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Metformin and carotid intima-media thickness in never-smokers with type 1 diabetes: The REMOVAL trial

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Abstract

Aim: To determine whether metformin's effects on carotid artery intima-media thickness (cIMT) in type 1 diabetes differ according to smoking status.

Methods: Regression model effect estimates for the effect of metformin versus placebo (double-blind) on carotid IMT were calculated as a subgroup analysis of the REMOVAL trial.

Results: In 428 randomized participants (227 never-smokers, 201 ever-smokers), averaged mean carotid IMT progression (per year) was reduced by metformin versus placebo in never-smokers (−0.012 mm, 95% CI −0.021 to −0.002; p = .0137) but not in ever-smokers (0.003 mm, 95% CI −0.008 to 0.014; p = .5767); and similarly in non-current smokers (−0.008 mm, 95% CI −0.015 to −0.00001; p = .0497) but not in

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current smokers (0.013 mm, 95% CI −0.007 to 0.032; p = .1887). Three-way interaction terms (treatment*time*smoking status) were significant for never versus ever smoking (p = .0373, prespecified) and non-current versus current smoking (p = .0496, exploratory). Averaged maximal carotid IMT progression (per year) was reduced by metformin versus placebo in never-smokers (−0.020 mm, 95% CI −0.034 to −0.006; p = .0067) but not in ever-smokers (−0.006 mm, 95% CI −0.020 to 0.008; p = .4067), although this analysis was not supported by a significant three-way interaction term.

Conclusions: This subgroup analysis of the REMOVAL trial provides additional support for a potentially wider role of adjunct metformin therapy in cardiovascular risk management in type 1 diabetes, particularly for individuals who have never smoked cigarettes.

KEYWORDS
cardiovascular disease, diabetes complications, metformin, type 1 diabetes

1 | INTRODUCTION

Although the prevalence of cigarette smoking has declined to around 15% of the general population in the UK and the United States, it remains the most significant cause of preventable premature mortality worldwide with an estimated 6 million deaths annually.[1,2] The proportion of people with type 1 diabetes reporting current smoking is at least as high (15%-20%), or higher in some populations, while an additional 20%-25% are former smokers.[3,4] As the commonest cause of premature death in type 1 diabetes is cardiovascular disease (CVD) and the detrimental effects of smoking on the vasculature are well documented, this is an unfortunate combination.[4–7] A meta analysis has shown that smoking is associated with a 50% increase in the risk of adverse cardiovascular outcomes in diabetes, although there are few data specific to type 1 diabetes.[4]

In the REducing with MetfOrmin Vascular Adverse Lesions (REMOVAL) trial (NCT01483560), a randomized, double-blind, placebo-controlled trial of metformin adjunct therapy in high cardiovascular-risk adults with type 1 diabetes, carotid intima-media thickness (IMT) was measured annually over 3 years as a surrogate marker of atherosclerosis progression strongly associated with CVD outcomes.[8,9] In the main analysis, progression of averaged mean far wall carotid IMT (the primary outcome) did not differ significantly between the metformin and placebo groups during follow-up. However, progression of averaged maximal far wall carotid IMT (a tertiary outcome) was significantly reduced by metformin.[9] Of note, the Mannheim Consensus favours mean carotid IMT as an outcome measure for studies in the general population,[10] but post-randomization follow-up analyses of the Diabetes Control and Complications Trial (DCCT) in type 1 diabetes reported maximal carotid IMT.[11,12]

Smoking is strongly associated with carotid IMT progression and adverse cardiovascular outcomes in the general population.[13] Its pro-atherosclerotic effects are mediated by a variety of mechanisms including release of pro-inflammatory cytokines, free radical formation, LDL oxidation, reduced bioavailability of nitric oxide, induction of a pro-thrombotic state and monocyte adhesion to vascular cells.[14–19] Smoking has been shown to interact with ageing and metabolic syndrome to accelerate carotid IMT progression. With the hypothesis that powerful adverse effects of smoking may attenuate protective vascular effects of metformin, we conducted a prespecified subgroup analysis of the REMOVAL trial with the aim of determining whether metformin's effects on carotid artery intima-media thickness in type 1 diabetes differ according to smoking status.

