

Review

Use of Metformin in Patients with Kidney and Cardiovascular Diseases

David Klachko^a Adam Whaley-Connell^{b, c}

Divisions of ^aEndocrinology and ^bNephrology, Department of Internal Medicine, University of Missouri-Columbia School of Medicine, and ^cHarry S. Truman VA Medical Center, Columbia, Mo., USA

Key Words

Cardiovascular benefits · Diabetes · Hyperglycemia · Metformin · Renal function

Abstract

Metformin is an insulin-sensitizing agent with anti-hyperglycemic properties that is widely used for the treatment of type-2 diabetes. The efficacy of metformin in reducing hyperglycemia is well established, and there is emerging evidence that its chronic use is associated with cancer and cardiovascular disease (CVD) risk reduction. While the hypoglycemic properties of metformin are largely attributed to suppression of hepatic glucose production and increases in peripheral tissue insulin sensitivity, the precise mechanism of the hypoglycemic action of metformin remains unclear. There is evidence that metformin use interrupts mitochondrial oxidative stress in the liver and corrects abnormalities of intracellular calcium metabolism in insulin-sensitive tissues (liver, skeletal muscle, and adipocytes) and cardiovascular tissue. However, the use of metformin in patients with kidney disease, a high-risk CVD state, is confounded by confusion regarding appropriate concerns about the development of lactic acidosis in this population. Thus, we will review current evidence on metformin use for improving CVD outcomes and its therapeutic use in kidney disease.

Copyright © 2011 S. Karger AG, Basel

Introduction

Metformin is an insulin-sensitizing biguanide used to treat patients with type-2 diabetes [1]. Use of metformin may have special benefits in overweight patients with polycystic ovary disease as well as those with type-2 diabetes [1]. Unlike traditional hypoglycemic agents such

as sulfonylureas, insulin, and thiazolidinediones, metformin does not increase body weight and does not cause hypoglycemia [1]. In this regard, significant reductions in total body fat and visceral fat have been observed in metformin-treated women with preexistent abdominal or visceral obesity [2]. This observation is important in the context that excess fat localized to the omental region is a major contributor to insulin resistance and thus is involved in the pathogenesis of the cardiorenal metabolic syndrome (CRS) [3]. The reduction in visceral adipose tissue may have additional cardiovascular disease (CVD) benefits in insulin-resistant persons with the CRS treated with metformin [1, 4, 5].

To elucidate the concerns about the use of metformin in patients with reduced renal function, it is important to understand the metabolism of this compound. At routine doses of 500–1,500 mg, metformin has an absolute oral bioavailability of 50–60% [6]. The drug is not protein bound and therefore has a wide volume of distribution with maximal accumulation in the small-intestine wall. Metformin undergoes no modifications in the body and is secreted unchanged by rapid kidney excretion (through glomerular filtration and, possibly, tubular secretion). Impaired kidney function slows elimination and may cause metformin accumulation, raising concerns regarding lactic acidosis [7]. However, recent data and reviews of large databases have suggested an increased use of metformin in those with diminished renal function with no increase in the occurrence of lactic acidosis [7–12].

Effect of Metformin on Obesity-Related Cardiovascular Morbidity and Mortality

In the United Kingdom Prospective Diabetes Study (UKPDS) 34, metformin therapy was compared with conventional treatment or treatment with sulfonylurea or insulin [3]. In this trial, which was designed to achieve a fasting glucose <6 mmol/l (<108 mg/dl), newly diagnosed obese type-2 diabetics were allocated to receive either metformin or treatment with chlorpropamide, glibenclamide, or insulin. During 10 years of follow-up, both groups achieved equal degrees of glycemic control. Compared with the conventional treatment, metformin-treated patients had a risk reduction of 32% for any diabetes-related end point and a 39% risk reduction for myocardial infarction. At the time when the results of this trial were published, the differences in myocardial infarction rates in the groups were thought to be partially explained by differences in the degree of glycemic control between the metformin group and the control group. Indeed, in the UKPDS 35 [13], the risk for CVD events, stroke, and all-cause death were again closely related to the degree of glycemia in diabetic patients. In that study, each 1% reduction in the hemoglobin A1c value was associated with a reduction in diabetes-related death, incident myocardial infarction, fatal and non-fatal strokes, as well as heart failure. Nevertheless, metformin was more effective than sulfonylureas or insulin in reducing rates of any diabetes-related end points, all-cause mortality, and stroke, even though both agents decreased hemoglobin A1c values equally. These observations suggest that metformin might have additional CVD and cancer-protective actions beyond its hypoglycemic properties.

