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 ever-smokers.
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.5-9 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.7 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/m², 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 was 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 −0.0001; p = .0497) but not in current smokers (0.013 mm per year, 95% CI −0.007 to 0.032; p = .1887) (Figure 1). The three-way (treatment*time*subgroup) interaction term was supported by a borderline significant three-way TABLE 1 Baseline characteristics of REMOVAL participants by smoking status

<table>
<thead>
<tr>
<th></th>
<th>Lifetime smoking (n = 428)</th>
<th>Current smoking (n = 428)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never smoked (n = 227)</td>
<td>Ever smoked(a) (n = 201)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.9 (8.6)</td>
<td>56.2 (8.6)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>127 (56)</td>
<td>126 (63)</td>
</tr>
<tr>
<td>Years of diabetes</td>
<td>33.9 (9.8)</td>
<td>33.7 (11.8)</td>
</tr>
<tr>
<td>Existing CVD(c) (%)</td>
<td>26 (11.5)</td>
<td>26 (12.9)</td>
</tr>
<tr>
<td>Averaged mean cIMT</td>
<td>0.752 (0.161)</td>
<td>0.815 (0.157)</td>
</tr>
<tr>
<td>Averaged maximal cIMT</td>
<td>0.883 (0.196)</td>
<td>0.958 (0.188)</td>
</tr>
<tr>
<td>Years of smoking</td>
<td>—</td>
<td>22.2 (13.2)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.0 (0.79)</td>
<td>8.1 (0.86)</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>64.3 (8.62)</td>
<td>64.6 (9.43)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.5 (4.0)</td>
<td>28.4 (4.7)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>129 (14.8)</td>
<td>131 (14.8)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.0 (0.87)</td>
<td>4.0 (0.95)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>92 (21.8)</td>
<td>92 (20.6)</td>
</tr>
<tr>
<td>BP-lowering treatment(Y/N)</td>
<td>162 (71)</td>
<td>151 (75)</td>
</tr>
<tr>
<td>Statin treatment (Y/N)</td>
<td>186 (82)</td>
<td>163 (81)</td>
</tr>
<tr>
<td>Aspirin treatment (Y/N)</td>
<td>79 (35)</td>
<td>72 (36)</td>
</tr>
<tr>
<td>Clopidogrel treatment (Y/N)</td>
<td>9 (4)</td>
<td>7 (4)</td>
</tr>
</tbody>
</table>

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 (%).

aFormer smokers and current smokers combined.

bNever-smokers and ex-smokers combined (see Figure S1).

CIs include heart failure, coronary artery bypass graft, stent, angina, transient ischaemic attack, peripheral vascular disease.
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.

### 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 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. Carotid IMT was selected as a surrogate vascular outcome because it is a well-validated, non-invasive marker of CVD 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).

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
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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Professor Michiel Bots was the external quality assurance adviser for carotid intima-media thickness. Elizabeth Douglas and Pamela Surtees (sponsor pharmacy, Glasgow, UK) specified manufacture and packaging of the study medication and liaised with Merck KGaA on drug supply management to trial sites. Sharon Kean (Robertson Centre for Biostatistics, University of Glasgow, Glasgow, UK) was responsible for data management. Maureen Travers (representative of the sponsor, NHS Greater Glasgow and Clyde, Glasgow, UK) was responsible for compliance of the Protocol and implementation of contracts. Lisa Jolly was responsible for project management. Jonathan Haw (deceased) was lay representative on the trial steering committee. These data were previously reported in abstract form: see European Association for the Study of Diabetes, September 2018, Diabetologia, 2018; 61 (Suppl 1): S555–S556 (Abstract 1138); https://link.springer.com/content/pdf/10.1007/s00125-018-4693-0.pdf, accessed 19 May 2020. The CONSORT statement was submitted with the main analysis paper. The REMOVAL trial was supported by JDRF (New York, NY, USA; SRA 17-2011-272). JDRF Australia provided additional funding for the Australian sites. Merck Germany KGaA (Darmstadt, Germany) donated study medication and shipped it to study sites.

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), and research grants and personal fees from Novo Nordisk.
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 AstraZeneca; 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 AstraZeneca, 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 Regeneron Pharmaceuticals, unrelated to the present work. She is also a shareholder in Roche Pharmaceuticals and Bayer. IF, MCGB, 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
The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14350.

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.

**APPENDIX A.**

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