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DIABETIC Medicine

RESEARCH: TREATMENT

Irbesartan treatment does not influence plasma levels of the dicarbonyls methylglyoxal, glyoxal and 3-deoxyglucosone in participants with type 2 diabetes and microalbuminuria: An IRMA2 sub-study

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Abstract

Aim: Angiotensin receptor blockers (ARBs) reduce vascular complications in diabetes independently of blood pressure. Experimental studies suggested that ARBs may restore the detoxifying enzyme glyoxalase 1, thereby lowering dicarbonyls such as methylglyoxal. Human data on the effects of ARBs on plasma dicarbonyl levels are lacking. We investigated, in individuals with type 2 diabetes, whether irbesartan lowered plasma levels of the dicarbonyls methylglyoxal, glyoxal, 3-deoxyglucosone and their derived advanced glycation end products (AGEs), and increased D-lactate, reflecting greater methylglyoxal flux.

Methods: We analysed a subset of the Irbesartan in Patients with T2D and Microalbuminuria (IRMA2) study. We measured plasma dicarbonyls methylglyoxal, glyoxal and 3-deoxyglucosone, free AGEs and D-lactate using ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS) in the treatment arm receiving 300 mg irbesartan ($n = 121$) and a placebo group ($n = 101$) at baseline and after 1 and 2 years. Effect of treatment was analysed with repeated measurements ANOVA.

Results: There was a slight, but significant difference in baseline median methylglyoxal levels [placebo 1119 (907–1509) nmol/l vs. irbesartan 300 mg 1053 (820–1427) nmol/l], but no significant changes were observed in any of the plasma dicarbonyls over time in either group and there was no effect of irbesartan treatment on plasma free AGEs or D-lactate levels at either 1 or 2 years.

Conclusion: Irbesartan treatment does not change plasma levels of the dicarbonyls methylglyoxal, glyoxal and 3-deoxyglucosone, free AGEs or D-lactate in type 2 diabetes. This indicates that increased dicarbonyls in type 2 diabetes are not targetable by ARBs, and other approaches to lower systemic dicarbonyls are needed in type 2 diabetes. (Clinical Trial Registry No: #NCT00317915).

1 | INTRODUCTION

Type 2 diabetes is associated with the development of cardiovascular disease (CVD), chronic kidney disease (CKD) and is a main cause of end-stage kidney disease (ESKD). The renin–angiotensin–aldosterone system plays an important role in the development of CVD and CKD in type 2 diabetes.[1,2] Inhibition of the renin–angiotensin–aldosterone system with ARBs reduces the incidence of CKD and CVD in type 2 diabetes.[3,4] However, the mechanisms through which ARBs attenuate renal and cardiovascular risk remain incompletely understood. This was highlighted by the seminal original Irbesartan in T2D With Microalbuminuria 2 (IRMA2) investigation, in which the ARB irbesartan reduced progression of diabetic CKD, independently of blood pressure.[4] Therefore, research into the mechanisms of action of ARBs is still needed to maximize the benefit of this vital class of medications for the prevention of diabetic complications. This is highly relevant because the incidence of CVD and CKD in type 2 diabetes remains high and many individuals still progress towards ESKD despite optimal treatment with ARBs.[5]

