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Metformin, lipids and atherosclerosis prevention.

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Table. 1

Purpose of Review

We provide an overview of recent publications that extend clinically relevant knowledge relating to metformin's effects on lipids and atherosclerotic vascular disease and/or provide insights into the drug's mechanisms of action on the heart and vasculature.

Recent findings

We focus on original research in humans or in human tissues. Several recently completed randomised clinical trials have reported effects of metformin on surrogate measures of atherosclerotic vascular disease, including carotid intima media thickness, vascular reactivity and calcification in people with Type 1 (T1D) and Type 2 (T2D) diabetes as well as non-diabetic dysglycemia. In addition, observational studies have provided novel insights into the mechanisms of metformin's effects on carotid plaque, monocytes/macrophages, vascular smooth muscle and endothelial cells, including via 5'-adenosine monophosphate-activated protein kinase (AMPK) activation.

Summary

Recent trials based on surrogate outcome measures have provided further data suggesting protective effects of metformin against vascular disease in youth and adults with Type 1 diabetes, as well as in adults with pre-diabetes and Type 2 diabetes. In parallel, human tissue and cell studies have provided new insights into pleiotropic effects of metformin and suggest novel drug targets. As metformin is an inexpensive agent with an established safety profile, larger scale clinical trials based on hard clinical outcomes [cardiovascular disease (CVD) events] are now indicated.

Keywords

Metformin, Lipoproteins, Atherosclerosis, Vascular Function, Diabetes Mellitus

Key points:

Metformin has been in clinical use for glucose control in T2D for over 60 years and has proven effects in reducing progression from pre-diabetes to T2D and in reducing rates of cardiovascular disease (CVD) events and death in T2D. These actions occur in part via glucose lowering, and in part via other "pleiotropic" effects.

Other than the recent REMOVAL trial, very few clinical trials have studied the effect of metformin on vascular complications in T1D.

Understanding of the mechanisms of metformin's apparently protective effects on vascular cells is gradually accumulating, including effects related to AMPK, scavenger receptors, hTERT, DNA methylation and mitochondrial biogenesis.

Surrogate end-point trials in high CVD risk adults (carotid intima media thickness) and in young people (vascular reactivity) with T1D support beneficial actions of metformin on the vasculature. These results highlight the need for CVD outcome trials.

In clinical studies of pre-diabetes and T2D, metformin has favourable effects including reduction of vascular calcification and novel vascular risk factors.

Introduction

The biguanide metformin has been used for over 60 years as the first-line oral agent for glucose lowering in Type 2 diabetes (T2D) [1]. Metformin also retards progression from pre-diabetes to T2D [2] and, as shown by the UK Prospective Diabetes Study (UKPDS), reduces cardiovascular disease (CVD) events and mortality in T2D [3,4]. Metformin lowers glucose by reducing glucose absorption from the gut and inhibiting hepatic glucose output [1]. In pre-diabetes and T2D metformin improves beta cell function and improves clustered metabolic risk factors [5]. Unlike most glucose control agents metformin induces visceral fat loss and reduces waist circumference [6]. Metformin is quite frequently used off-label as an insulin-sparing glucose control agent in overweight/obese people with Type 1 diabetes (T1D), though this is based on relatively small short duration studies [7].

Metformin's beneficial effects have been postulated to extend beyond glycemia, to effects on multiple other pathways mediating complications, including lipoproteins, inflammation, thrombosis and oxidative stress ("pleiotropic" effects) [1,8]. Metformin is low cost with an excellent safety profile, other than appreciable rates of gastrointestinal upset [1], which can be reduced with meal-time dosing and extended release preparations [8]. Metformin reduces Vitamin B12 absorption, which may increase homocysteine levels but is currently thought to have only marginal clinical relevance in terms of neuropathy risk [9]. Lactic acidosis is a rare side-effect, the risk of which is increased with renal impairment [1], hence recommendations to avoid use in late-stage CKD [10, 11]. Pleiotropic effects and mechanisms of action of metformin are still being identified: recent studies are reviewed herein.

Metformin is therefore a candidate for more widespread clinical use, particularly in an aging and obese population. Recent publications report surrogate measures of vascular disease in

randomized controlled trials (RCTs) of metformin in T1D, pre-diabetes and T2D. Other papers report effects on novel vascular risk factors and provide insights into metformin's cellular actions.