Cardiovascular Protective Actions of Metformin

Insulin resistance is a cornerstone in the pathogenesis of the CRS and is commonly associated with hypertension, abdominal obesity, diminished kidney function, atherogenic dyslipidemia, and vascular dysfunction, all of which play a significant part in the development of accelerated atherosclerosis [1, 4, 14, 15]. The increased levels of free fatty acids that occur in obesity and poorly controlled diabetes contribute not only to the development of

insulin insensitivity, but also to increased synthesis and secretion of very-low-density lipoprotein [16]. Elevated triglyceride levels inhibit degradation of apolipoprotein B in the liver and lead to increased assembly of small, dense, low-density lipoprotein (LDL) particles [17, 18]. Excess generation of cytokines and reactive oxygen species (such as peroxynitrates) in cardiovascular and kidney tissue in combination with increased non-enzymatic glycation of lipoproteins (glyco-oxidation) leads to the formation of atypical glyco-oxidized LDL particles. These particles bind poorly to classic LDL receptors but have high affinity for 'scavenger' receptors, which are located predominantly on macrophages. Accumulation of glyco-oxidized small, dense, LDL particles converts macrophages into foam cells, which are essential participants in the early steps of atherosclerotic plaque formation.

Metformin has been shown to have significant beneficial effects on lipid metabolism in patients with insulin resistance. Further, several studies have shown that metformin improves the lipoprotein profile in diabetic patients [14, 15]. Metformin decreases plasma levels of triglycerides, very low-density lipoproteins and free fatty acids [19, 20], and oxidation of free fatty acids by tissue [21]. Metformin reduces tissue oxidative stress, inflammation, and lipid oxidation, in part by lowering plasma glucose levels [15, 22]. Collectively, these observations suggest that the beneficial effects of metformin on lipoprotein metabolism may contribute to its protective effects against CVD. Since, unlike other oral hypoglycemia agents [23], metformin does not cause hypoglycemia, this may further contribute to its CVD benefits.

Metformin has also been shown to improve alterations in coagulation and fibrinolytic pathways in insulin resistance by decreasing levels of plasminogen activator inhibitor-1 and increasing tissue plasminogen activator activity [24–26]. Therapy with metformin also reduces the thrombogenic propensity by decreasing levels of tissue plasminogen activator antigen and von Willebrand factor [27]. In BIGPRO1 (Biguanides and the Prevention of the Risk of Obesity study), treatment with metformin was associated with a reduction in plasminogen activator inhibitor-1 activity and decreases in von Willebrand factor levels [27]. Furthermore, the observation that metformin therapy results in decreased platelet aggregation in diabetic patients [28] suggests metformin improves hypercoagulability and exaggerated platelet aggregation/adhesion in diabetic patients.

