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Effects of gemfibrozil treatment on vascular reactivity of streptozotocin-diabetic rat aorta

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Abstract

The effects of gemfibrozil treatment on plasma lipids, lipid peroxides and vascular reactivity of aorta were investigated in diabetic rats. Rats were divided randomly into two groups: control and diabetic. Diabetes was induced by a single intraperitoneal injection of streptozotocin (45 mg kg^{-1}). Twelve weeks after the induction of diabetes, some of the control and diabetic rats were started treatment with gemfibrozil (100 mg kg^{-1} daily; gavage) for 2 weeks. Blood glucose, plasma triglyceride, cholesterol, low-density lipoprotein (LDL) cholesterol and thiobarbituric acid reactive substances (TBARS) levels were markedly increased and gemfibrozil treatment restored these parameters in diabetic rats. However high-density lipoprotein (HDL) cholesterol levels did not differ in all experimental groups. In diabetic rats, the endothelium-dependent relaxations to acetylcholine were decreased when compared with control rats. Gemfibrozil treatment restored the endothelium-dependent responses to acetylcholine in diabetic rats. The endothelium-independent relaxation responses to sodium nitroprusside were not altered in all groups. These findings suggest that gemfibrozil treatment has beneficial effects against cardiovascular and metabolic complications of diabetes via its hypolipidaemic and antioxidant properties.

Introduction

Vascular diseases are well recognized complications in diabetes mellitus (Colwell et al 1979; Regan 1983). Reduced endothelium-dependent relaxation in patients with type I and type II diabetes is mentioned in several studies (McVeigh et al 1992; Johnstone et al 1993). Similar observations have been documented in a variety of blood vessels in experimental diabetes mellitus, suggesting that this abnormality is not unique to man (Taylor et al 1992, 1995; Pieper et al 1996). It is generally associated with reduction in nitric oxide (NO) bioavailability, and increased expression of cytoadhesive molecules and components involved in thrombosis and fibrinolysis (Pieper et al 1996; Hwang et al 1997; Abe et al 1998). Factors for reducing NO bioavailability are unknown. Several mechanisms have been proposed, among which is the suggestion that oxidative stress and dyslipidaemia could play a vital role (Wolf 1993; Tribe & Poston 1996). Hyperglycaemia induced oxidative stress by glucose auto-oxidation, non-enzymatic protein glycation, and dyslipidaemia also caused an augmentation in lipid peroxidation by increasing the susceptibility of lipoproteins to oxidation (Baynes 1991). Increased plasma lipids and lipid peroxides, oxidative modifications of lipoproteins and altered antioxidant enzyme activity are markers of oxidative stress (Kakkar et al 1995). Oxidative modification of both lipoproteins, especially low-density lipoprotein (LDL), and other free radicals, such as hydroxyl radicals and superoxide anions, is responsible for reduction in the expression of endothelial NO synthesis and impairment of endothelium-dependent relaxation in arteries (Liao et al 1995). Recent reports have shown that treatment of dyslipidaemia with hypolipidaemic drugs restores both oxidative stress markers and improves endothelial function in cardiovascular disease with or without diabetes (Keaney et al 1995; Kamata et al 1996a, b).

Although gemfibrozil and other fibric acid derivates have been used in clinical practice for over 4 decades as cholesterol-lowering therapy (Frick et al 1987), their mechanism is not clearly clarified. Recently, a clinical trial clearly demonstrated that

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fibrates reduced the incidence of death from cardiovascular disease (Rubins 1998). Reasons for reduction in these diseases could not only be associated with their reducing effect on the concentration of plasma lipids but may also be the result of improved vasomotor function, a reduction in inflammation, oxidative changes and some beneficial alterations in the metabolism of lipoproteins (Vamecq & Latruffe 1999; Schiffrin 2003).

Only a few studies have indicated the improvement of endothelial function by a fibrate. The effect of gemfibrozil on endothelial function in the experimentally-induced diabetic aorta has not been investigated (Evans 2000; Malik 2001). Therefore, in this study, we investigated the effects of gemfibrozil on endothelium-dependent relaxation of diabetic aorta and evaluated whether there was a relationship between the restorative effect on lipid metabolism and the vascular reactivity of gemfibrozil.

