

## Review Article

# The Cardiovascular Effects of Metformin: Conventional and New Insights

Battsetseg Batchuluun<sup>1</sup>, Noriyuki Sonoda<sup>1,2\*</sup>, Ryoichi Takayanagi<sup>1</sup> and Toyoshi Inoguchi<sup>1,2</sup>

<sup>1</sup>Department of Internal Medicine and Bioregulatory Science, Kyushu University, Japan

<sup>2</sup>Innovation Center for Medical Redox Navigation, Kyushu University, Japan

## INTRODUCTION

Metformin, a first-line agent for type 2 diabetes pharmacotherapy, is one of the most prescribed drugs worldwide. Its glucose lowering effect is mediated through suppressing the hepatic production of glucose, decreasing intestinal glucose absorption and improving glucose uptake and utilization [1]. Despite the emergence of new glucose lowering medications, metformin is still reported to be the agent most widely used to treat diabetes. This is because of its safety record over past decades and its various beneficial outcomes, as well as its glucose lowering action. One of the most favorable effects of metformin is cardiovascular protection in diabetes. This protective action is vital in diabetic patients because microvascular and macrovascular diseases are responsible for most diabetes-associated morbidities and mortalities [2]. Identifying the molecular targets of metformin is likely to enable the development of second-generation drugs with similar properties, including the prevention and management of diabetic vascular complications. In this review, we summarize conventional and new understandings of the effects of metformin on cardiovascular outcomes and its mechanisms of action involving cardiovascular protection.

## Metformin and cardiovascular outcomes

The history of metformin is linked to the use of *Galega officinalis* as an herbal medicine in medieval Europe. Metformin was first synthesized in 1929 and the first clinical trial of metformin as a treatment for type 2 diabetes was published in 1957 [3,4]. Since then, metformin has been thoroughly researched in diabetes, with large clinical trials investigating the outcomes of treatment with this agent. The UK Prospective Diabetes Study (UKPDS) showed that early intervention with metformin in patients with type 2 diabetes decreased the incidence of diabetes-related vascular endpoints by 32%, myocardial infarction by 39%, diabetes-related deaths by 42% and all-cause mortality by 36% [5]. In addition, the rates of diabetes-related endpoints and all-cause mortality were significantly lower in patients receiving metformin than in patients treated with sulfonylureas or insulin. Subsequent trials yielded similar findings, confirming that metformin has a protective effect against cardiovascular out-

Special Issue on

## Antidiabetic Drug-Metformin

\*Corresponding author

Noriyuki Sonoda, Department of Internal Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan, Tel: +81-92-642-5284; Fax: +81-92-642-5287; Email: noriyuki@intmed3.med.kyushu-u.ac.jp

Submitted: 03 April 2014

Accepted: 26 May 2014

Published: 28 May 2014

ISSN: 2333-6692

Copyright

© 2014 Sonoda et al.

OPEN ACCESS

comes in type 2 diabetes [6-10]. A trial in diabetic patients with a previous myocardial infarction showed that metformin treatment resulted in a lower mortality rate than sulfonylurea treatment [11,12]. In addition, metformin treatment of type 2 diabetic patients for 2-3 years reduced carotid intima-media thickness (CIMT) [13,14], and an observational study showed that the metformin-associated reduction in cardiovascular morbidity and mortality was independent from its glucose lowering properties [15]. The ability of metformin to provide similar cardiovascular benefits to non-diabetic individuals was also tested [16]. In that study, metformin did not significantly affect the progression of CIMT, carotid plaque score, and several other markers of cardiovascular disease, indicating that metformin has no effect on CIMT in non-diabetic patients with high cardiovascular risk. However, metformin was shown to reduce CIMT in non-diabetic women with polycystic ovary syndrome [17] and to increase lower arterial flow in non-diabetic patients with peripheral vascular disease [18]. Thus, although metformin treatment clearly benefits diabetic patients, its benefits in non-diabetic patients are unclear.