A potential underlying mechanism of the protective effects of ARBs is a reduction in the accumulation of methylglyoxal, a highly reactive glucose metabolite and the major precursor in the rapid formation of advanced glycation end products (AGEs).[6] Methylglyoxal levels are increased in type 2 diabetes,[7] and higher plasma methylglyoxal levels were associated with CVD, albuminuria and a decline in eGFR in type 2 diabetes.[8–10] Methylglyoxal is detoxified by the glyoxalase system to D-lactate, with glyoxalase 1 as the rate-limiting enzyme.[11] Glyoxalase 1 expression is lower in kidneys affected by CKD.[12] In line with this, glyoxalase 1 overexpression attenuated albuminuria in diabetic rats,[13] while glyoxalase 1 knockdown caused a CKD-like phenotype even in normoglycaemic mice.[14] These findings imply that glyoxalase 1 is a major protective factor against CKD. Interestingly, it has been shown in an experimental study that the ARB candesartan attenuates the formation of methylglyoxal levels through enhanced glyoxalase 1 expression.[15] These observations led to the hypothesis that the health benefits of ARBs are due, at least in part, to increased expression of glyoxalase 1 and a decrease in methylglyoxal levels. However, human studies about the effect of ARBs on systemic methylglyoxal levels are lacking. To explore whether irbesartan lowers dicarbonyl stress, we have now investigated plasma levels of methylglyoxal and two other major dicarbonyls, glyoxal and 3-deoxyglucosone, as well as D-lactate and free levels of the major dicarbonyl-derived AGEs N^{ε}-carboxymethyllysine (CML), N^{ε}-carboxyethyllysine (CEL) and methylglyoxal-derived hydroimidazolone (MG-H1) in the IRMA2 sub-study with a follow-up period of 2 years.

What's new?

- The angiotensin receptor blocker irbesartan reduces progression of diabetic kidney disease independently of blood pressure in individuals with type 2 diabetes.
- Methylglyoxal is a major driver of diabetic kidney disease and may be lowered by angiotensin receptor blockers.
- Irbesartan (300 mg) did not lower plasma levels of methylglyoxal, or any of the additional glycation markers measured in this study.
- This indicates that increased dicarbonyls in type 2 diabetes are not targetable by irbesartan, and other approaches to lower systemic dicarbonyls are needed in type 2 diabetes.

2 | METHODS

2.1 | Ethical approval

The study protocol was in accordance with the Declaration of Helsinki and approved by the institutional review board at each centre. All participants gave written informed consent.

2.2 | Statistical analyses

Variables with skewed distribution (plasma dicarbonyls) were ln-transformed prior to further analyses. Changes in plasma dicarbonyl levels over the 2-year follow-up time were examined according to the intention-to-treat principle and with the use of repeated measurement ANOVA. As a sensitivity analysis, we stratified our data set on the median baseline HbA_{1c} value of the current data set to investigate whether the effect of irbesartan differed by glycaemic control. For this analyses 19 individuals were initially excluded because of missing baseline HbA_{1c} values. When we added these individuals to the above median HbA_{1c} group, reasoning that individuals with missing values on average have poorer control, the results did not change. All statistical analyses were performed using SPSS version 23.

2.3 | Study design and participants

The IRMA 2 study was a 2-year multicentre, double-blind, parallel randomized controlled trial in individuals with type 2 diabetes and microalbuminuria in which the main goal was to compare the effects of irbesartan (150 or 300 mg once daily) vs. placebo, in addition to conventional

TABLE 1 Baseline characteristics of the study participants

	Placebo ($n = 101$)	300 mg Irbesartan ($n = 121$)
Sex (% women)	32.1	30.8
Age (years)	57.7 ± 9.3	57.3 ± 7.8
Known duration of diabetes (years)	7.0 (3.0–14.5)	7.0 (4.0–12.0)
HbA_{1c} (mmol/mol)	53 ± 18	52 ± 19
HbA_{1c} (%)	7.0 ± 1.6	6.9 ± 1.7
BMI (kg/m^2)	30.4 ± 4.3	29.9 ± 4.3
Total cholesterol (mmol/l)	5.7 ± 1.1	5.8 ± 1.1
Current smoking (%)	19.3	14.6
Systolic blood pressure (mmHg)	153.1 ± 14.3	154.5 ± 13.2
Diastolic blood pressure (mmHg)	89.8 ± 8.4	91.9 ± 9.7
Creatinine clearance (ml min^{-1} 1.73 m^{-2})	110.0 (89.5–130.5)	106.0 (92.5–124.3)
Urinary albumin excretion (mg per 24 h)	51.0 (33.0–82.0)	51.0 (35.0–87.0)