Materials and Methods

Drugs

All chemicals used in experiments were purchased from Sigma Chemical Co. (St Louis, MO).

Experimental protocols

Wistar albino male rats, 200–250 g, were used and were randomly divided into two groups: control and diabetic. Diabetes was induced by a single injection of streptozotocin (STZ; 45 mg kg^{-1}, i.p.). Three days after the injection, development of diabetes was confirmed by measuring blood glucose levels from the tail vein. Rats with blood glucose levels of $\geq$250 mg dL^{-1} were considered to be diabetic. At 12 weeks after the STZ injection, both groups were divided as untreated and treated with gemfibrozil (100 mg kg^{-1} daily; gavage in carboxymethylcellulose) for 14 days (Haubenwallner et al 1995). During the experimental period (14 weeks), the rats had free access to food and water. The Animal Care Ethics Committee of Ankara University approved the study.

Blood analysis

Blood samples were taken from the rats by cardiac puncture and plasma was obtained for determination of lipids and thiobarbituric acid reactive substance (TBARS) and stored at $-70\,^{\circ}C$ until assayed. Blood glucose concentration was measured by an Accutrend Glucometer (Boehringer, Manheim, Germany). Plasma triglyceride, total cholesterol and HDL cholesterol concentrations were measured with an automatic analyser by using a commercially available enzyme kit (Wako, Osaka, Japan). LDL cholesterol concentrations were calculated according to the Friedewald's formula. Plasma TBARS levels were measured according to the method of Yagi (1987).

Vascular studies

The thoracic aorta was excised, rapidly placed in cold physiological solution (PSS), carefully cleaned from excess connective tissue and cut into transverse rings of approximately 3–4 mm length each. The rings were suspended horizontally between stainless hooks in 10-mL organ baths, filled with PSS of the following composition (in mM): NaCl 118.0; KCl 7.4; $CaCl_2$ $2H_2O$ 2.5; KH_2PO_4 1.2; $MgSO_4$ $7H_2O$ 1.2; $NaHCO_3$ 25 and glucose 10.0. The PSS was aerated with 95% O_2–5% CO_2 at 37 °C. Each ring was connected to a force displacement transducer (Grass). Changes in isometric tension were recorded on a data acquisition system equilibrated for 60 min under a resting tension of 2 g (Mikroelektrik Ltd, Ankara, Turkey). During this period, the bath solution was replaced every 10 min. At the end of the equilibration period, concentration–response curves to cumulative concentrations of phenylephrine were performed on each ring. After reaching plateau, each ring was serially washed to baseline and equilibrated. Rings were contracted with a submaximal concentration of phenylephrine, which produced approximately 80% of the maximum response. This concentration was usually 1 μM but was occasionally varied between 10^{-6} and 3×10^{-6} M to obtain equieffective agonist activity. After reaching plateau of contraction, both cumulative concentration–response curves to acetylcholine (10^{-8} to 10^{-5} M) and then sodium nitroprusside (10^{-10} to 10^{-7} M) were obtained to evaluate endothelium-dependent and -independent relaxation, respectively.

Data and statistical analysis

Relaxation responses to acetylcholine and sodium nitroprusside were expressed as a percentage of the maximum contractile response to phenylephrine. The sensitivity to the agonists was evaluated as the pD_2 ($-\log EC50$) value. Results are expressed as the mean $\pm$ s.e.m. They were first subjected to Bartlett's test for homogeneity of variances and were given a log transformation if necessary. One-way analysis of variance was then performed, followed by the Student–Newman–Keul's test to estimate the significance of differences for individual between-group comparisons. Results were considered significantly different if $P < 0.05$.

Results

Metabolic parameters

Blood glucose, plasma triglyceride, total and LDL cholesterol levels of diabetic rats were significantly increased compared with controls, although HDL cholesterol levels were unchanged in all experimental groups. Plasma triglyceride, cholesterol and LDL cholesterol levels of diabetic groups were reduced by treatment with gemfibrozil. Gemfibrozil treatment did not affect the severity of hyperglycaemia in diabetic rats (Table 1). Induction of diabetes caused a marked augmentation of lipid peroxidation and gemfibrozil treatment normalized plasma TBARS levels

Table 1 Some characteristics of control, gemfibrozil-treated control, diabetic and gemfibrozil-treated diabetic rats.