## Mechanism of the cardiovascular effects of metformin

Clinical evidence of the protective effects of metformin prompted evaluations of its mechanism of action and molecular targets. Metformin was reported to improve lipoprotein profiles in diabetic patients by decreasing plasma concentrations of free fatty acid, triglycerides, total cholesterol and LDL cholesterol and by increasing HDL cholesterol [1,19]. Moreover, metformin was shown to decrease hypercoagulation and to increase fibrinolysis in insulin resistant states, apparently by reducing plasminogen activator inhibitor-1 (PAI-1) [20]. In addition, metformin reduced platelet sensitivity to aggregation [21] and chronic low-grade inflammation in the atherogenic process [22]. In addition to its anti-atherogenic effects, metformin was reported to ameliorate cardiac functional abnormalities in rats with diabetic cardio-

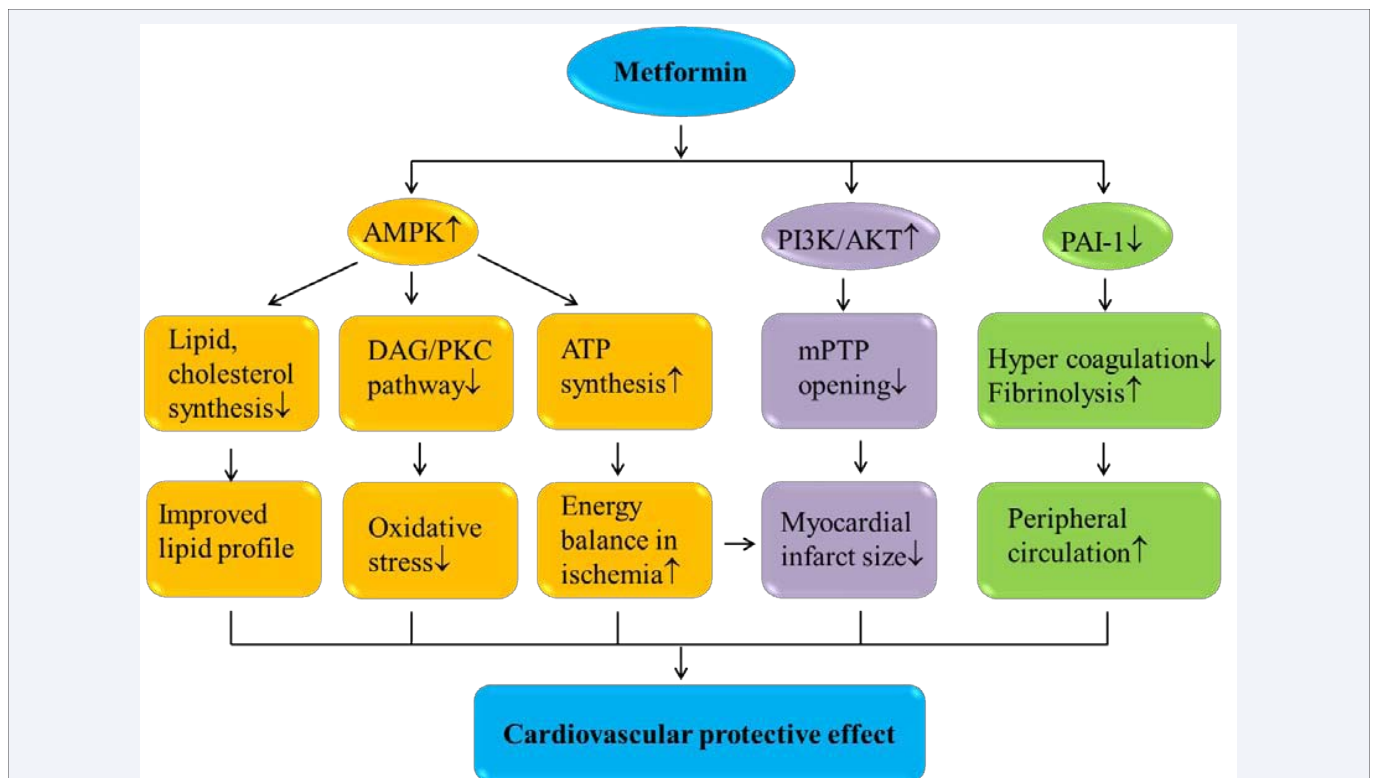
myopathy [23]. Because oxidative stress plays a pivotal role in vascular tissue damage, the cardiovascular protective effects of metformin may be because of its ability to prevent diabetes-induced oxidative stress, [24-27]. Hyperglycemia-induced protein kinase C (PKC) activation and subsequent activation of NAD (P) H oxidase is a pathway causing the overproduction of Reactive Oxygen Species (ROS) in diabetes [28-31]. PKC is associated with vascular alterations, such as increases in permeability, contractility, extracellular matrix synthesis, cell growth and apoptosis, angiogenesis, leukocyte adhesion, and cytokine activation and inhibition [30]. The PKC $\beta$ 2 isoform is preferentially elevated in diabetic heart and aorta [28], whereas a specific inhibitor of PKC $\beta$  improved vascular dysfunction in diabetic rats [32] and clinical parameters of diabetic nephropathy in patients with type 2 diabetes [33], suggesting the significance of PKC $\beta$  activation in diabetic complications. A recent trial showed that metformin reduced oxidative stress parameters in patients newly diagnosed with type 2 diabetes [34], consistent with results showing that metformin inhibited diabetes-induced oxidative stress in endothelial cells [35,36]. Our recent study revealed that metformin suppressed high glucose-induced oxidative stress in human aortic endothelial cells by inhibiting the PKC-NAD (P)H oxidase pathway [37]. The ability of metformin to protect against cardiovascular outcomes may be because, at least in part, of these properties.