Metformin's cardiometabolic effects in Type 1 diabetes

Interest in metformin as an insulin adjunct in T1D has arisen because of the benefits and challenges of optimizing glycemia [12] and an increased rate of adiposity, which is associated with increased vascular complications [13,14]. In the 2000s a series of small short-term trials of metformin reported modest benefits for weight, insulin dose and LDL-cholesterol (LDL-C); meta-analyses confirmed a significant reduction in insulin dose requirement (6.6 IU/day, $p < 0.001$) with metformin, and weight reduction in some trials [7,15]. Although there was no consistent evidence for HbA1c reduction, the UK National Institute for Health and Care Excellence (NICE) and the American Diabetes Association (ADA) recommended metformin for overweight/obese T1D patients for improving glycemia while limiting insulin dose [15,16]. Subsequently in 140 overweight/obese adolescents with high HbA1c and insulin doses, 26 weeks of metformin treatment induced only a small (transient) reduction in HbA1c, and small reductions in BMI and insulin dose, with no change in lipids. No vascular measurements were made in this trial. [17]

The REversing with MetfOrmin Vascular Adverse Lesions (REMOVAL) Trial addressed cardiometabolic health in T1D adults at high CVD risk [18-21]. REMOVAL is the largest and longest trial of metformin in T1D and the first to evaluate a CVD end-point, albeit a surrogate measure of carotid intima media thickness (cIMT). REMOVAL also included a vascular reactivity substudy. In this multicentre international RCT 428 adults aged ≥ 40 years, with ≥ 5 -years T1D and ≥ 3 of 10 CVD risk factors were randomized to placebo or metformin (1000 mg b.d. or lower if not tolerated) with insulin titrated towards HbA1c 7%

(53 mmol/mol) [18,19]. Participants were followed for a mean of three years with annual assessments of cIMT, vascular reactivity, renal and retinal status, CVD risk factors and side-effects [18,19]. cIMT was chosen as a surrogate CVD measure for several reasons: the need for a shorter smaller clinical trial than that required by clinical vascular end-point studies; because cIMT predicts CVD in the general population [22]; and because in the (T1D) Diabetes Control and Complications Trial (DCCT) intensive management reduced cIMT [23], which over 30 years follow-up was associated with better CVD outcomes [12]. cIMT is increased even in children with T1D [24].

REMOVAL participants had a mean age of 55 years, with 33 years T1D, blood pressure 130/72 mmHg, LDL-C 2.1 mmol/l. BMI indicated overweight/obesity in 71% whilst 82% were on statins, 73% were on anti-hypertension agents, and 39% were on antiplatelet agents [19].

Relative to placebo the primary end-point of mean far wall cIMT (as per the Mannheim consensus, which excludes IMT measures >1.5 mm and plaque) was not significantly reduced. However, maximal cIMT (tertiary end-point), which includes plaque, was reduced by metformin (-0.01mm, $p=0.0093$) [19]. Vascular reactivity and (retinal photo-based) retinopathy progression did not differ between treatment arms. Renal function, assessed by eGFR increased (mean 4 ml/min/1.73m²) soon after metformin commencement then declined in parallel with the placebo group, resulting in apparently better renal function with metformin [19]. Further renal measures are being assessed, but interestingly a similar trend (of borderline statistical significance) was recently observed in a one year pilot feasibility study for a planned CV outcome trial with metformin in prediabetes (the Glucose Lowering In Non-diabetic hyperglycaemia Trial (GLINT) [25]. Metformin was associated with a transient 0.24% (2.6 mmol/mol) HbA1c reduction, with no difference in severe hypoglycemia or DKA rates [19]. Metformin allocation was associated with sustained small weight loss

(1.17 kg), LDL-C reduction (0.13 mmol/l) and a 2 U/day reduction in insulin dose. Relative to placebo, about double the number of participants allocated metformin discontinued treatment; mean metformin dosage was 1.4 g/day, with high rates of gastrointestinal upset, and a higher rate (12% vs. 5%) of Vitamin B12 deficiency. There were only three CVD (of seven) deaths, two with metformin and one with placebo [19]. Overall, the results were in keeping with non-glucose mediated atheroprotective effects of metformin in T1D and supported the need for CVD event end-point studies.

Metformin improves vascular function in youth with Type 1 diabetes.

Vascular reactivity, such as ultrasound measured brachial artery dilatation (flow mediated dilatation (FMD) and/or by GTN), is associated with and predictive of CVD [26] and coronary artery calcification (CAC) [27], and is abnormal in T2D [28] and T1D [29]. Changes in FMD- and GTN-induced reactivity can be discordant, as FMD is dependent on NO released from endothelium while GTN, a NO donor, directly affects vascular smooth muscle and is therefore endothelium independent [30].