Groups	Control (n = 7)	Gemfibrozil-treated control (n = 5)	Diabetic (n = 8)	Gemfibrozil-treated diabetic (n = 9)
Blood glucose (mg dL^{-1})	125 ± 4	129 ± 6	375 ± 12*	325 ± 22*
Plasma cholesterol (mg dL^{-1})	63 ± 4	70 ± 6	135 ± 6*	69 ± 7*
Plasma triglyceride (mg dL^{-1})	84 ± 6	60 ± 10	291 ± 30#	107 ± 19$,†
Plasma LDL cholesterol (mg dL^{-1})	17 ± 8	15 ± 9	52 ± 9#	34 ± 10$,†
Plasma HDL cholesterol (mg dL^{-1})	55 ± 5	62 ± 7	52 ± 5	60 ± 4
Plasma TBARS (nmol (mg protein)$^{-1}$)	1.51 ± 0.09	1.61 ± 0.02	2.53 ± 0.03*	1.43 ± 0.04§

Data are means ± s.e.m., n, no. of rats. *$P < 0.001$, #$P < 0.01$, $$P < 0.05$ vs control rats; †$P < 0.05$, §$P < 0.001$ vs diabetic rats.

(Table 1). There was no difference in this parameter between control and gemfibrozil-treated control groups (Table 1).

Vascular studies

Acetylcholine and sodium nitroprusside resulted in concentration-dependent relaxation in all aorta rings pre-contracted with phenylephrine. The acetylcholine-induced relaxation was significantly decreased in diabetic rats compared with control rats. Gemfibrozil treatment partially restored the defective endothelium-dependent relaxation of diabetic aorta rings. The endothelium-independent vasodilatation induced by the sodium nitroprusside relaxation did not significantly differ between experimental groups. Concentration–response curves for both acetylcholine and sodium nitroprusside are represented in Figure 1. On the other hand, the sensitivity of aorta from all experimental groups to acetylcholine and sodium nitroprusside was unchanged (Table 2).

Discussion

This study indicates that gemfibrozil treatment of diabetic rats restores the diabetes-induced impaired endothelium-dependent relaxation. Furthermore, it reduced the increased plasma lipids, lipoproteins and lipid peroxidation although it did not have any effect on high blood glucose levels in diabetic rats.

As in this study, it has been shown that diabetes is associated with an increase in plasma cholesterol, LDL cholesterol and triglyceride levels (Kobayashi & Kamata 1999; Kobayashi et al 2000). In addition, the formation of TBARS was markedly increased in the plasma of diabetic rats. The high plasma TBARS levels confirmed the previous studies and there was evidence of increased oxidative stress (Kubow et al 1996; Çinar 2001).

Our findings showed that there was a reduction in endothelium-dependent relaxation to acetylcholine but there was no alteration seen in its sensitivity in the diabetic rats, similar to the results of several studies (Keaney et al 1995; Kamata et al 1996a, b). This indicates that the mechanisms of diabetes-induced decrease in endothelium-dependent relaxation do not occur at the receptor level, but maybe there is a link with post-receptor events. There are several possible mechanisms that were postulated for the attenuation of endothelium-dependent relaxation that was seen in diabetes. Reduction of the bioavailability of nitric oxide (NO) could be a major factor in the attenuation in endothelium-dependent relaxation to acetylcholine in diabetic vessels. NO is an important substance for free radicals, especially superoxide radicals produced from a variety of sources, which can react with NO and produce peroxynitrite, nitrite and nitrate, which are less potent vasodilators. These radicals could reduce endothelium-dependent relaxation in diabetic rat aorta. Peroxynitrite also breaks down to produce highly reactive hydroxyl radicals (Gryglewski et al 1986; Mügge et al 1991; Beckman et al 1994). Lipid peroxidation is a potential source of hydroxyl radicals. Lipoproteins, especially LDL, are increased, the lipid content undergoes oxidation by peroxidation chain reactions and then can cause endothelial damage in diabetes (Beckman et al 1990; Baynes & Thorpe 1999). Furthermore, the response of endothelium-independent relaxation and the sensitivity to sodium nitroprusside was similar in all groups. This indicates that the activity of soluble guanylate cyclase in smooth muscle of aorta was not altered in diabetic rats (Özçelikay et al 2000).