### Molecular Targets of metformin

Identifying the molecular targets underlying the protective effects of metformin is important in the development of second

generation drugs that may improve the management of diabetic cardiovascular complications. AMP-activated kinase (AMPK) is a target for the anti-hyperglycemic action of metformin, as well as playing a critical role in metformin-mediated cardiovascular protection (Figure 1). AMPK is a major regulator of cellular energy and is activated by an increase in the cellular AMP: ATP ratio during energy stress. AMPK was shown to maintain the energy balance in cells during ischemia by increasing ATP levels [38]. Metformin increased AMPK activation in both ischemic and non-ischemic hearts but failed to protect cardiac-specific AMPK $\alpha$ 2 dominant-negative transgenic mice [39], suggesting that AMPK is essential for the protective effects of metformin. Similarly, rats treated with metformin showed a significant reduction in infarct size compared with untreated animals [40]. However, the metformin mediated reduction of infarct size also involves other potential target molecules, including the phosphatidylinositol-3-kinase (PI3K)-Akt pathway and adenosine concentration [41]. The mitochondrial permeability transition pore (mPTP) opens, leading to necrosis and apoptosis in reperfusion. It was reported that metformin reduces myocardial infarct size in both the non-diabetic and diabetic heart through preventing the opening of mPTP by activation of PI3K and Akt [42].

Metformin was also shown to increase fatty acid oxidation through AMPK activation, decreasing the amounts of intracellular free fatty acids available for the production of Diacylglycerol (DAG) and triacylglycerol. In diabetes, hyperglycemia increases the *de novo* synthesis of intracellular DAG, which consists of two fatty acid chains covalently bound to a glycerol molecule. *De novo*



**Figure 1** Molecular targets and mechanisms of metformin effect on cardiovascular protection. AMPK activation mediated by metformin treatment improves lipid profile, energy balance and decreases oxidative stress. Metformin is also shown to decrease myocardial infarct size through PI3K/AKT activation, and increase peripheral blood circulation by decreasing PAI-1. These properties of metformin may contribute to its positive impact on cardiovascular protection.

synthesized DAG acts upstream of the PKC pathway; therefore, increased DAG induces PKC activation, resulting in increased oxidative stress [28-31]. Our recent study showed that metformin treatment significantly increased AMPK phosphorylation and greatly reduced intracellular DAG subgroups, including 1-palmitoyl-2-oleoyl-glycerol, 1,2-dipalmitoyl-glycerol and 1,2-dioleoyl-glycerol. Moreover, metformin inhibited the PKC-NAD (P)H oxidase pathway, subsequently reducing high glucose-induced oxidative stress in aortic endothelial cells [37]. Thus, AMPK is likely to be crucial for the ability of metformin, not only to maintain energy homeostasis in ischemia but to prevent diabetes-induced oxidative stress.

### Metformin and incretin combinations

In recent years, Glucagon-Like Peptide-1 (GLP-1) has emerged as a new potential anti-diabetic treatment option. GLP-1, an incretin hormone secreted by distal intestinal L cells, is important for the regulation of glucose homeostasis. In addition to its multi-organ targeted anti-diabetic effects, such as improved insulin secretion, reduced food intake, decreased glucagon secretion and increased mass of pancreatic  $\beta$ -cells [43-47], GLP-1 has been found to have protective effects on cardiovascular function [46-48]. Metformin increases the endogenous secretion of GLP-1 and decreases the activity of DPP-4, an enzyme that degrades GLP-1, resulting in greater glucose lowering in type 2 diabetes [49-52]. We recently found that, in addition to this glucose-lowering effect, metformin, at a therapeutic concentration, enhances the ability of a GLP-1 analog to prevent oxidative stress caused by high glucose concentrations [37]. Hyperglycemia-induced PKC $\beta$ 2 activation was recently shown to reduce the expression of GLP-1 receptor protein in the glomerular endothelium, blunting the effect of GLP-1 [45]. Because metformin inhibits PKC $\beta$ 2 signaling [37], the combination of metformin and GLP-1 analog may be preferable clinically because metformin prevents GLP-1 receptor degradation by inhibiting PKC $\beta$ 2, improving the glucose lowering and cardiovascular protective effects of GLP-1 in patients with diabetes.