In a single paediatric centre experienced in FMD- and GTN-mediated vascular reactivity (CVs \leq 4%) a one year double-blind placebo-controlled trial of metformin (with weight appropriate doses up to 1000 mg b.d.) was conducted in 90 youth aged 8-18 years, \geq 6-months T1D and above average weight (BMI $>$ 50th percentile) [31]. Youth on or with a contraindication to metformin, or on statins, blood pressure drugs or multivitamins, or with recent severe hypoglycemia, DKA, or serious comorbidities were excluded. Vascular reactivity was measured at baseline, 3-, 6- and 12-months. Whilst the primary end-point, FMD, was unchanged, metformin improved GTN-induced reactivity (3.3%, p=0.03) independent of improved HbA1c. As expected, given the short duration, there were no changes in carotid or aortic IMT. Metformin reduced HbA1c (1%, p=0.001), with greater

benefit at 3-months, when adherence was greater, and lowered insulin doses, and leptin over 12-months. BMI, body composition, blood pressure, renal function, lipids, homocysteine, CRP, and adiponectin did not change significantly [31]. Ten subjects discontinued treatment (six on metformin) and mean tablet adherence was 75%, similar in both study arms. Lactate levels did not change and there were no episodes of lactic acidosis, severe hypoglycemia or DKA. Metformin slightly reduced Vitamin B12, though levels were still within normal range, and homocysteine did not rise [31].

FMD results were negative in this paediatric cohort [31], as was the Reactive Hyperemia Index in the REMOVAL Study [19], but GTN-induced hyperemia was improved by metformin in T1D youth [31]. Important study differences include the methodology of assessment (GTN was not used in REMOVAL), and subject demographics, including age, T1D duration, complications and medications. The positive (albeit non-primary) end-points in both studies provide clinicians with additional evidence of a potential vascular benefit of adjunct metformin therapy in T1D. These data are corroborated by recent studies in the STZ-diabetic mouse model in which metformin improved endothelial function and increased bone marrow derived endothelial progenitor cells (EPCs) [32], with clinical confirmation of the latter in the T1D MERIT study [33].

Surrogate endpoint data from recent trials therefore support the need for clinical CVD endpoint trials with metformin in T1D. The likelihood of such trials being funded either by research charities, governmental agencies or pharmaceutical companies currently seems low given that thousands of patients would need to be randomised over several years of follow-up and metformin has long been available in generic form.

Metformin vascular effects in pre-diabetes and Type 2 diabetes

As mentioned above, a beneficial effect of metformin on CVD outcomes in people with T2D is often considered quite established, on the basis of the UKPDS [3,4] in 1998 and also (in insulin-treated patients) the HOME study (n=390) in 2009 in which the hazard ratio for reduction of CVD events over 4.3 years of follow-up was 0.61 (95% CI, 0.40-0.94, p=0.02) [34]. However, when all available data are subjected to meta-analysis some uncertainty remains [35]. One recent small but interesting clinical trial (based on surrogate measures) aimed to examine some of the relevant mechanisms and gain insights into "first-line" use of metformin as opposed to other agents in T2D.

The Sapporo Athero-Incretin Study 3 in T2D patients on 500-750 mg/day metformin evaluated if doubling the dose of metformin or adding a DPP-4 inhibitor (vildagliptin), which has glucose lowering, anti-inflammatory and anti-atherosclerotic actions, improved brachial artery FMD (primary end-point) and novel vascular risk factors [36]. The study randomised 97 patients with T2D (mean (SD) age 58.7(11.0) years, HbA1c 7.5 (0.3) %) to vildagliptin or higher dose metformin for 12-weeks. There was no significant FMD change in either group. Metformin/vildagliptin lowered HbA1c more (-0.80 (0.38)% vs. -0.40 (0.47)%; p<0.01) than high dose metformin. Reductions in ApoB/A1 were significant, but similar with both treatments [36]. Thus, whilst combination therapy was effective in terms of glycaemia, no differences in effects on vascular or lipid parameters could be discerned between the two strategies.

In people without diabetes, the effect of metformin on CVD is much less certain. For example, in the double-blind, placebo-controlled Carotid Atherosclerosis MEtformin for Insulin ResistAnce Study (CAMERA) trial in people with established CVD but without diabetes, metformin treatment for 18 months had no effect on progression of mean cIMT (the

primary endpoint) or on total cholesterol, HDL-cholesterol, non-HDL-cholesterol, or triglycerides [37]. However, data suggesting an effect of metformin on vascular calcification in dysglycemic subjects have been reported [38, 39].

The Diabetes Prevention Program (DPP) is the longest (1996-2001) and largest (n=3234) trial of lifestyle or metformin for T2D prevention in adults with pre-diabetes and is in follow-up (*DPP/Diabetes Prevention Program Outcomes Study (DPPOS)*), with an emphasis on cardiometabolic outcomes, cancer and long-term safety. An excellent review of the DPP/DPPOS was published in 2017 [40]. In brief, the DPP demonstrated that relative to placebo, over a mean 3.2 years follow-up, metformin 850 mg b.d. reduced progression to T2D by 31%, with greater benefit in the obese and in women with prior gestational diabetes. Intensive lifestyle reduced progression by 58%. Ten and 15-year follow-up (DPPOS) demonstrated 18% T2D reduction with metformin. HbA1c, fasting glucose, hepatic glucose output, beta cell function and insulin/proinsulin improved significantly. Most benefit (64%) was explained by weight reduction and decreased central adiposity (DPP) [38,40]. Metformin did not improve lipid (triglycerides, LDL-C or HDL-C) levels or blood pressure, but improved CRP and tissue plasminogen activator (tPA) levels [40]. To date no reductions in microvascular complications have been reported and CVD events are still being monitored [40].