We found that gemfibrozil did not have any effect on blood glucose in diabetic rats and these findings are in agreement with those of previous studies (Shen et al 1991; Fashing et al 1996). As expected, gemfibrozil treatment decreased plasma lipid levels – this result is similiar to the lipid-reducing effect of gemfibrozil on experimental and clinical diabetic status (Schonfeld 1994; Fashing et al 1996; Wiklund et al 1996). Gemfibrozil is a peroxisome proliferator-activated receptor agonist and acts principally on nuclear receptors to increase the expression of a variety of genes that regulate the synthesis of certain key proteins that are integral to the metabolism of lipoproteins (Fruchart et al 1999; Pineda-Torra et al 2001). One of the major effects of gemfibrozil is to reduce triglyceride levels by increasing lipoprotein lipase expression, which catalyses the hydrolysis and decreases apolipoprotein C-III expression, which normally inhibits both the lipolysis

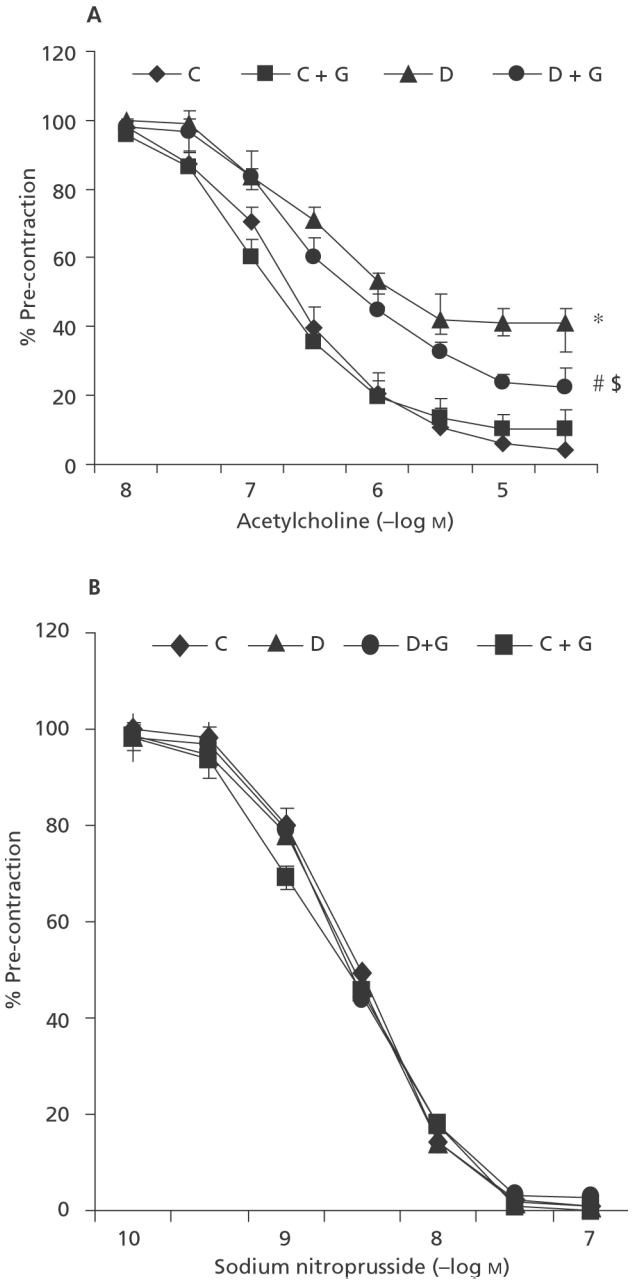

Figure 1 Cumulative concentration–response curves to acetylcholine (A) and sodium nitroprusside (B) in aorta rings pre-contracted with phenylephrine from control (C; n = 7), gemfibrozil-treated control (C + G; n = 5), diabetic (D; n = 8) and gemfibrozil-treated diabetic (D + G; n = 9) groups of rats. #$P < 0.01$, *$P < 0.001$, vs control rats; \$$P < 0.01$, significantly different from diabetic rats.