2 | METHODS

The REMOVAL trial was undertaken at 23 hospital diabetes clinics in five different countries (UK, Canada, Australia, the Netherlands and Denmark); 428 adults aged 40 years and older with type 1 diabetes of at least 5 years' duration and at least three of 10 specified risk factors for CVD were randomized from December 2011 to June 2014 to either metformin 1000 mg twice daily (or maximum dose tolerated) or placebo in addition to usual insulin therapy and were followed up over 3 years. The primary objective (reported elsewhere) was to investigate whether adding metformin to standard titrated insulin therapy reduced progression of atherosclerosis as measured by carotid IMT at 12, 24 and 36 months.[9] Cigarette smoking status was ascertained by self-report at baseline (never, former or current; duration where applicable).

2.1 | Statistical analysis

The 'ever' smoking group consisted of those reporting 'current' or 'former' smoking (independent of duration) (Figure S1). 'Never' versus 'ever' smoking status was one of 11 subgroup analyses prespecified in the statistical analysis plan for the primary carotid IMT outcome; the others were age, sex, baseline carotid IMT, history of CVD, duration of diabetes, baseline HbA1c, body mass index (BMI), LDL-cholesterol, systolic blood pressure and insulin pump use.

Baseline data in each of the groups according to smoking status were summarized using means and standard deviations for continuous variables and by number and percentages for categorical variables. Three-way interaction terms (treatment*time*subgroup) were calculated for all prespecified subgroups (Table S1). Where appropriate, repeated-measures random effects regression (as previously described for the main analysis) was used to assess the effect of metformin within subgroups.[9] Following review of carotid IMT results by 'never' versus 'ever' smoking status, the steering committee requested a further exploratory analysis by 'non-current' versus 'current' smoking status. As the 'non-current' smoking group consisted of 'never' and 'former' smokers combined (Figure S1), a further exploratory analysis was conducted according to never versus former versus current smoking. Analyses were performed with SAS (version 9.3) with a two-sided significance level of 5%. No adjustments for multiple comparisons were prespecified.

3 | RESULTS

Of 428 randomized participants ([mean ± SD] age 55.5 ± 8.6 years, HbA1c 8.1% ± 0.82% (64.5 ± 9.0 mmol/mol), BMI 28.5 ± 4.3 kg/m^2, duration of diabetes 34 ± 10.8 years), 227 (53%) were never-smokers and 201 (47%) were ever-smokers. In further analyses, 371 (87%) were non-current smokers and 57 (13%) were current smokers. Smoking duration was 22.2 ± 13.2 years for ever-smokers and 31.6 ± 12.4 years for current smokers. Other baseline demographic characteristics by smoking status are shown in Table 1.

Carotid IMT was higher at baseline in ever-smokers versus never-smokers (0.815 ± 0.157 vs. 0.752 ± 0.161 mm; $p < .0001$) but not in current versus non-current smokers (0.801 ± 0.163 vs. 0.779 ± 0.162 mm; $p = .3283$).

The three-way (treatment*time*subgroup) interaction term for the prespecified subgroup analysis of the primary outcome (averaged mean carotid IMT) for never versus ever smoking was significant ($p = .0373$). Progression of averaged mean carotid IMT was reduced by metformin in never-smokers (−0.012 mm per year, 95% CI −0.021 to −0.002; $p = .0137$) but not in ever-smokers (0.003 mm per year, 95% CI −0.008 to 0.014; $p = .5767$) (Figure 1). The three-way (treatment*time*subgroup) interaction term was also significant in exploratory analysis of the same outcome by non-current versus current smoking ($p = .0496$). Thus, averaged mean carotid IMT progression was reduced in non-current smokers (−0.008 mm per year, 95% CI −0.015 to −0.00001; $p = .0497$) but not in current smokers (0.013 mm per year, 95% CI −0.007 to 0.032; $p = .1887$) (Figure 2). The three-way (treatment*time*subgroup) interaction term for exploratory analysis according to never versus former versus current smoking was supported by a borderline significant three-way