In a DPP/DPPOS substudy (n=2029) a mean 14-years post-randomization, CAC was quantified in men [mean age 67 years] and women (mean age 63 years) [38]. T2D had developed in 54%, 51% and 59% of the metformin, lifestyle and placebo groups respectively. Relative to placebo, metformin (for 9.6 (9.4) years) was associated with lower presence of CAC (i.e. CAC score above zero) (75 vs. 84%, p=0.02) and severity (39.5 vs. 66.9 Agatston units, p=0.04) in men only. This metformin benefit was independent of age, BMI, progression to diabetes or statin use [38]. Long-term safety and tolerability was good, with

(predominantly gastrointestinal) side-effects in 9.8% of metformin-allocated patients (vs. 1.1% placebo) and lower Vitamin B12, but no lactic acidosis with >15,000 patient years of metformin [40]. Rates of clinical CVD events from the ongoing follow-up study are awaited.

Calcium scores in peripheral vascular disease. In a cross-sectional study of 198 people with T2D those on metformin (81%) had a 41% lower prevalence of below knee vascular calcification and lower serum IL-6 levels than non-metformin users (independent of age, diabetes duration, gender, smoking, lipids, HbA1c, eGFR, prior CVD, retinopathy, neuropathy, insulin and IL-6 levels) [39]. This observation is supported by lower rates of lower limb amputation rates in T2D patients treated with metformin vs. sulfonylurea or insulin monotherapy [41].

These two studies suggest that metformin may protect against vascular calcification in dysglycemia. Potential mechanisms include anti-inflammatory, antioxidant and specific anti-calcification effects which may relate to AMPK activation [1,8,42,43].

We believe that there is sufficient evidence to support the commissioning of a cardiovascular endpoint trial of metformin in people without diabetes and have recently contributed to a feasibility trial (GLINT) [25]. If commissioned, this will provide more definitive evidence of metformin's effects on CVD and the results will have implications for people with and without diabetes (whether T1D or T2D).

Metformin effects on novel vascular risk factors

Glucagon-like peptide (GLP)-1. Mechanism(s) of metformin delaying glucose uptake from the gut have not been fully elucidated. GLP-1 receptor agonist trial data in T2D support CVD safety and in some cases efficacy against CVD, with further evidence awaited [44]. In a substudy (n=173) of the above-mentioned CAMERA trial of patients without diabetes

metformin sustainably (at least 18 months) increased ($\approx 23\%$) total GLP-1 levels, independent of changes in glycemia or weight,] but did not reduce cIMT [45]. This is the largest and longest metformin study evaluating GLP-1. In a DIRECT consortium (n=775 T2D subjects) study metformin use was associated with 14.1% higher total GLP-1 and 39.1% higher fasting active GLP-1, independent of HbA1c, weight or gender, but post-prandial levels did not differ from other therapies [46]. These exploratory studies support a role for the incretin axis as a component of the mechanism of metformin's cardiometabolic effects.

Neutrophil Gelatinase Associated Lipocalin (NGAL). In an observational study, circulating neutrophil-derived acute phase protein was measured in 67 T2D and 69 non-diabetic subjects and also in their carotid endarterectomy samples (mRNA expression) [47]. Relative to non-diabetic control samples, circulating NGAL levels were $>50\%$ higher in T2D, and significantly higher in those with carotid artery stenosis (CAS), whether symptomatic or asymptomatic. NGAL mRNA was present in 95% of T2D endarterectomy tissues vs. 5% from non-diabetic subjects ($p < 0.0001$). Those (n=17) who were prescribed metformin had lower NGAL (60.7 vs. 121.7 ng/ml, $p < 0.0001$) and carotid tissues from these individuals had less leukocyte infiltration and more complex and vulnerable plaques [47].

Metformin effects in vascular cells

Uptake of lipids, including Oxidized LDL (Ox-LDL) by macrophages and their subsequent apoptosis are key steps in atherosclerosis; there is evidence that metformin can inhibit both of these. In human THP-1 cells, a monocyte-like cell line, it was demonstrated that metformin can attenuate: (i) Ox-LDL uptake by reducing scavenger receptor (SRA and CD36) expression by suppression of β -catenin, activating protein (AP)-1 and PPAR- γ ; (ii) Ox-LDL induced endoplasmic reticulum stress and reactive oxygen species generation and (iii) Ox-LDL induced mitochondrial membrane depolarisation and cyto-C release [48]. In the same

cell line, an independent group reported that metformin-induced activation of AMPK, inhibits Sterol Regulatory Element-Binding Protein 2 (SREBP2)-mediated cholesterol uptake [49].