Effects of Metformin on Diabetic Heart Disease

Individuals with insulin resistance and diabetes have an increased risk for heart failure [29] secondary to hypertensive, myopathic and ischemic changes within the myocardium. Diabetic cardiomyopathy is characterized by structural changes such as myocardial fibrosis, and it is also characterized by functional alterations that lead to impairments in diastolic relaxation and ventricular compliance [30–33]. The observed impaired diastolic relaxation in diabetes is related to diminished removal of intracellular ionized calcium ($[Ca^{2+}]_i$) from cardiomyocytes after systolic contraction [31–33]. Treatment of diabetic rats with metformin corrects these functional cardiac abnormalities [33–36], probably through improvement of tyrosine kinase-dependent increases in $[Ca^{2+}]_i$ removal after systole [33]. This myocardial protective action of metformin was shown to be insulin independent. Moreover, treatment of spontaneously hypertensive rats with metformin has been reported to decrease heart rate (a sympatho-inhibitory effect) more than placebo [36, 37]. Although these findings are of interest, there is little direct clinical evidence of the effect of metformin on the development and course of congestive heart failure in diabetic and CRS patients.

Metformin and Vascular Reactivity

Insulin resistance is associated with endothelial dysfunction that is associated with impaired insulin-induced vasorelaxation [4]. Recently, our group and others have demonstrated that impairments in insulin metabolic signaling may contribute to increased vascular resistance, the hallmark of hypertension in patients with the CRS and type-2 diabetes [4, 18]. Insulin normally acts through the phosphoinositol 3-kinase/protein kinase B (Akt) pathway to activate nitric oxide (NO) synthase, enhance sodium pump activity in vascular smooth muscle tissue, and increase glucose transport in cardiac and skeletal muscle tissue [38]. Moreover, insulin is responsible for the normal handling of divalent cations in vascular smooth muscle, and this function is diminished in insulin resistance [38]. Impairments in insulin action in vascular tissue may result in impaired NO-dependent vascular relaxation, decreased sodium pump activity, and increased levels of $[Ca^{2+}]_i$ in vascular smooth muscle in patients with type-2 diabetes [4, 14, 38]. These abnormalities in NO metabolism play a role in the vascular resistance that characterizes endothelial dysfunction in insulin resistance states such as the CRS and type-2 diabetes [38].

Several reports indicate metformin may have a moderate anti-hypertensive effect in animals [37, 39–41] and humans [42]. Potential mechanisms of the anti-hypertensive action of metformin are complex and include both insulin-dependent and insulin-independent vasodilatory actions. Short-term metformin administration has been shown to increase vascular smooth muscle cell repolarization and lead to subsequent vascular relaxation [43] via a reduction in the agonist-induced increase in $[Ca^{2+}]_i$ in vascular smooth muscle cells [40, 44]. This attenuation of $[Ca^{2+}]_i$ responses may be secondary to increased NO production by vascular smooth muscle during exposure to metformin. Indeed, NO has been shown to decrease vascular smooth muscle cell $[Ca^{2+}]_i$ responses to vasoconstrictor agonists through activation of the cyclic guanosine monophosphate pathway [45]. Metformin may also reduce $[Ca^{2+}]_i$ by increasing the activity of the sodium-adenosine triphosphatase pump and enhancing adenosine triphosphate-sensitive K^+ channels [44–46]. The ability of metformin to stimulate sodium pump activity is probably linked to increased lactate production in vascular smooth muscle tissue [46, 47].

Even a small elevation in blood pressure significantly increases the risk for myocardial infarction, stroke, congestive heart failure and death from CVD in diabetic persons [48]. Therefore, even a minimal reduction in blood pressure during treatment with metformin may contribute to a significant decrease in diabetes-related morbidity and mortality. Thus, in addition to its beneficial metabolic effects, the blood pressure-lowering effects of metformin may be important in reducing CVD risk in patients with CRS and diabetes mellitus.

Use of Metformin in Chronic Kidney Disease

As noted earlier, metformin is a biguanide related to phenformin, which was removed from the market in 1977 due to several cases of fatal lactic acidosis. Metformin is renally cleared and excreted in the urine, and there is concern that decreased renal clearance can lead to toxic levels. Therefore, many physicians discontinue its use whenever there is any increase in serum creatinine, and it is currently contraindicated in patients with serum creatinine levels >1.4 mg/dl in women and >1.5 mg/dl in men. However, phenformin has a chemical structure substantially different from that of metformin. Unlike metformin, phenformin can impair oxidative phosphorylation in the liver, thereby increasing lactate production by anaerobic pathways [49–51]. In contrast to phenformin, metformin inhibits hepatic gluconeogenesis without altering lactate turnover or lactate oxidation [51]. Studies in rats

with doses equivalent to high doses in humans showed no elevation in lactate levels in contrast to high lactate levels with phenformin administration. Thus, the concern about the development of lactic acidosis with metformin therapy is likely overblown.