of triglycerides by LPL and uptake of remnants by the liver (Hertz & Bishara-Shieban 1995; Schoonjans et al 1995). Gemfibrozil also possibly affects HDL metabolism (Fruchart et al 1999; Pineda-Torra et al 2001). It has been shown that gemfibrozil increases HDL-mediated reverse cholesterol transport in hypertriglyceridaemic patients (Hertz & Bishara-Shieban 1995). This process, whereby

Table 2 pD_2 values to acetylcholine and sodium nitroprusside in aorta from control and gemfibrozil-treated rats.

Groups	Acetylcholine	Sodium nitroprusside
Control (n = 7)	6.76 ± 0.21	8.53 ± 0.06
Gemfibrozil-treated control (n = 5)	6.58 ± 0.18	8.61 ± 0.12
Diabetic (n = 8)	6.53 ± 0.25	8.55 ± 0.08
Gemfibrozil-treated diabetic (n = 9)	6.85 ± 0.26	8.54 ± 0.23

Data are means ± s.e.m.

HDL removes cholesterol from arterial macrophage foam cells to be transported to the liver, involves increased hepatic uptake of HDL cholesterol and increased biliary secretion and increased cholesterol excretion (Leiss et al 1985; Chianale et al 1996; Chinetti et al 2001). However, in this study we could not find any change in the HDL cholesterol levels. It is possible that the short duration of treatment was not suitable to observe the changes in the HDL cholesterol levels. A 4-week treatment is a minimally sufficient duration (Vinik & Colwell 1993). On the other hand, it has been reported that the metabolism of HDL in rodents is different from that in man. In rodents, gemfibrozil results in a considerable lowering of plasma HDL concentration because of a marked decrease in the expression of the apoA-I and Apo-AII genes (Fruchart et al 1999; Pineda-Torra et al 2001). In addition, we found that gemfibrozil treatment restored the plasma lipid peroxidation level in diabetic rats. This effect may be related to its hypolipidaemic activity by reducing the amount of substrate for lipid peroxidation (Yoshida et al 1998; Ozansoy et al 2001). Furthermore, it has been shown that there is a shift in the type of fatty acid from those susceptible to lipid peroxidation to fatty-acid species less subject to lipid peroxidation (Vasquez et al 1996; Aviram et al 1998). In a previous study we showed that gemfibrozil treatment improved catalase activity, which was increased in diabetic aorta (Ozansoy et al 2001). Thus, the lipid-peroxidation-reducing effect of gemfibrozil may, at least in part, be related to it, or its metabolites, having an antioxidant property.

This study has provided the first evidence that gemfibrozil restored vascular dysfunction of diabetic aorta. This effect of gemfibrozil might be associated with the improvement of the bioavailability of NO by reduction of lipid peroxidation and with its antioxidant property. An additional possible effect of gemfibrozil treatment is its pleiotropic metabolic action in the restoration of endothelium-dependent responses. It has been demonstrated that gemfibrozil has a variety of anti-inflammatory actions in the arterial wall that relate to the inhibition of pro-inflammatory cytokines, adhesion molecules for leucocytes and macrophage infiltration (Fujii et al 1992; Fruchart et al 1999; Neve et al 2001).

This study shows that gemfibrozil treatment partially normalized the vascular reactivity that is associated with reduced dyslipidaemia and oxidative stress, whereas it did not affect hyperglycaemia. The beneficial effect of gemfibrozil treatment on aorta of diabetic rats is due to its antioxidant and hypolipidaemic properties and may be directly related to treatment duration.

Conclusion

Our findings suggest that gemfibrozil is able to partly reverse endothelial dysfunction in diabetic rats after 2 weeks' administration. This effect is associated with a reduction in plasma lipids and TBARS levels. These findings may provide a new approach for therapy of diabetic cardiovascular complications.