In experiments using THP-1 cells and macrophages from T2D patients cultured in high glucose, metformin activated AMPK by reducing (proinflammatory) cyclophilin A expression, and suppressed scavenger receptors and lipid uptake, foam cell formation, reactive oxygen species toxicity, and inflammatory cytokine release [50].

Two studies evaluated the role of AMPK activation by metformin in other vascular cells. In cultured human aortic smooth muscle cells metformin activated AMPK, upregulating p53 and IF116, inhibiting cell proliferation and migration [51]. In cultured (early passage) human aortic endothelial cells metformin activated AMPK α and induced telomere extending hTERT, delaying cell senescence [52]. Chronic metformin reduced mitochondrial biogenesis, which was dependent on H3K79 methylation in the SIRT3 promoter. In complementary experiments in ApoE^{-/-} mice, metformin decreased vascular aging and plaque formation [52].

Results of human studies with metformin are summarized (Table 1).

Conclusions

Recent studies reveal new knowledge of an old drug's cardiometabolic effects, supporting the case for cardiovascular outcome trials in individuals with T1D and non-diabetic dysglycemia. At the same time, molecular and cellular studies are providing insights into the mechanisms of metformin's actions on the vasculature, suggesting novel biomarkers that require validation in independent cohorts. These studies may guide development and targeting of novel agents for the treatment and prevention of cardiovascular and metabolic disease.

Acknowledgements and Conflicts of Interest:

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References

* Of special interest

** Of outstanding interest

1. Anabtawi A, Miles JM. Metformin: Nonglycemic Effects and Potential Novel Indications. *Endocr Pract.* 2016;22(8):999-1007.
2. Knowler WC, Barrett-Connor E, Fowler SE et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.* 2002;346(6):393-403.
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(9131):854-865.
4. Holman RR, Paul SK, Bethel MA et al. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med.* 2008;359(15):1577-1589.
5. Orchard TJ, Temprosa M, Goldberg R et al. The effect of metformin and intensive lifestyle intervention on the metabolic syndrome: the Diabetes Prevention Program randomized trial. *Ann Intern Med.* 2005;142(8):611-619.
6. Yanovski JA, Krakoff J, Salaita CG et al. Effects of metformin on body weight and body composition in obese insulin-resistant children: a randomized clinical trial. *Diabetes.* 2011 ;60(2):477-485.
7. Vella S, Buetow L, Royle P et al. The use of metformin in type 1 diabetes: a systematic review of efficacy. *Diabetologia.* 2010;53(5):809-820.
8. Fujita Y, Inagaki N. Metformin: New Preparations and Nonglycemic Benefits. *Curr Diab Rep.* 2017;17(1):5.

9. Crandall JP. Metformin and vitamin B12-What's missing from this picture? J Diabetes Complications. 2018;32(2):129.
10. Imam TH. Changes in metformin use in chronic kidney disease. Clinical Kidney Journal. 2017;10(3):301-304.
11. US Food and Drug Administration, Silver Spring, MD. FDA Drug Safety Communication: FDA Revises Warnings Regarding Use of the Diabetes Medicine Metformin in Certain Patients with Reduced Kidney Function 2016 <http://www.fda.gov/downloads/Drugs/DrugSafety/UCM494140.pdf>
12. Nathan DM; DCCT/EDIC Research Group. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. Diabetes Care. 2014;37(1):9-16.
13. de Boer IH, Sibley SD, Kestenbaum B et al. Central obesity, incident microalbuminuria, and change in creatinine clearance in the epidemiology of diabetes interventions and complications study. J Am Soc Nephrol. 2007;18(1):235-243.
14. Purnell JQ, Braffett BH, Zinman B et al. Impact of Excessive Weight Gain on Cardiovascular Outcomes in Type 1 Diabetes: Results From the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study. Diabetes Care. 2017;40(12):1756-1762 .
15. NICE (2016) Type 1 diabetes in adults: diagnosis and management of type 1 diabetes. www.nice.org.uk/guidance (accessed 20th May 2018)
16. American Diabetes Association. Standards of Medical Care in Diabetes. Diabetes Care 2017 Suppl 1 (S1-135).

17. Libman IM, Miller KM, DiMeglio LA et al. Effect of Metformin Added to Insulin on Glycemic Control Among Overweight/Obese Adolescents With Type 1 Diabetes: A Randomized Clinical Trial. JAMA. 2015;314(21):2241-2250.

18. Petrie JR, Chaturvedi N, Ford I et al. Metformin in adults with type 1 diabetes: Design and methods of Reducing with MetfOrmin Vascular Adverse Lesions (REMOVAL): An international multicentre trial. Diabetes Obes Metab. 2017;19(4):509-516.

** 19. Petrie JR, Chaturvedi N, Ford I et al. Cardiovascular and metabolic effects of metformin in patients with type 1 diabetes (REMOVAL): a double-blind, randomised, placebo-controlled trial. Lancet Diabetes Endocrinol. 2017;5(8):597-609.