### CONCLUSION

Metformin is a well-established component of diabetic management. In addition to its glucose-lowering effect, metformin has been found to provide various benefits, enhancing cardiovascular protection in diabetic patients as shown in large clinical trials, whereas the action of metformin in non-diabetic patients may be specific to certain conditions or individuals. For example, metformin has protective effects against cardiovascular risk in patients with polycystic ovarian syndrome, with these effects being important in patients with advanced cardiovascular risk. The cardiovascular protective mechanisms of action of metformin may include improved lipid profiles, anti-atherogenic effects, decreased ischemic injury and amelioration of oxidative stress. Combining metformin and GLP-1 may be more effective in patients with type 2 diabetes than either alone.

### REFERENCES

- DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. *N Engl J Med.* 1995; 333: 541-549.
- Hanefeld M, Fischer S, Julius U, Schulze J, Schwanebeck U, Schmechel H, et al. Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up. *Diabetologia.* 1996; 39: 1577-1583.
- Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. *Diabetes Care.* 1989; 12: 553-564.
- Bailey CJ, Day C. Metformin: its botanical background. *Practical Diabetes Int.* 2004; 21: 115-117.
- 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.
- Selvin E, Bolen S, Yeh HC, Wiley C, Wilson LM, Marinopoulos SS, et al. Cardiovascular outcomes in trials of oral diabetes medications: a systematic review. *Arch Intern Med.* 2008; 168: 2070-2080.
- Saenz A, Fernandez-Esteban I, Mataix A, Ausejo M, Roque M, Moher D. Metformin monotherapy for type 2 diabetes mellitus. *Cochrane Database Syst Rev.* 2005; CD002966.
- Lamanna C, Monami M, Marchionni N, Mannucci E. Effect of metformin on cardiovascular events and mortality: a meta-analysis of randomized clinical trials. *Diabetes Obes Metab.* 2011; 13: 221-228.
- Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med.* 2008; 359: 1577-1589.
- Kooy A, de Jager J, Lehert P, Bets D, Wulffélé MG, Donker AJ, et al. Long-term effects of metformin on metabolism and microvascular and macrovascular disease in patients with type 2 diabetes mellitus. *Arch Intern Med.* 2009; 169: 616-625.
- Schramm TK, Gislason GH, Vaag A, Rasmussen JN, Folke F, Hansen ML, et al. Mortality and cardiovascular risk associated with different insulin secretagogues compared with metformin in type 2 diabetes, with or without a previous myocardial infarction: a nationwide study. *Eur Heart J.* 2011; 32: 1900-1908.
- Jorgensen CH, Gislason GH, Andersson C, Ahlehoff O, Charlot M, Schramm TK, et al. Effects of oral glucose-lowering drugs on longterm outcomes in patients with diabetes mellitus following myocardial infarction not treated with emergent percutaneous coronary intervention--a retrospective nationwide cohort study. *Cardiovasc Diabetol.* 2010; 9: 54.
- Katakami N, Yamasaki Y, Hayaishi-Okano R, Ohtoshi K, Kaneto H, Matsuhisa M, et al. Metformin or gliclazide, rather than glibenclamide, attenuate progression of carotid intima-media thickness in subjects with type 2 diabetes. *Diabetologia.* 2004; 47: 1906-1913.
- Matsumoto K, Sera Y, Abe Y, Tominaga T, Yeki Y, Miyake S. Metformin attenuates progression of carotid arterial wall thickness in patients with type 2 diabetes. *Diabetes Res Clin Pract.* 2004; 64: 225-228.
- Roussel R, Travert F, Pasquet B, Wilson PW, Smith SC Jr, Goto S, et al. Metformin use and mortality among patients with diabetes and atherosclerosis. *Arch Intern Med.* 2010; 170: 1892-1899.
- Preiss D, Lloyd SM, Ford I, McMurray JJ, Holman RR, Welsh P, et al. Metformin for non-diabetic patients with coronary heart disease (the CAMERA study): a randomised controlled trial. *Lancet Diabetes Endocrinol.* 2014; 2: 116-124.
- Orio F Jr, Palomba S, Cascella T, De Simone B, Manguso F, Savastano S, et al. Improvement in endothelial structure and function after metformin treatment in young normal-weight women with polycystic ovary syndrome: results of a 6-month study. *J Clin Endocrinol Metab.* 2005; 90: 6072-6076.