TABLE 1 Baseline characteristics of REMOVAL participants by smoking status

	Lifetime smoking (n = 428)		Current smoking (n = 428)	
	Never smoked (n = 227)	Ever smoked[a] (n = 201)	Non-smoker[b] (n = 371)	Current smoker (n = 57)
Age (years)	54.9 (8.6)	56.2 (8.6)	55.8 (8.7)	53.5 (7.9)
Male (%)	127 (56)	126 (63)	215 (58)	38 (67)
Years of diabetes	33.9 (9.8)	33.7 (11.8)	34.4 (10.6)	29.9 (11.6)
Existing CVD[c] (%)	26 (11.5)	26 (12.9)	47 (12.7)	5 (8.8)
Averaged mean cIMT	0.752 (0.161)	0.815 (0.157)	0.779 (0.162)	0.801 (0.163)
Averaged maximal cIMT	0.883 (0.196)	0.958 (0.188)	0.915 (0.196)	0.938 (0·196)
Years of smoking	—	22.2 (13.2)	18.5 (11.6)	31.6 (12.4)
HbA1c (%)	8.0 (0.79)	8.1 (0.86)	8.0 (0.80)	8.2 (0.95)
HbA1c (mmol/mol)	64.3 (8.62)	64.6 (9.43)	64.3 (8.77)	65.7 (10.40)
BMI (kg/m^2)	28.5 (4.0)	28.4 (4.7)	28.8 (4.3)	26·3 (3.5)
Systolic BP (mmHg)	129 (14.8)	131 (14.8)	130 (15.2)	126 (11.9)
Cholesterol	4·0 (0.87)	4·0 (0.95)	4·0 (0.91)	4·0 (0.87)
eGFR (mL/min/1.73m^2)	92 (21.8)	92 (20.6)	92 (21.3)	91 (21.1)
BP-lowering treatment (Y/N)	162 (71)	151 (75)	273 (74)	40 (70)
Statin treatment (Y/N)	186 (82)	163 (81)	305 (82)	44 (77)
Aspirin treatment (Y/N)	79 (35)	72 (36)	129 (35)	22 (39)
Clopidogrel treatment (Y/N)	9 (4)	7 (4)	16 (4)	0 (0)

Abbreviations: BMI, body mass index; BP, blood pressure; cIMT, carotid intima-media thickness; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate.

Mean (SD) or number (%).

[a]Former smokers and current smokers combined.

[b]Never-smokers and ex-smokers combined (see Figure S1).

[c]Includes heart failure, coronary artery bypass graft, stent, angina, transient ischaemic attack, peripheral vascular disease.

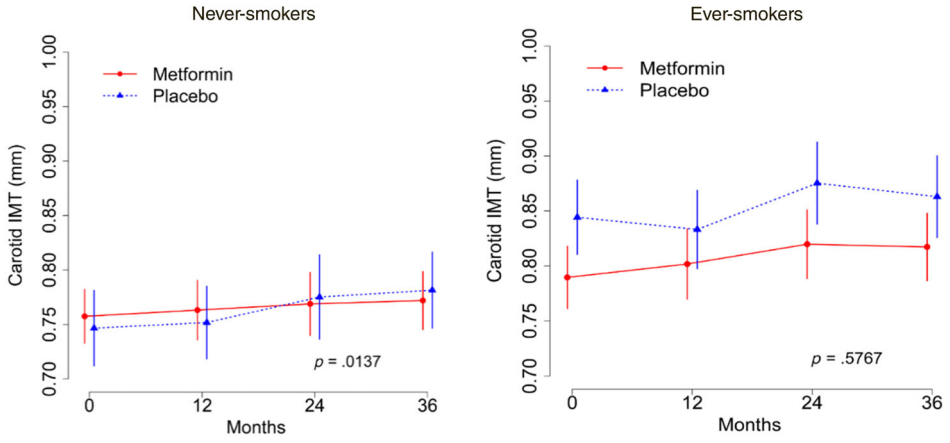

FIGURE 1 Primary carotid outcome (averaged mean carotid IMT) by lifetime smoking status. In never smokers [left panel, n = 227] progression of averaged mean carotid IMT by repeated-measures regression was reduced by metformin (red) vs. placebo (blue) (p = .0137). In ever smokers [right panel, n = 201] there was no effect of metformin (p= .5767). Error bars represent 95% confidence intervals