Study results of the REMOVAL Trial – the largest and longest trial of metformin in Type 1 diabetes and the first to address vascular end-points in Type 1 diabetes. Results demonstrated metformin benefit on maximal cIMT and small, but significant effects on vascular risk factors. Further non-primary end-point study results are emerging.

* 20. Standl E. Metformin in type 1 diabetes. Lancet Diabetes Endocrinol. 2017;5(8):567-569. *Commentary related to metformin use in Type 1 diabetes related to REMOVAL Study results by an independent author.*

* 21. Livingstone R, Boyle JG, Petrie JR; REMOVAL Study Team. A new perspective on metformin therapy in type 1 diabetes. Diabetologia. 2017;60(9):1594-1600.

Excellent summary and commentary related to metformin use in Type 1 diabetes related to REMOVAL Study results by the REMOVAL Study investigators.

22. Lorenz MW, Polak JF, Kavousi M et al. Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data. Lancet. 2012;379(9831):2053-2062.

23. Nathan DM, Lachin J, Cleary P et al. Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N Engl J Med*. 2003;348(23):2294-2303.
24. Heilman K, Zilmer M, Zilmer K et al. Arterial stiffness, carotid artery intima-media thickness and plasma myeloperoxidase level in children with type 1 diabetes. *Diabetes Res Clin Pract*. 2009;84(2):168-173.
25. Griffin SJ, Bethel MA, Holman RR et al. Metformin in non-diabetic hyperglycaemia: the GLINT feasibility RCT. *Health Technol Assess*. 2018;22(18):1-64.
26. Matsuzawa Y, Kwon TG, Lennon RJ et al. Prognostic Value of Flow-Mediated Vasodilation in Brachial Artery and Fingertip Artery for Cardiovascular Events: A Systematic Review and Meta-Analysis. *J Am Heart Assoc*. 2015;4(11).
27. Kullo IJ, Malik AR, Bielak LF et al. Brachial artery diameter and vasodilator response to nitroglycerine, but not flow-mediated dilatation, are associated with the presence and quantity of coronary artery calcium in asymptomatic adults. *Clin Sci (Lond)*. 2007;112(3):175-182.
28. Williams SB, Cusco JA, Roddy MA, et al. Impaired nitric oxide mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* . 1996;27:567–574.
29. Clarkson P, Celermajer DS, Donald AE et al. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. *J Am Coll Cardiol*. 1996;28(3):573-579.
30. Creager MA, Lüscher TF, Cosentino F et al. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. *Circulation*. 2003;108(12):1527-1532.
- **31. Anderson JJA, Couper JJ, Giles LC et al. Effect of Metformin on Vascular Function in Children With Type 1 Diabetes: A 12-Month Randomized Controlled Trial. *J Clin Endocrinol Metab*. 2017;102(12):4448-4456.

Well conducted trial of metformin on vascular reactivity in youth with Type 1 diabetes showing metformin benefit on GTN-induced vascular reactivity, but not FMD and on some vascular risk factors. Results complement and extend REMOVAL trial results, and (as does REMOVAL), uses a surrogate vascular end-point.

32. Yu JW, Deng YP, Han X et al. Metformin improves the angiogenic functions of endothelial progenitor cells via activating AMPK/eNOS pathway in diabetic mice. *Cardiovasc Diabetol.* 2016;15:88.

33. Ahmed FW, Rider R, Glanville M et al. Metformin improves circulating endothelial cells and endothelial progenitor cells in type 1 diabetes: MERIT study. *Cardiovasc Diabetol.* 2016;15(1):116.

34. Kooy A, de Jager J, Lehert P 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.

*35. Griffin SJ, Leaver JK, Irving GJ. Impact of metformin on cardiovascular disease: a meta-analysis of randomised trials among people with type 2 diabetes. *Diabetologia.* 2017;60(9):1620-1629.

A meta-analysis of RCTs of metformin in over 2000 people with Type 2 diabetes reveals trends, but no statistical significant reductions in CVD events.

36. Kitao N, Miyoshi H, Furumoto T et al. The effects of vildagliptin compared with metformin on vascular endothelial function and metabolic parameters: a randomized, controlled trial (Sapporo Athero-Incretin Study 3). *Cardiovasc Diabetol.* 2017;16(1):125.

37. Preiss D, Lloyd SM, Ford I et al. Metformin for non-diabetic patients with coronary heart disease (the CAMERA study): a randomised controlled trial. *Lancet Diabetes Endocrinol.* 2014;2(2):116-124.

**38. Goldberg RB, Aroda VR, Bluemke DA et al. Effect of Long-Term Metformin and Lifestyle in the Diabetes Prevention Program and Its Outcome Study on Coronary Artery Calcium. *Circulation*. 2017;136(1):52-64.