18. Sirtori CR, Franceschini G, Gianfranceschi G, Sirtori M, Montanari G, Bosisio E, et al. Metformin improves peripheral vascular flow in nonhyperlipidemic patients with arterial disease. *J Cardiovasc Pharmacol.* 1984; 6: 914-923.
19. Reaven GM, Johnston P, Hollenbeck CB, Skowronski R, Zhang JC, Goldfine ID, et al. Combined metformin-sulfonylurea treatment of patients with noninsulin-dependent diabetes in fair to poor glycemic control. *J Clin Endocrinol Metab.* 1992; 74: 1020-1026.
20. Chakrabarti R, Hocking ED, Fearnley GR. Fibrinolytic effect of metformin in coronary-artery disease. *Lancet.* 1965; 2: 256-259.
21. Wiernsperger NF. Metformin: intrinsic vasculoprotective properties. *Diabetes Technol Ther.* 2000; 2: 259-272.
22. Libby P. Inflammation in atherosclerosis. *Nature.* 2002; 420: 868-874.
23. Verma S, McNeill JH. Metformin improves cardiac function in isolated streptozotocin-diabetic rat hearts. *Am J Physiol.* 1994; 266: H714-719.
24. Inoguchi T, Sonta T, Tsubouchi H, Etoh T, Kakimoto M, Sonoda N, et al. Protein kinase C-dependent increase in reactive oxygen species (ROS) production in vascular tissues of diabetes: role of vascular NAD(P)H oxidase. *J Am Soc Nephrol.* 2003; 14: S227-232.
25. Inoguchi T, Nawata H. NAD(P)H oxidase activation: a potential target mechanism for diabetic vascular complications, progressive beta-cell dysfunction and metabolic syndrome. *Curr Drug Targets.* 2005; 6: 495-501.
26. Sasaki S, Inoguchi T. The role of oxidative stress in the pathogenesis of diabetic vascular complications. *Diabetes Metab J.* 2012; 36: 255-261.
27. Inoue T, Kobayashi K, Inoguchi T, Sonoda N, Maeda Y, Hirata E, et al. Downregulation of adipose triglyceride lipase in the heart aggravates diabetic cardiomyopathy in db/db mice. *Biochem Biophys Res Commun.* 2013; 438: 224-229.
28. Inoguchi T, Battan R, Handler E, Sportsman JR, Heath W, King GL. Preferential elevation of protein kinase C isoform beta II and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycemic control by islet cell transplantation. *Proc Natl Acad Sci USA.* 1992; 89: 11059e63.
29. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes.* 2000; 49: 1939-1945.
30. Geraldès P, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. *Circ Res.* 2010; 106: 1319-1331.
31. King GL, Kunisaki M, Nishio Y, Inoguchi T, Shiba T, Xia P. Biochemical and molecular mechanisms in the development of diabetic vascular complications. *Diabetes.* 1996; 45 Suppl 3: S105-108.
32. Ishii H, Jirousek MR, Koya D, Takagi C, Xia P, Clermont A, et al. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor. *Science.* 1996; 272: 728-731.
33. Tuttle KR, Bakris GL, Toto RD, McGill JB, Hu K, Anderson PW. The effect of ruboxistaurin on nephropathy in type 2 diabetes. *Diabetes Care.* 2005; 28: 2686-2690.
34. Gallo A, Ceolotto G, Pinton P, Iori E, Murphy E, Rutter GA, et al. Metformin prevents glucose-induced protein kinase C-beta2 activation in human umbilical vein endothelial cells through an antioxidant mechanism. *Diabetes.* 2005; 54: 1123-1131.
35. Ouslimani N, Peynet J, Bonnefont-Rousselot D, Théron P, Legrand A, Beaudoux JL. Metformin decreases intracellular production of reactive oxygen species in aortic endothelial cells. *Metabolism.* 2005; 54: 829-834.
36. Esteghamati A, Eskandari D, Mirmiranpour H, Noshad S, Mousavizadeh M, Hedayati M, et al. Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. *Clin Nutr.* 2013; 32: 179-185.
37. Batchuluun B, Inoguchi T, Sonoda N, Sasaki S, Inoue T, Fujimura Y, et al. Metformin and liraglutide ameliorate high glucose-induced oxidative stress via inhibition of PKC-NAD(P)H oxidase pathway in human aortic endothelial cells. *Atherosclerosis.* 2014; 232: 156-164.