Risk of Lactic Acidosis

In two recent Cochrane database analyses, metformin therapy was associated with lactic acidosis only when there was an underlying condition such as hypotension, hypoxemia, acute kidney injury, or cirrhosis [49, 50]. However, lactic acidosis (pH <7.37 and/or plasma lactate levels >4 mmol/l) continues to be discussed in the literature [52] even though the absolute risk appears to be low, with incidence rates of lactic acidosis associated with metformin use ranging from 1 to 16.7 cases per 100,000 patient-years [52–55]. In the Cochrane analysis, investigators have identified all trials and cohort studies conducted between 1959 and 2002 and have not found a single case of lactic acidosis in 36,893 person-years of metformin exposure [10, 49, 50, 55]. Further, in 49 individual cases of lactic acidosis associated with metformin use, overall mortality was not found to correlate with plasma lactate concentrations [56]. In this same study, circulating metformin concentrations were three times higher in those who survived. It was observed that in subjects who developed fatal lactic acidosis, there were acute co-morbidities predisposing to lactic acidosis. These compelling data suggest that lactic acidosis may be coincidental rather than causally associated with metformin use.

Several studies have shown no increase in the frequency of lactic acidosis provided that the dosage is adjusted appropriately in patients with impaired renal function [50]. In a meta-analysis of 347 studies in type-2 diabetes mellitus with 70,490 patient-years in the metformin group and 55,451 patient-years in the non-metformin group, there was ‘no evidence that metformin is associated with an increased risk of lactic acidosis or with increased levels of lactate compared to other anti-hyperglycemic treatments’. Among 50,000 patients with type-2 diabetes mellitus in the UK General Practice Research Database, 6 cases of lactic acidosis were identified [11]. The crude incidence rate in cases per 100,000 patient-years was 3.3 for patients taking metformin and 4.8 for those taking sulfonylureas. The authors largely concluded that the incidence of lactic acidosis in patients with diabetes does not appear to be influenced by the use of metformin, and the relevant co-morbidities known as risk factors for lactic acidosis could be identified in all cases. Similarly, in a review of all reported cases of ‘metformin-associated lactic acidosis’ from May 1995 through January 2000, 22 patients met the criteria for lactic acidosis [56, 57]. Plasma metformin concentration was measured in only 4 and was normal in 1 patient. The most common precipitating cause of lactic acidosis was acute renal failure. None of the 10 deaths in this cohort was directly related to the metformin therapy [57].

Understanding Renal Clearance of Metformin

In a few studies, measurements of the renal clearance of metformin have been reported. Following administration of a single 850-mg tablet of metformin hydrochloride, renal clearance in 12 elderly healthy subjects was 35–40% lower than in 6 young healthy adults [56, 58]. In 15 adults with varying degrees of chronic kidney disease (CKD), renal clearance was compared between the 6 young and 3 middle-aged subjects. Those with mild renal impairment had clearance decreased by 23–33%, while the decrease was 74–78% in those with moderate-to-severe renal insufficiency. In another study [59], 26 patients aged 70–88 years with poorly controlled type-2 diabetes mellitus were given 850 or 1,700 mg metformin dependent on

Table 1. Modification of metformin doses with respect to diminished renal function

CKD stage	eGFR ml/min/1.73 m ²	Dose	% max.
0–1	≥90	2,500 mg daily	100
2	≥60	1,000 mg b.i.d.	80
3	≥45	500 mg b.i.d.	40
3	≥30	500 mg daily	20
4–5	<30	0	0

creatinine clearance levels of 30–60 or >60 ml/min/1.73 m², respectively. Blood levels of metformin remained within the expected values for both groups. Blood lactate levels remained unchanged in the group receiving the higher dosage and were lower in the group with the lower dose.