References

Abe, Y., El-Masri, B., Kimball, K. T. (1998) Soluble cell adhesion molecules in hyperglycemia and potential significance on monocyte adhesion. *Arterioscler. Thromb. Vasc. Biol.* **18**: 723–731

Aviram, M., Rosenblat, M., Bisgaier, C. L., Newton, R. S. (1998) Atorvastatin and gemfibrozil metabolites, but not parent drugs are potent antioxidant against lipoprotein oxidation. *Atherosclerosis* **138**: 271–280

Baynes, J. W. (1991) Perspectives in diabetes: role of oxidative stress in development of complications in diabetes. *Diabetes* **40**: 405–412

Baynes, J. W., Thorpe, S. R. (1999) Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* **48**: 1–9

Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., Freeman, B. A. (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl Acad. Sci. USA* **87**: 1620–1624

Beckman, J. S., Chen, J., Ischiropoulos, H., Crow, J. P. (1994) Oxidative chemistry of peroxy nitrite. *Methods Enzymol.* **233**: 229–240

Chianale, J., Vollrath, V., Wielandt, A. M. (1996) Fibrates induce mdr2 gene expression and biliary phospholipid secretion in the mouse. *Biochem J.* **314**: 781–786

Chinetti, G., Lestavel, S., Bocher, V., Remaley, A. T., Neve, B., Torra, I. P., Teissier, E., Minnich, A., Jaye, M., Duverger, N., Brewer, H. B., Fruchart, J. C., Clavey, V., Staels, B. (2001) PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA 1 pathway. *Nat. Med.* **7**: 53–58

Çinar, M. G., Ülker, S., Alper, G., Evinç, A. (2001) Effect of dietary vitamin E supplementation on vascular reactivity of thoracic aorta in streptozotocin-diabetic rats. *Pharmacology* **62**: 56–64

Colwell, J. A., Halushka, P. V., Sarji, K. E., Lopes-Virella, M. F. (1979) Vascular disease in diabetes. *Arch. Intern. Med.* **139**: 225–230

Evans, M., Anderson, R. A., Graham, J., Ellis, G. R., Morris, K., Davies, S., Jackson, S. K., Lewis, M. J., Frenneaux, M. P., Rees, A. (2000) Ciprofibrate therapy improves endothelial function and reduces postprandial lipidemia and oxidative stress in type 2 diabetes mellitus. *Circulation* **101**: 1773–1779

Fashing, P., Rohac, M., Liener, K., Schneider, B., Nowotny, P., Waldhausl, W. (1996) Fish oil supplementation versus gemfibrozil treatment in hyperlipidemic NIDDM. *Horm. Metab. Res.* **28**: 230–236

Frick, M. H., Elo, O., Haapa, K., Heinonen, O. P., Heinsalmi, P., Helo, P., Huttunen, J. K., Kaitaniemi, P., Koskinen, P., Manninen, V. (1987) Helsinki Heart Study Primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. *N. Engl. J. Med.* **317**: 1237–1245

Fruchart, J. C., Duriez, P., Staels, B. (1999) Peroxisome proliferator-activated receptor-alpha activators regulate genes governing lipoprotein metabolism, vascular inflammation and atherosclerosis. *Curr. Opin. Lipidol.* **10**: 245–257

Fujii, S., Sobel, B. E. (1992) Direct effects of gemfibrozil on the fibrinolytic system. Diminution of synthesis of plasminogen activator inhibitor type1. *Circulation* **85**: 1888–1893

Gryglewski, R. J., Palmer, R. M. J., Moncada, S. (1986) Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature* **320**: 454–456

Haubenwallner, S., Essenburg, A. D., Barnett, B. C., Pape, M. E., DeMattos, R. B., Krause, B. R., Minton, L. L., Aurebach, B. L., Newton, R. S., Leff, T. (1995) Hypolipidemic activity of select fibrates corralates to changes in hepatic apolipoprotein C-III expression: a potential physiologic basis for their mode of action. *J. Lipid Res.* **36**: 2541–2551