This prospective DPP/DPPOS based trial and follow-up reports metformin (but not intensive lifestyle) benefit on coronary artery calcification, and supports prior CVD end-point benefit in pre-diabetes and Type 2 diabetes. These clinical findings support pleiotropic effects of metformin, including of inhibition of vascular calcification.

*39. Mary A, Hartemann A, Liabeuf S et al. Association between metformin use and below-the-knee arterial calcification score in type 2 diabetic patients. *Cardiovasc Diabetol*. 2017;16(1):24. doi:10.1186/s12933-017-0509-7.

A small observational study in Type 2 diabetes showing metformin associations with lower lower limb vascular calcification, which is supported by observational studies of reduced leg amputation risk in clinical practice. Additional studies are desirable.

40. Aroda VR, Knowler WC, Crandall JP et al. Metformin for diabetes prevention: insights gained from the Diabetes Prevention Program/Diabetes Prevention Program Outcomes Study. *Diabetologia*. 2017;60(9):1601-1611.

41. Hippisley-Cox J, Coupland C. Diabetes treatments and risk of amputation, blindness, severe kidney failure, hyperglycaemia, and hypoglycaemia: open cohort study in primary care. *BMJ*. 2016;352:i1450.

42. Cai Z, Ding Y, Zhang M et al. Ablation of Adenosine Monophosphate-Activated Protein Kinase $\alpha 1$ in Vascular Smooth Muscle Cells Promotes Diet-Induced Atherosclerotic Calcification In Vivo. *Circ Res*. 2016;119(3):422-433.

43. Fadini GP. A reappraisal of the role of circulating (progenitor) cells in the pathobiology of diabetic complications. *Diabetologia*. 2014;57(1):4-15.

44. Chatterjee S, Davies MJ, Khunti K. What have we learnt from "real world" data, observational studies and meta-analyses. *Diabetes Obes Metab.* 2018;20 Suppl 1:47-58.

45. Preiss D, Lloyd SM, Ford I et al. Metformin for non-diabetic patients with coronary heart disease (the CAMERA study): a randomised controlled trial. *Lancet Diabetes Endocrinol.* 2014 ;2(2):116-124.

46. Preiss D, Dawed A, Welsh P et al. Sustained influence of metformin therapy on circulating glucagon-like peptide-1 levels in individuals with and without type 2 diabetes. *Diabetes Obes Metab.* 2017;19(3):356-363.

*47. Eilenberg W, Stojkovic S, Piechota-Polanczyk A et al. Neutrophil gelatinase associated lipocalin (NGAL) is elevated in type 2 diabetics with carotid artery stenosis and reduced under metformin treatment. *Cardiovasc Diabetol.* 2017;16(1):98

NGAL is an emerging risk factor for diabetic renal and vascular complications, and this study of carotid end-arterectomy patients demonstrates elevated NGAL in blood and atheroma in Type 2 diabetes patients, and lower levels in association with metformin use.

*48. Huangfu N, Wang Y, Cheng J et al. Metformin protects against oxidized low density lipoprotein-induced macrophage apoptosis and inhibits lipid uptake. *Exp Ther Med.* 2018;15(3):2485-2491.

Extends knowledge of mechanisms of metformin's inhibition of lipid uptake by human monocytes.

*49. Gopaju R, Panangipalli S, Kotamraju S. Metformin treatment prevents SREBP2-mediated cholesterol uptake and improves lipid homeostasis during oxidative stress-induced atherosclerosis. *Free Radic Biol Med.* 2018 118:85-97.

Extends knowledge of mechanisms of metformin's inhibition of lipid uptake by human monocytes.

**50. Ramachandran S, Anandan V, Kutty VR et al. Metformin attenuates effects of cyclophilin A on macrophages, reduces lipid uptake and secretion of cytokines by repressing decreased AMPK activity. Clin Sci (Lond). 2018;132(6):719-738.

Comprehensive series of studies in both a human monocyte cell line and cultured monocytes/macrophages from Type 2 diabetes patients delineating mechanisms of metformin's effects on AMPK and pro-inflammatory cyclophilin A, with inhibition of Ox-LDL uptake and adverse cellular responses related to inflammation and oxidative stress. Results extend prior knowledge and point to additional targets as surrogate end-points and therapeutic targets to retard CVD.

* 51. Hao B, Xiao Y, Song F et al. Metformin-induced activation of AMPK inhibits the proliferation and migration of human aortic smooth muscle cells through upregulation of p53 and IFI16. Int J MolMed. 2018;41(3):1365-1376.

Details the mechanisms by which metformin inhibits AMPK and adverse proatherogenic responses of human aortic smooth muscle cells.

*52. Karnewar S, Neeli PK, Panuganti D et al. Metformin regulates mitochondrial biogenesis and senescence through AMPK mediated H3K79 methylation: Relevance in age-associated vascular dysfunction. Biochim Biophys Acta. 2018;1864(4 Pt A):1115-1128.