The authors have collectively concluded that the term ‘metformin-associated lactic acidosis’ is commonly used to depict all situations of lactic acidosis in metformin therapy. Nevertheless, true metformin-associated lactic acidosis, i.e. one which refers to metformin and concurrent pathologies as co-precipitating factors, has not been observed in the studied reports. As there was no mortality due to metformin alone, it is important that physicians are familiar with the range of other risk factors that contribute to lactic acidosis in patients treated with metformin, such as hypotension, hypoxemia, acute kidney injury, or other acute pathophysiological insults [60].

Metformin Use and Dosage in Clinical Practice

The increasing recognition that individuals with CRS and type-2 diabetes may benefit from metformin treatment despite the presence of CKD and CVD has led many to reconsider its use in this high-risk population. A recent consensus statement from the United Kingdom National Institute for Health and Clinical Excellence [61], clinical guideline 38, suggests clinicians review the dose of metformin if the estimated glomerular filtration rate (eGFR) is <45 ml/min/1.73 m², and discontinue metformin treatment if the eGFR is <30 ml/min/1.73 m². The committee does suggest prescribing metformin with caution for those at risk of a sudden deterioration in kidney function and those at risk of eGFR falling <45 ml/min/1.73 m². In this context, some investigators have reported safe use of metformin in large CKD populations where other oral hypoglycemic agents are unavailable and have proposed a protocol for adjustment of dose with diminishing renal function (table 1) [57].

Conclusions

In summary, metformin is a biguanide commonly used for the treatment of type-2 diabetes acting primarily on hepatic glucose production, but has additional effects on peripheral insulin sensitivity. The major actions of metformin are mediated via a reduction in hepatic gluconeogenesis and perhaps through modulation of mitochondrial [Ca²⁺]_i handling. Over time, metformin has proven to have an excellent safety profile. However, there have been concerns regarding the development of lactic acidosis in certain populations. These

concerns are increasingly being questioned. Metformin has shown a substantial beneficial effect on lipid metabolism, clotting factors, and platelet function in clinical and preclinical states. In laboratory animals, metformin has been shown to improve cardiac diastolic dysfunction and vascular relaxation. These effects may contribute to the cardiovascular risk reduction observed during the UKPDS. After years of use and extensive database and case report analysis, use of metformin in CKD appears to be safe provided there is dose adjustment based on the level of kidney function and cessation in those with an eGFR <30 ml/min/1.73 m². Thus, it is important to consider its use in patients with CKD who are at risk for CVD.

Acknowledgment

Dr. Whaley-Connell is supported by the Department of Veteran Affairs through a Career Development Award-2.

Disclosure Statement

There are no conflicts to disclose.