38. Boyle JG, Salt IP, McKay GA. Metformin action on AMP-activated protein kinase: a translational research approach to understanding a potential new therapeutic target. *Diabet Med.* 2010; 27: 1097-1106.
39. Calvert JW, Gundewar S, Jha S, Greer JJ, Bestermann WH, Tian R, et al. Acute metformin therapy confers cardioprotection against myocardial infarction via AMPK-eNOS-mediated signaling. *Diabetes.* 2008; 57: 696-705.
40. Paiva MA, Gonçalves LM, Providência LA, Davidson SM, Yellon DM, Mocanu MM. Transitory activation of AMPK at reperfusion protects the ischaemic-reperfused rat myocardium against infarction. *Cardiovasc Drugs Ther.* 2010; 24: 25-32.
41. El Messaoudi S, Rongen GA, Riksen NP. Metformin therapy in diabetes: the role of cardioprotection. *Curr Atheroscler Rep.* 2013; 15: 314.
42. Bhamra GS, Hausenloy DJ, Davidson SM, Carr RD, Paiva M, Wynne AM, et al. Metformin protects the ischemic heart by the Akt-mediated inhibition of mitochondrial permeability transition pore opening. *Basic Res Cardiol.* 2008; 103: 274-284.
43. Maida A, Lamont BJ, Cao X, Drucker DJ. Metformin regulates the incretin receptor axis via a pathway dependent on peroxisome proliferator-activated receptor- $\alpha$  in mice. *Diabetologia.* 2011; 54: 339-349.
44. Komatsu R, Matsuyama T, Namba M, Watanabe N, Itoh H, Kono N, et al. Glucagonostatic and insulinotropic action of glucagonlike peptide 1-(7-36)-amide. *Diabetes.* 1989; 38: 902-905.
45. Mima A, Hiraoka-Yamamoto J, Li Q, Kitada M, Li C, Geraldès P, et al. Protective effects of GLP-1 on glomerular endothelium and its inhibition by PKC $\beta$  activation in diabetes. *Diabetes.* 2012; 61: 2967-2979.
46. Nagashima M, Watanabe T, Terasaki M, Tomoyasu M, Nohtomi K, Kim-Kaneyama J, Miyazaki A. Native incretins prevent the development of atherosclerotic lesions in apolipoprotein E knockout mice. *Diabetologia.* 2011; 54: 2649-2659.
47. Arakawa M, Mita T, Azuma K, Ebato C, Goto H, Nomiyama T, et al. Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4. *Diabetes.* 2010; 59: 1030-1037.
48. Koska J, Schwartz EA, Mullin MP, Schwenke DC, Reaven PD. Improvement of postprandial endothelial function after a single dose of exenatide in individuals with impaired glucose tolerance and recent-onset type 2 diabetes. *Diabetes Care.* 2010; 33: 1028-1030.
49. Cuthbertson J, Patterson S, O'Harte FP, Bell PM. Addition of metformin to exogenous glucagon-like peptide-1 results in increased serum glucagon-like peptide-1 concentrations and greater glucose lowering in type 2 diabetes mellitus. *Metabolism.* 2011; 60: 52-56.
50. Yasuda N, Inoue T, Nagakura T, Yamazaki K, Kira K, Saeki T, et al. Enhanced secretion of glucagon-like peptide 1 by biguanide compounds. *Biochem Biophys Res Commun.* 2002; 298: 779-784.
51. Mulherin AJ, Oh AH, Kim H, Grieco A, Lauffer LM, Brubaker PL. Mechanisms underlying metformin-induced secretion of glucagon-like peptide-1 from the intestinal L cell. *Endocrinology.* 2011; 152: 4610-4619.

52. Pratley RE, Nauck M, Bailey T, Montanya E, Cuddihy R, Filetti S, et al. Liraglutide versus sitagliptin for patients with type 2 diabetes who did not have adequate glycaemic control with metformin: a 26-week, randomised, parallel-group, open-label trial. *Lancet*. 2010; 375: 1447-1456.

**Cite this article**

Batchuluun B, Sonoda N, Takayanagi R, Inoguchi T (2014) The Cardiovascular Effects of Metformin: Conventional and New Insights. *J Endocrinol Diabetes Obes* 2(2): 1035.