Note: Data are given as mean ± SD, median (IQR) or percentages as appropriate and stratified according to treatment group (300 mg irbesartan or placebo).

anti-hypertensive treatment, on the development of overt nephropathy.[4] Briefly, we enrolled individuals with type 2 diabetes (diagnosed according to the WHO criteria) and hypertension (defined as mean SBP >135 mmHg and/or mean DBP >85 mmHg in two of three consecutive measurements 1 week apart) and persistent microalbuminuria (defined as an albumin excretion rate of 20–200 mg/min in two of three consecutive overnight urine samples) and a serum creatinine concentration of 133 μmol/l for men and 97 μmol/l for women. A total of 590 (186 women) individuals, aged 30–70 years, were included and randomly assigned to receive 150 mg irbesartan once daily ($n = 195$), 300 mg irbesartan once daily ($n = 194$) or matching placebo ($n = 201$) (Figure S1). Results regarding the primary end point, and other secondary end points such as markers of inflammation and endothelial dysfunction, have been reported previously.[4] The aim of this study was to evaluate the effect of irbesartan treatment on glycation, using several state-of-the art panels to quantify plasma levels of the plasma dicarbonyls methylglyoxal, glyoxal and 3-deoxyglucosone, the free AGE levels of CML, CEL, MG-H1 and D-lactate. Samples for the assessment of these markers at baseline and at 1- and 2-year follow-up were available from 121 participants in the treatment arm receiving 300 mg irbesartan and from 101 participants receiving placebo. In the initial analyses of this study, no significant effects of 150 mg irbesartan treatment vs. placebo were found on the development of nephropathy, creatinine clearance or blood pressure. The 150 mg irbesartan samples were not included in the current analyses because they are no longer available as they unfortunately had been discarded by mistake in the past. Individuals included in the current analyses had similar baseline characteristics to the original investigation (data not shown).[4]

2.4 | Biochemical measurements

HbA_{1c} was measured by ion-exchange high-performance liquid chromatography. Serum creatinine concentration was originally measured by the Jaffe reaction with the use of a Hoffmann LaRoche kit and creatinine clearance (in ml min^{-1} 1.73 m^{-2} body surface area) was estimated using the Cockcroft–Gault equation. These variables were evaluated at the time of randomization, 2 and 4 weeks after randomization and at 3, 6, 12, 18 and 22–24 months.

2.5 | Measurement of plasma dicarbonyls

Plasma samples were stored at −80C° until analyses. Plasma dicarbonyls were measured with ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS).[16] In brief, EDTA plasma samples (25 μl) were mixed with 75 μl d_8-*O*-phenylenediamine (oPD; 10 mg oPD in 10 ml 1.6 mol/l perchloric acid) in an Eppendorf cup. After an overnight (20 h) reaction at room temperature and shielded from light, 10 μl of internal standard solution was added. Samples were mixed and subsequently centrifuged for 20 min at 21 000 *g* at a temperature of 4°C; 10 μl was injected for UPLC/MSMS analysis. The interassay variations for methylglyoxal, glyoxal and 3-deoxyglucosone were 4.3%, 5.1% and 2.2%, respectively.[16]

Plasma D-lactate levels were measured with UPLC-MS/MS.[17] Some 25 μl of plasma was derivatized with diacetyl-L-tartaric anhydride and separated on a C_{18}-reversed phase column. D-Lactate inter- and intra-assay variations were between 2% and 9%.