Hertz, R., Bishara-Shieban, J. (1995) Mode of action of peroxisome proliferators as hypolipidemic drugs, supression of apolipoprotein C-III. *J. Biol. Chem.* **270**: 13470–13475

Hwang, S. J., Ballantyne, C. M., Sharret, A. R., Smith, L. C., Davis, C. E., Gotto, A. M., Boerwinkle, E. (1997) Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. *Circulation* **96**: 4219–4225

Johnstone, M. T., Creager, S. J., Scales, K. M., Cusco, J. A., Lee, B. K., Creager, M. A. (1993) Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* **88**: 2510–2516

Kakkar, R., Kalra, J., Mantha, S. V., Prasad, K. (1995) Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. *Mol. Cell. Biochem.* **151**: 113–119

Kamata, K., Sugiura, M., Kojima, S., Kasuya, Y. (1996a) Preservation of endothelium-dependent relaxation in both cholesterol-fed and streptozotocin-induced-diabetic mice by chronic administration of cholestyramine. *Br. J. Pharmacol.* **118**: 385–391

Kamata, K., Sugiura, M., Kojima, S., Kasuya, Y. (1996b) Restoration of endothelium-dependent relaxation in both hypercholesterolemia and diabetes by chronic taurine. *Eur. J. Pharmacol.* **303**: 47–53

Keaney, J. F., Xu, A., Cunningham, D., Jackson, T., Frei, B., Vita, J. A. (1995) Dietary probucol preserves endothelial function in cholesterol fed rabbits by limiting vascular oxidative stress and superoxide generation. *J. Clin. Invest.* **96**: 2520–2529

Kobayashi, T., Kamata, K. (1999) Relationship among cholesterol, superoxide anion and endothelium-dependent relaxation in diabetic rats. *Eur. J. Pharmacol.* **367**: 213–222

Kobayashi, T., Matsumoto, T., Kamata, K. (2000) Mechanisms underlying the chronic pravastatin treatment-induced improvement in the impaired endothelium-dependent aortic relaxation seen in streptozotocin-induced diabetic rats. *Br. J. Pharmacol.* **131**: 231–238

Kubow, S., Goyette, N. N., Kermasha, S., Stewart-Phillip, J., Koshi, K. G. (1996) Vitamin E inhibits fish-oil induced hyperlipidemia and tissue lipid peroxidation in hamsters. *Lipid* **31**: 839–847

Leiss, O., von Bergmann, K., Gnasso, A., Augustin, J. (1985) Effect of gemfibrozil on biliary lipid metabolism in normolipidemic subjects. *Metabolism* **34**: 74–82

Liao, J. K., Shin, W. S., Lee, W. Y., Clark, K. (1995) Oxidised low-density lipoprotein decreases the expression of endothelial nitric oxide synthase. *J. Biol. Chem.* **270**: 319–324

Malik, J., Melenoysky, V., Wichterle, D., Haas, T., Simek, J., Ceska, R., Hradec, J. (2001) Both fenofibrate and atorvastatin improve vascular reactivity in combined hyperlipidemia (fenofibrate versus atorvastatin trial–FAT). *Cardiovasc. Res.* **52**: 290–298

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

Mügge, A., Elwell, J. H., Peterson, T. E., Harrison, D. G. (1991) Release of intact endothelium-derived relaxing factor depends on endothelial superoxide dismutase activity. *Am. J. Physiol.* **260**: C219–C225

Neve, B. P., Seaux, D., Chinetti, G. (2001) PPAR-α agonists inhibit tissue factor expression in human monocytes and macrophages. *Circulation* **103**: 207–212

Ozansoy, G., Akin, B., Aktan, F., Karasu, Ç. (2001) Short-term gemfibrozil treatment reverses lipid profile and peroxidation but does alter blood glucose and tissue antioxidant enzymes in chronically diabetic rats. *Mol. Cell. Biochem.* **216**: 59–63

Özçelikay, A. T, Tay, A., Güner, Ş., Taşayaran, V., Yildizoğlu-Ari, N., Dinçer, Ü. D., Altan, M. V. (2000) Reversal effects of L-Arginine treatment on blood pressure and vascular responsiveness of streptozotocin-diabetic rats. *Pharmacol. Res.* **41**: 201–209

