance and resistance arteries: differential effects of insulin resistance. Can. J. Physiol. Pharmacol. 76, 72–76.

Okuno, A., Ikeda, K., Shiota, M., Fujimara, T., Yoshioka, S., Sugano, T., Horikoshi, H., 1997. Acute effect of troglitazone on glucose metabolism in the absence or presence of insulin in perfused rat hindlimb. Metabolism 46, 716–721.

Pickavance, L.C., Tadayyon, M., Widdowson, P.S., Buckingham, R.E., Wilding, J.P.H., 1999. Therapeutic index for rosiglitazone in dietary obese rats: separation of efficacy and haemodilution. Br. J. Pharmacol. 128, 1570–1576.

Pinkney, J.H., Stehouwer, C.D.A., Coppack, S.W., Yudkin, J.S., 1997. Endothelial dysfunction: cause of the insulin resistance syndrome. Diabetes 46, S9–S13.

Reaven, G.M., 1988. Role of insulin resistance in human disease. Diabetes 37, 1595–1607.

Russell, J.C., Dolphin, P.J., Graham, S.E., Amy, R.M., Brindley, D.N., 1998. Improvement of insulin sensitivity and cardiovascular outcomes in the JCR:LA-cp rat by D-fenfluramine. Diabetologia 41, 380–389.

Shimabukuro, M., Zhou, Y.T., Lee, Y., Unger, R.H., 1998. Troglitazone lowers islet fat and restores beta cell function of Zucker diabetic fatty rats. J. Biol. Chem. 273, 3547–3550.

Shimokawa, H., 1999. Primary endothelial dysfunction: atherosclerosis. J. Mol. Cell. Cardiol. 31, 23–37.

Song, J., Walsh, M.F., Igwe, R., Ram, J.L., Barazi, M., Dominguez, L.J., Sowers, J.R., 1997. Troglitazone reduces contraction by inhibition of vascular smooth muscle cell $Ca2+$ currents and not endothelial nitric oxide production. Diabetes 46, 659–666.

Steinberg, H.O., Brechtel, G., Johnson, A., Fineberg, N., Baron, A.D., 1994. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. J. Clin. Invest. 94, 1172–1179.

Steinberg, H.O., Chacker, H., Leaming, R., Johnson, A., Brechtel, G., Baron, A.D., 1996. Obesity/insulin resistance is associated with endothelial dysfunction: implications for the syndrome of insulin resistance. J. Clin. Invest. 97, 2601–2610.

Suter, S.L., Nolan, J.J., Wallace, P., Gumbiner, B., Olefsky, J.M., 1992. Metabolic effects of new oral hypoglycemic agent CS-045 in NIDDM subjects. Diabetes Care 15, 193–203.

Tack, C.J., Ong, M.K., Lutterman, J.A., Smits, P., 1998. Insulin-induced vasodilatation and endothelial function in obesity/insulin resistance. Effects of troglitazone. Diabetologia 41, 569–576.

Walker, A.B., Dores, J., Buckingham, R.E., Savage, M.W., Williams, G., 1997. Impaired insulin-induced attenuation of noradrenaline-mediated vasoconstriction in insulin-resistant obese Zucker rats. Clin. Sci. 93, 235–241.

Walker, A.B., Naderali, E.K., Chattington, P.D., Buckingham, R.E., Williams, G., 1998. Differential vasoactive effects of the insulin sensitizers rosiglitazone (BRL 49653) and troglitazone on human small arteries in vitro. Diabetes 47, 810–814.

Watts, G.F., O'Brien, S.F., Silvester, W., Millar, J.A., 1996. Impaired endothelium-dependent and independent dilatation of forearm arteries in men with diet-treated non-insulin-dependent diabetes: role of dyslipidaemia. Clin. Sci. 91, 567–573.

Williams, S.B., Cusco, J.A., Roddy, M.A., Johnstone, M.T., Creager, M.A., 1996. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. J. Am. Coll. Cardiol. 27, 567–574.

Yoshimoto, T., Naruse, M., Nishikawa, M., Naruse, K., Tanabe, A., Seki, T., Imaki, T., Demura, R., Aikawa, E., Demura, H., 1997. Antihypertensive and vasculo- and renoprotective effects of pioglitazone in genetically obese diabetic rats. Am. J. Physiol. 272, E989–E996.

Yoshioka, S., Nishino, H., Shiraki, T., Ikeda, K., Koike, H., Okuno, A., Wada, M., Fijiware, T., Horikoshi, H., 1993. Antihypertensive effects of CS-045 treatment in obese Zucker rats. Metabolism 42, 75–80.