References

- 1 Kirpichnikov D, McFarlane SI, Sowers JR: Metformin: an update. *Ann Intern Med* 2002;137:25–33.
- 2 Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D, Fiorini S, Cognigni GE, Filicori M, Morselli-Labate AM: Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2000;85:2767–2774.
- 3 Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998;352:854–865.
- 4 Sowers JR, Whaley-Connell A, Hayden MR: The role of overweight and obesity in the cardiorenal syndrome. *Cardiorenal Med* 2011;1:5–12.
- 5 Sowers JR: Obesity and cardiovascular disease. *Clin Chem* 1998;44:1821–1825.
- 6 McFarlane SI, Banerji M, Sowers JR: Insulin resistance and cardiovascular disease. *J Clin Endocrinol Metab* 2001;86:713–718.
- 7 Scheen AJ: Clinical pharmacokinetics of metformin. *Clin Pharmacokinet* 1996;30:359–371.
- 8 Lalau JD, Andrejak M, Morinière P, Coevoet B, Debussche X, Westeel PF, Fournier A, Quichaud J: Hemodialysis in the treatment of lactic acidosis in diabetics treated by metformin: a study of metformin elimination. *Int J Clin Pharmacol Ther Toxicol* 1989;27:285–288.
- 9 Vasisht KP, Chen SC, Peng Y, Bakris GL: Limitations of metformin use in patients with kidney disease: are they warranted? *Diabetes Obes Metab* 2010;12:1079–1083.
- 10 Salpeter S, Greyber E, Pasternak G, Salpeter E: Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2006;25:CD002967.
- 11 Bodmer M, Meier C, Krähenbühl S, Jick SS, Meier CR: Metformin, sulfonylureas, or other antidiabetes drugs and the risk of lactic acidosis or hypoglycemia: a nested case-control analysis. *Diabetes Care* 2008;31:2086–2091.
- 12 Mani MK: Nephrologists sans frontières: preventing chronic kidney disease on a shoestring. *Kidney Int* 2006;70:821–823.
- 13 Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR: Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000;321:405–412.
- 14 Wu MS, Johnston P, Sheu WH, Hollenbeck CB, Jeng CY, Goldfine ID, Chen YD, Reaven GM: Effect of metformin on carbohydrate and lipoprotein metabolism in NIDDM patients. *Diabetes Care* 1990;13:1–8.
- 15 Robinson AC, Burke J, Robinson S, Johnston DG, Elkeles RS: The effects of metformin on glycemic control and serum lipids in insulin-treated NIDDM patients with suboptimal metabolic control. *Diabetes Care* 1998;21:701–705.
- 16 Howard BV: Insulin resistance and lipid metabolism. *Am J Cardiol* 1999;84:28J–32J.
- 17 Kirpichnikov D, Sowers JR: Diabetes mellitus and diabetes-associated vascular disease. *Trends Endocrinol Metab* 2001;12:225–230.