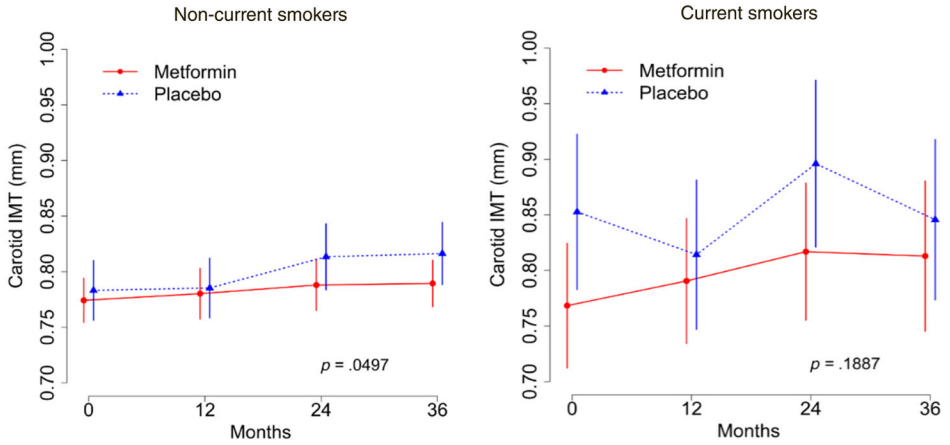

FIGURE 2 Primary carotid outcome (averaged mean carotid IMT) by current smoking status. In non-current smokers [left panel, n = 371], progression of averaged mean carotid IMT by repeated-measures regression was reduced by metformin (p = .0497). In current smokers (right panel, n = 57) there was no effect of metformin (p = .1887). Error bars represent 95% confidence intervals

interaction term (p = .0544). There was no attenuation of carotid IMT progression with metformin versus placebo in former smokers (n = 144; p = .9185) (Figure S2).

Progression of the tertiary carotid outcome, averaged maximal carotid IMT, was also reduced by metformin in never-smokers (−0.020 mm per year, 95% CI −0.034 to −0.006; p = .0067) but not in ever-smokers (−0.006 mm per year, 95% CI −0.020 to 0.008; p = .4067), and in non-current (−0.014 mm per year, 95% CI −0.025 to −0.003; p = .0102) but not in current (−0.006 mm per year, 95% CI −0.032 to 0.020; p = .6543) smokers (data not shown). These analyses were not supported by statistically significant interaction terms (p = .1764 and p = .5280, respectively).

Three-way (treatment*time*subgroup) interaction terms for the other 10 prespecified subgroup analyses, including sex, were not statistically significant for the primary outcome (Table S1). As 97% of participants self-reported as White, a subgroup analysis by ethnicity could not be performed.

4 | DISCUSSION

While subgroup analyses must be interpreted with caution, in a prespecified analysis of the REMOVAL trial, we observed that the effect of metformin on carotid IMT in type 1 diabetes differed according to smoking status. In individuals who had never smoked, treatment with metformin for 3 years attenuated progression of this well-validated surrogate measure of CVD[9] despite an average duration of diabetes—in the majority of cases with associated hypertension and dyslipidaemia—of more than 30 years. This was broadly consistent whether carotid IMT was measured as averaged mean (primary outcome) or averaged maximal (tertiary outcome).