Details the cellular mechanisms by which metformin retards cellular senescence in human aortic endothelial cells, which include effects on DNA methylation in the SIRT pathway, which is linked with metabolic memory.

53. Wu T, Xie C, Wu H, Jones KL, Horowitz M, Rayner CK. Metformin reduces the rate of small intestinal glucose absorption in type 2 diabetes. Diabetes Obes Metab. 2017;19(2):290-293.

54. Takahara M, Kaneto H, Katakami N, Matsuhisa M, Shimomura I. Effect of metformin on hepatic glucose production in Japanese patients with type 2 diabetes mellitus. *Endocr J*. 2012;59(9):845-7.
55. Natali A, Ferrannini E. Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose uptake in type 2 diabetes: a systematic review. *Diabetologia*. 2006 Mar;49(3):434-41.
56. Wang C, Liu F, Yuan Y, Wu J, Wang H, Zhang L, Hu P, Li Z, Li Q, Ye J. Metformin suppresses lipid accumulation in skeletal muscle by promoting fatty acid oxidation. *Clin Lab*. 2014;60(6):887-96.
57. Wulffelé MG, Kooy A, de Zeeuw D, Stehouwer CD, Gansevoort RT. The effect of metformin on blood pressure, plasma cholesterol and triglycerides in type 2 diabetes mellitus: a systematic review. *J Intern Med*. 2004 Jul;256(1):1-14.
58. Orio F, Manguso F, Di Biase S, et al. Metformin administration improves leukocyte count in women with polycystic ovary syndrome: a 6-month prospective study. *Eur J Endocrinol*. 2007 Jul;157(1):69-73.
59. Goldberg RB, Temprosa MG, Mather KJ, Orchard TJ, Kitabchi AE, Watson KE; Diabetes Prevention Program Research Group. Lifestyle and metformin interventions have a durable effect to lower CRP and tPA levels in the diabetes prevention program except in those who develop diabetes. *Diabetes Care*. 2014 Aug;37(8):2253-60. doi: 10.2337/dc13-2471.
60. Xin G, Wei Z, Ji C, et al. Metformin Uniquely Prevents Thrombosis by Inhibiting Platelet Activation and mtDNA Release. *Sci Rep*. 2016 Nov 2;6:36222.

Table 1. Effects of metformin.

Metformin Effect	Evidence Level	Reference
Glucose lowering / insulin resistance		
Inhibits glucose absorption from gut	II	53
Decreases hepatic glucose output	III-3	54
Increases peripheral glucose uptake	I	55
Increases fatty acid oxidation	Basic science	56
Lowers HbA1c and glucose levels in pre-diabetes and T2D	II	31
<i>Moderate HbA1c reduction in T1D</i>	II	17, 19
<i>Moderate weight loss and leptin reduction in T1D</i>	II	19, 31
<i>Small insulin dose reduction in T1D</i>	II	19
<i>Increases GLP-1 levels</i>	II	45
	III	46
Lipid related		
<i>Small LDL-C and total cholesterol reductions</i>	I	19,57
Novel risk factors:		
<i>Lowers NGAL levels in circulation and atheroma in T2D</i>	III-2	47
<i>Lowers CRP and WBC</i>	II	40, 58
<i>Lowers tPA</i>	II	40, 59
Reduces platelet aggregation	Basic science	60
<i>Increased circulating EPC</i>	III-2	33
Clinical vascular events		
Controversy regarding reduction in CVD events (including myocardial infarction, heart failure, stroke and atrial fibrillation) in T2D	I II	35 3,4
Surrogate vascular outcomes		
<i>Retards maximum CIMT progression in T1D</i>	II	19
<i>Retards CAC in pre-diabetes and T2D</i>	II	38
<i>Associated with lower CAC in lower limb arteries</i>	III-2	39
Associated with lower lower limb amputations in T2D	III-2	41
<i>Associated with less advanced, complex and inflamed carotid atheroma</i>	III-2	47
<i>Reduces renal filtration loss in T1D</i>	II	19
<i>Improves GTN mediated vascular dysfunction in T1D youth</i>	II	31
<i>Does not improve FMD</i>	II	19, 31
Vascular cell biology		
Activates AMPK	Basic Science	49-52
Increases vascular cell eNOS	Basic Science	32
<i>Inhibits Ox-LDL and TG uptake by macrophages, reducing foam cell formation and apoptosis</i>	Basic Science	48
<i>Inhibits endothelial cell senescence</i>	Basic Science	52
<i>Reduces mitochondrial biogenesis</i>	Basic Science	52
Side-effects		
Gastrointestinal upset: Metallic taste, nausea, vomiting, diarrhoea	II	19,31,40
<i>Lowers Vitamin B12 levels, including in T1D</i>	II	19

Newly documented effects with potentially vascular effects are in *italics and underlined*.