- 18 Whaley-Connell A, Pulakat L, DeMarco VG, Hayden MR, Habibi J, Henriksen EJ, Sowers JR: Overnutrition and the cardiorenal syndrome: use of a rodent model to examine mechanisms. *Cardiorenal Med* 2011;1:23–30.
- 19 Abbasi F, Kamath V, Rizvi AA, Carantoni M, Chen YD, Reaven GM: Results of a placebo-controlled study of the metabolic effects of the addition of metformin to sulfonylurea-treated patients. Evidence for a central role of adipose tissue. *Diabetes Care* 1997;20:1863–1869.
- 20 Reaven GM, Johnston P, Hollenbeck CB, Skowronski R, Zhang JC, Goldfine ID, Chen YD: Combined metformin-sulfonylurea treatment of patients with noninsulin-dependent diabetes in fair to poor glycemic control. *J Clin Endocrinol Metab* 1992;74:1020–1026.
- 21 Lalau JD, Race JM: Lactic acidosis in metformin therapy. *Drugs* 1999;58(suppl 1):55–60, discussion 75–82.
- 22 Landin K, Tengborn L, Smith U: Treating insulin resistance in hypertension with metformin reduces both blood pressure and metabolic risk factors. *J Intern Med* 1991;229:181–187.
- 23 Holstein A, Egberts EH: Risk of hypoglycemia with oral antidiabetic agents in patients with type 2 diabetes. *Exp Clin Endocrinol Diabetes* 2003;111:405–414.
- 24 Tessier D, Maheux P, Khalil A, Fülöp T: Effects of gliclazide versus metformin on the clinical profile and lipid peroxidation markers in type 2 diabetes. *Metabolism* 1999;48:897–903.
- 25 Grant PJ, Stickland MH, Booth NA, Prentice CR: Metformin causes a reduction in basal and post-venous occlusion plasminogen activator inhibitor-1 in type 2 diabetic patients. *Diabet Med* 1991;8:361–365.
- 26 Nagi DK, Yudkin JS: Effects of metformin on insulin resistance, risk factors for cardiovascular disease, and plasminogen activator inhibitor in NIDDM subjects. A study of two ethnic groups. *Diabetes Care* 1993;16:621–629.
- 27 Charles MA, Morange P, Eschwege E, André P, Vague P, Juhan-Vague I: Effect of weight change and metformin on fibrinolysis and the von Willebrand factor in obese nondiabetic subjects: the BIGPRO1 Study. Biguanides and the Prevention of the Risk of Obesity. *Diabetes Care* 1998;21:1967–1972.
- 28 Marfella R, Acampora R, Verrazzo G, Ziccardi P, De Rosa N, Giunta R, Giugliano D: Metformin improves hemodynamic and rheological responses to L-arginine in NIDDM patients. *Diabetes Care* 1996;19:934–939.
- 29 Shindler DM, Kostis JB, Yusuf S, Quinones MA, Pitt B, Stewart D, Pinkett T, Ghali JK, Wilson AC: Diabetes mellitus, a predictor of morbidity and mortality in the Studies of Left Ventricular Dysfunction (SOLVD) Trials and Registry. *Am J Cardiol* 1996;77:1017–1020.
- 30 Fiordaliso F, Li B, Latini R, Sonnenblick EH, Anversa P, Leri A, Kajstura J: Myocyte death in streptozotocin-induced diabetes in rats in angiotensin II-dependent. *Lab Invest* 2000;80:513–527.
- 31 Ren J, Davidoff AJ: Diabetes rapidly induces contractile dysfunctions in isolated ventricular myocytes. *Am J Physiol* 1997;272:H148–H158.
- 32 Ren J, Dominguez LJ, Sowers JR, Davidoff AJ: Troglitazone attenuates high-glucose-induced abnormalities in relaxation and intracellular calcium in rat ventricular myocytes. *Diabetes* 1996;45:1822–1825.
- 33 Ren J, Dominguez LJ, Sowers JR, Davidoff AJ: Metformin but not glyburide prevents high glucose-induced abnormalities in relaxation and intracellular Ca²⁺ transients in adult rat ventricular myocytes. *Diabetes* 1999;48:2059–2065.
- 34 Ren J, Sowers JR, Walsh MF, Brown RA: Reduced contractile response to insulin and IGF-I in ventricular myocytes from genetically obese Zucker rats. *Am J Physiol Heart Circ Physiol* 2000;279:H1708–H1714.
- 35 Verma S, McNeill JH: Metformin improves cardiac function in isolated streptozotocin-diabetic rat hearts. *Am J Physiol* 1994;266:H714–H719.
- 36 Muntzel MS, Hamidou I, Barrett S: Metformin attenuates salt-induced hypertension in spontaneously hypertensive rats. *Hypertension* 1999;33:1135–1140.
- 37 Petersen JS, DiBona GF: Acute sympathoinhibitory actions of metformin in spontaneously hypertensive rats. *Hypertension* 1996;27:619–625.
- 38 Whaley-Connell A, Sowers JR: Hypertension and insulin resistance. *Hypertension* 2009;54:462–464.
- 39 Katakam PV, Ujhelyi MR, Hoenig M, Miller AW: Metformin improves vascular function in insulin-resistant rats. *Hypertension* 2000;35:108–112.
- 40 Bhalla RC, Toth KF, Tan E, Bhatti RA, Mathias E, Sharma RV: Vascular effects of metformin. Possible mechanisms for its antihypertensive action in the spontaneously hypertensive rat. *Am J Hypertens* 1996;9:570–576.
- 41 Verma S, Yao L, Dumont AS, McNeill JH: Metformin treatment corrects vascular insulin resistance in hypertension. *J Hypertens* 2000;18:1445–1450.
- 42 Giugliano D, Quatraro A, Consoli G, Minei A, Ceriello A, De Rosa N, D'Onofrio F: Metformin for obese, insulin-treated diabetic patients: improvement in glycaemic control and reduction of metabolic risk factors. *Eur J Clin Pharmacol* 1993;44:107–112.
- 43 Chen XL, Panek K, Rembold CM: Metformin relaxes rat tail artery by repolarization and resultant decreases in Ca²⁺ influx and intracellular [Ca²⁺]. *J Hypertens* 1997;15:269–274.
- 44 Dominguez LJ, Davidoff AJ, Srinivas PR, Standley PR, Walsh MF, Sowers JR: Effects of metformin on tyrosine kinase activity, glucose transport, and intracellular calcium in rat vascular smooth muscle. *Endocrinology* 1996;137:113–121.
- 45 Trovati M, Anfossi G: Insulin, insulin resistance and platelet function: similarities with insulin effects on cultured vascular smooth muscle cells. *Diabetologia* 1998;41:609–602.
- 46 Peuler JD, Miller JA, Bourghli M, Zammam HY, Soltis EE, Sowers JR: Disparate effects of antidiabetic drugs on arterial contraction. *Metabolism* 1997;46:1199–1205.
- 47 Ofenstein JP, Dominguez LJ, Sowers JR, Sarnaik AP: Effects of insulin and metformin on glucose metabolism in rat vascular smooth muscle. *Metabolism* 1999;48:1357–1360.