REMOVAL is the largest trial examining the role of metformin in type 1 diabetes.[9] Carotid IMT was selected as a surrogate vascular outcome because it is a well-validated, non-invasive marker of CVD that predicts clinical events in the general population[8,13,20]; indeed, since REMOVAL was completed and the main results were published, a large and robust meta-analysis has shown that the extent to which an intervention reduces progression of mean carotid IMT is closely associated with the degree of CVD reduction observed.[8] Mean far wall carotid IMT is a measurement of IMT over 10-mm arterial segments proximal to the carotid bifurcation (in three planes for each artery). In REMOVAL, following the Mannheim Consensus, individual carotid IMT measurements greater than 1.5 mm—potentially indicative of atherosclerotic plaque—were excluded from the primary outcome analysis. Maximal carotid IMT is the mean of the maximum IMT measured in each of these carotid segments that is inclusive of areas of plaque.[20] The main trial results showed that metformin reduced

averaged maximal carotid IMT (tertiary outcome) but not averaged mean carotid IMT (primary outcome).[9,21] In revealing an interaction with smoking status, and that progression of both carotid IMT outcomes was significantly reduced by metformin in never-smokers, the present subgroup analyses provide important and potentially clinically significant insights into the previously reported findings.[9]

Of note, two previous trials that examined the effect of metformin on carotid IMT in other populations reported no effect.[22,23] However, both were smaller than REMOVAL and had half the duration of follow-up: indeed, the latter acknowledged a lack of statistical power. No previous studies have examined the impact of metformin by smoking status on either surrogate or clinical cardiovascular outcomes in type 1 diabetes. One large observational cohort study in type 2 diabetes concluded that metformin has a protective effect against CVD specifically in smokers. However, this was a non-randomized study, in which only 17% of smokers were taking metformin therapy and demographic information was not presented by metformin treatment status. While findings in type 2 diabetes cannot be directly extrapolated to type 1 diabetes, it seems possible using this design that metformin operated as a marker of absence of co-morbidity, that is, there may have been residual confounding by indication.[24]

Smoking rates are decreasing globally but many people with type 1 diabetes continue to smoke.[1,2,4,25] Smoking, diabetes and hypertension are all major contributors to increased carotid IMT and the development and progression of atherosclerotic plaque.[17–19,25,26] The mechanisms involved are complex[15] but include induction of pro-inflammatory cytokines and recruitment of leukocytes to the vascular wall.[4,15] In type 1 diabetes, dysglycaemia (including both hyper- and hypoglycaemia) additionally acts to increase vascular inflammation, promote thrombosis, increase deleterious lipids and impair nitric oxide availability.[4,27]

A variety of lines of evidence exist to support an antiatherosclerotic effect of metformin and a reduced risk of adverse cardiovascular outcomes, mainly in type 2 diabetes.[28,29] The principal mechanisms invoked include inhibition of vascular pro-inflammatory pathways[30–34] and reduction of differentiation of monocytes to macrophages at the endothelial level (thereby inhibiting foam cell formation).[32,33] Reduction in HbA1c with metformin in the REMOVAL trial was only statistically but not clinically significant, hence effects on carotid IMT progression were most probably glycaemia independent.[9] To account for our findings in the present analysis we speculate that metformin was unable to mitigate the multiple deleterious vascular mechanisms in play at the vascular wall in individuals who had a history of many decades of both type 1 diabetes and cigarette smoking. Studies in murine models suggest that metformin may prevent early atherogenesis but not reverse more established disease[33]: by analogy, cigarette-smoking participants in REMOVAL may have had more established atherosclerotic plaques that were less susceptible to metformin's vascular effects.

Against this conjecture, other agents that reduce vascular risk in type 1 diabetes (e.g. antihypertensives, statins) have not been shown to be less effective in smokers. It could also be argued that higher baseline carotid IMT should have provided greater scope for a metformin-related reduction over time to be shown (although such a reduction may have been more difficult to detect because of greater measurement variability). Alternatively, there may have been unmeasured behavioural or other differences according to smoking status to account for the observed differences in carotid IMT progression between groups, for example, other health behaviours or lower adherence to prescribed therapies.