Pieper, G. M., Siebeneich, W., Roza, A. M., Jordan, M., Adams, M. B. (1996) Chronic treatment in vivo with dimethylthiourea, a hydroxyl radical scavenger, prevents diabetes-induced endothelial dysfunction. *J. Cardiovasc. Pharmacol.* **28**: 741–745

Pineda Torra, I., Chinetti, G., Duval, C. (2001) Peroxisome proliferator-activated receptors: from transcriptional control to clinical practice. *Curr. Opin. Lipidol.* **12**: 245–254

Regan, T. J. (1983) Congestive heart failure in the diabetic. *Annu. Rev. Med.* **34**: 161–168

Rubins, H. B. (1998) *Veterans Affairs High Density Lipoprotein Cholesterol Intervention Trial (VA-HIT): scientific sessions of the American Heart Association.* Dallas, USA: Plenary session XII Late breaking clinical trials

Schiffrin, E. L., Amiri, A., Benkirane, K., Iglarz, M., Diep, Q. N. (2003) Peroxisome proliferator-activated receptors vascular and cardiac effects in hypertension. *Hypertension* **42**: 1–5

Schonfeld, G. (1994) The effcts of fibrates on lipoprotein and hemostatic coronary risk factors. *Atherosclerosis* **111**: 161–174

Schoonjans, K., Staels, B., Deeb, S. (1995) Fibrates and fatty acids induce lipprotein lipase gene expression via the peroxisome proliferator-activated receptors. *Circulation* **92** (Suppl. 1): I495

Shen, D. C., Fuh, M. M. T., Shieh, S. M., Chen, Y. D. I., Reaven, G. M. (1991) Effect of gemfibrozil treatment in sulfonurea-treated patients with non-insulin dependent diabetes mellitus. *J. Clin. Endocrinol. Metab.* **73**: 503–510

Taylor, P. D., Mc Carthy, A. L., Thomas, C. R., Poston, L. (1992) Endothelium-dependent relaxation and noradrenaline sensivity in mesenteric arteries of streptozotocin-induced diabetic rats. *Br. J. Pharmacol.* **107**: 393–399

Taylor, P. D., Graves, J. E., Poston, L. (1995) Selective impairment of acetylcholine-mediated endothelium-dependent relaxation in isolated resistance arteries of streptozotocin-induced diabetic rat. *Clin. Sci.* **88**: 1–6

Tribe, R. M., Poston, L. (1996) Oxidative stress and lipids in diabetes: a role in endothelium vasodilatator dysfunction. *Vasc. Med.* **1**: 195–206

Vamecq, J., Latruffe, N. (1999) Medical significance of peroxisome proliferator-activated receptors. *Lancet* **354**: 141–148

Vasquez, M., Merlos, M., Adzet, T., Laguna, J. C. (1996) Decreased susceptibility to copper-induced oxidation of rat-lipoproteins after fibrate treatment: Influence of fatty acid composition. *Br. J. Pharmacol.* **117**: 1155–1162

Vinik, A. I., Colwell J. A. (1993) Effects of gemfibrozil on triglyceride levels in patients with NIDDM: hyperlipidemia in diabetes investigators. *Diabetes Care* **16**: 37–44

Wiklund, O., Bondjers, G., Wright, I., Camejo, G. (1996) Insoluble complex formation between LDL and arterial proteoglycans in relation to serum lipid levels and effects of lipid lowering drugs. *Atherosclerosis* **119**: 57–67

Wolf, S. P. (1993) Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus complications. *Br. Med. Bull.* **49**: 642–652

Yagi, K. (1987) Lipid peroxidases and human disease. *Chem. Phys. Lipids* **45**: 337–351

Yoshida, H., Ishikawa, T., Ayaori, M., Shige, H., Ito, T., Suzukawa, M., Nakamura, H. (1998) Beneficial effect of gemfibrozil on the chemical composition and oxidative susceptibility of low density lipoprotein. A randomized, double-blind placebo-controlled study. *Atherosclerosis* **139**: 179–187