- 48 Campbell JD, Paul RJ: The nature of fuel provision for the Na⁺, K⁽⁺⁾-ATPase in porcine vascular smooth muscle. *J Physiol* 1992;447:67–82.
- 49 Salpeter SR, Greyber E, Pasternak GA, Salpeter EE: Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2010;4:CD002967.
- 50 Salpeter SR, Greyber E, Pasternak GA, Salpeter EE: Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2010;1:CD002967.
- 51 Bando K, Ochiai S, Kunimatsu T, Deguchi J, Kimura J, Funabashi H, Seki T: Comparison of potential risks of lactic acidosis induction by biguanides in rats. *Regul Toxicol Pharmacol* 2010;58:155–160.
- 52 Tahrani AA, Varughese GI, Scarpello JH, Hanna FW: Metformin, heart failure, and lactic acidosis: is metformin absolutely contraindicated? *BMJ* 2007;335:508–512.
- 53 Brown JB, Pedula K, Barzilay J, Herson MK, Latare P: Lactic acidosis rates in type 2 diabetes. *Diabetes Care* 1998;21:1659–1663.
- 54 Stang M, Wysowski DK, Butler-Jones D: Incidence of lactic acidosis in metformin users. *Diabetes Care* 1999;22:925–927.
- 55 Salpeter S, Greyber E, Pasternak G, Salpeter E: Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2002;2:CD002967.
- 56 Lalau JD, Race JM: Lactic acidosis in metformin-treated patients. Prognostic value of arterial lactate levels and plasma metformin concentrations. *Drug Saf* 1999;20:377–384.
- 57 Mani MK: Metformin in renal failure – weigh the evidence. *Nephrol Dial Transplant* 2009;24:2287–2288.
- 58 Sambol NC, Chiang J, Lin ET, Goodman AM, Liu CY, Benet LZ, Cogan MG: Kidney function and age are both predictors of pharmacokinetics of metformin. *J Clin Pharmacol* 1995;35:1094–1102.
- 59 Lalau JD, Vermersch A, Hary L, Andrejak M, Isnard F, Quichaud J: Type 2 diabetes in the elderly: an assessment of metformin (metformin in the elderly). *Intl J Clin Pharmacol Ther Toxicol* 1990;28:329–332.
- 60 Lalau JD, Race JM: Lactic acidosis in metformin therapy: searching for a link with metformin in reports of ‘metformin-associated lactic acidosis’. *Diabetes Obes Metab* 2001;3:195–201.
- 61 National Institute for Health and Clinical Excellence: NICE Clinical Guidelines CG87 (May 2009): Type 2 diabetes – newer agents (partial update of CG66) (<http://www.nice.org.uk/CG87>).