The strengths of this analysis are the evaluation of well-characterized adults with type 1 diabetes in the setting of a rigorous placebo-controlled randomized trial with prestated hypotheses. Subgroup analysis by smoking status was supported by significant interaction terms, although as for other subgroups prespecified in the statistical analysis plan, these were not adjusted for multiple comparisons. Other limitations relate to use of a surrogate outcome for CVD, self-reporting of smoking status and the lack of ongoing data on smoking exposure during the trial.

In conclusion, the present subgroup analysis of the REMOVAL trial provides further support for a potentially wider role of adjunct metformin therapy in cardiovascular risk reduction in type 1 diabetes, particularly for individuals who have never smoked cigarettes. Cardiovascular outcome trials are required to elucidate whether metformin (or other adjunct agents) may offer cardiovascular risk reduction in this high-risk population.

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CONFLICT OF INTEREST

JRP has received research grants from JDRF for the present work. He has also received personal fees and travel support from Novo Nordisk, research grants and personal fees from Sanofi Aventis, Quintiles and Janssen unrelated to the present work, non-financial support (donation of study medication for the present trial) from Merck KGaA (Germany),

personal fees from Lilly and ACI Clinical unrelated to the present work and non-financial support (donation of EndoPAT equipment, reading services and quality assurance support for the present trial) from Itamar Medical. JGB has received speaker fees from Sanofi Aventis and travel support from Napp Pharmaceuticals and Novo Nordisk. JGT has received travel support from Napp Pharmaceuticals. NC has received research grants from JDRF for the present work and personal fees from AstraZeneca, unrelated to the present work. NG has received research grants from JDRF for the present work. IH has received research grants from JDRF/Federal Development Funding for the present work. She has also received personal fees from Amgen, Boehringer Ingelheim, Hoffmann-La Roche, Insulet and Takeda; research grants and personal fees from AstraZeneca/Bristol-Myers Squibb, GlaxoSmithKline, Janssen-Ortho (Johnson & Johnson/JNJ), Merck Frosst, Novo Nordisk and Sanofi-Aventis; research grants, personal fees and travel support from Eli Lilly; and research grants from Lexicon and Medtronic, unrelated to the present work. TCO has received research grants from JDRF for the present work. Related to the REMOVAL trial, AJJ has received grants from JDRF International and from JDRF Australia. Unrelated to this trial she has received grants from Medtronic, the NHMRC (Australia) and Medical Research Future Fund, JDRF Australia, JDRF International, the Helmsley Trust, Sanofi-Aventis, Mylan and Abbott. PR has received research grants from JDRF for the present work. He has also received research grants, personal fees and travel support from Novo Nordisk; research grants and personal fees from Astra Zeneca; and personal fees from Astellas, Boehringer Ingelheim, Bayer and Eli Lilly, unrelated to the present work. NS has received research grants and personal fees from Boehringer Ingelheim; personal fees from Novo Nordisk, Janssen and Eli Lilly; and research grants from Astra Zeneca, unrelated to the present work. HMC has received research grants, personal fees and lecture and consultation support from Sanofi; consultation support from Sanofi Aventis and Novartis; research grants, personal fees and travel support from Eli Lilly; research grants from Pfizer, Boehringer Ingelheim, AstraZeneca and Roche Pharmaceuticals; and personal fees and lecture and consultation support from Regeron Pharmaceuticals, unrelated to the present work. She is also a shareholder in Roche Pharmaceuticals and Bayer. IF, MCGJB, TT, ADH, BEKK, RK and CDAS declare no competing interests.

AUTHOR CONTRIBUTIONS

This analysis was initiated by the Trial Steering Committee (JRP, HMC, NC, IF, IH, ADH, AJJ, BEKK, TCO, PR, NS, CDAS and RK [deceased]). JGT wrote the first draft of the manuscript. NG carried out the statistical analyses. JGB assisted with early drafts of the manuscript. JRP (Chief Investigator) supervised development of the manuscript and is guarantor for the contents of the article. All the other authors were involved in data collection and/or reading centres and provided comments during the development of the manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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APPENDIX A.

The REMOVAL study group.

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