TABLE 2 Plasma dicarbonyls, D-lactate and free advanced glycation end product levels at baseline and at 1- and 2-year follow-up

	Baseline		Year 1		Year 2	
	Placebo	300 mg Irbesartan	Placebo	300 mg Irbesartan	Placebo	300 mg Irbesartan
Methylglyoxal (nmol/l)	1119 (907–1590)	1053 (821–1448)	1131 (873–1488)	1147 (824–1539)	1078 (876–1448)	1142 (839–1565)
Glyoxal (nmol/l)	6079 (4967–7164)	6003 (5153–6939)	6067 (5108–7959)	6304 (5230–7642)	6036 (4928–7758)	6238 (5169–7332)
3-Deoxyglucosone (nmol/l)	5499 (4446–6555)	5211 (4204–6491)	5582 (4548–6846)	5311 (4304–6305)	5319 (4279–7111)	5657 (4184–6573)
D-Lactate (mmol/l)	13.8 (9.2–22.3)	14.0 (9.9–20.3)	14.8 (10.0–19.4)	15.2 (10.6–21.2)	15.4 (10.3–21.2)	14.9 (10.8–22.4)
Free CML (nmol/l)	116.8 (98.4–158.1)	107.0 (84.6–134.9)	116.5 (92.5–154.8)	111.9 (92.6–134.5)	125.2 (98.8–155.3)	121.1 (96.5–154.7)
Free CEL (nmol/l)	63.3 (49.1–81.1)	52.6 (42.4–70.4)	63.4 (43.4–80.7)	58.1 (47.6–77.6)	66.2 (51.0–84.7)	63.5 (50.2–82.9)
Free MG-H1 (nmol/l)	198.0 (138.7–284.7)	175.9 (114.7–265.0)	198.0 (138.7–284.7)	175.9 (114.7–265.0	201.2 (137.4–298.5)	198.6 (127.3–285.2)

Note: Data are expressed as median (IQR) and stratified according to treatment group (300 mg irbesartan or placebo).

Abbreviations: CEL, N^{ε}-carboxyethyllysine; CML, N^{ε}-carboxymethyllysine; MG-H1, methylglyoxal-derived hydroimidazolone.

Plasma free MG-H1 levels were measured with UPLC-MS/MS.[18] Some 50 µl plasma was used, samples were derivatized with butanolic hydrochloric acid and subsequently detected in multiple reaction monitoring mode using a Xevo TQ MS (Waters, Milford, MA, USA). Quantification of free MG-H1 was performed by calculating the ratio of each unlabelled peak area to the corresponding internal standard peak area. In plasma, the intra- and interassay variations of protein-bound CML, CEL and MG-H1 were between 4.8% and 18.8%, and for free CML, CEL and MG-H1 were between 2.8% and 7.1%.

3 | RESULTS

Baseline characteristics of the current subset of the IRMA2 trial are shown in Table 1. An in-depth description of the complete study has been provided elsewhere.[4]

3.1 | Effects of 300 mg irbesartan treatment on plasma levels of methylglyoxal, glyoxal and 3-deoxyglucosone

Plasma methylglyoxal levels were significantly lower at baseline in the group randomized to 300 mg irbesartan (Table 2 and Figure 1). Plasma levels of methylglyoxal, glyoxal and 3-deoxyglucosone did not change significantly over the 1- and 2-year follow-up time in either group, and did not differ between groups (Table 2 and Figure 1). These results did not change when we adjusted the repeated measurements in ANOVA for sex, age, HbA_{1c}, duration of diabetes and BMI (data not shown).

3.2 | Effects of 300 mg irbesartan treatment on plasma levels of D-lactate

Plasma levels of D-lactate did not change significantly in either group after 1- and 2-year follow-up time and no difference was found between groups (Table 2 and Figure 2).

3.3 | Effects of 300 mg irbesartan treatment on plasma levels of free CML, CEL and MG-H1

We next analysed levels of the free plasma AGEs CML, CEL and MG-H1 (Table 2 and Figure 3). At baseline, prior to treatment, plasma CML, CEL and MG-H1 appeared to be higher in the group randomised to placebo treatment, and this was statistically significant for CML and CEL (Table 2 and Figure 3). However, at 1- and 2-year follow-up we observed no differences between the placebo or irbesartan groups.

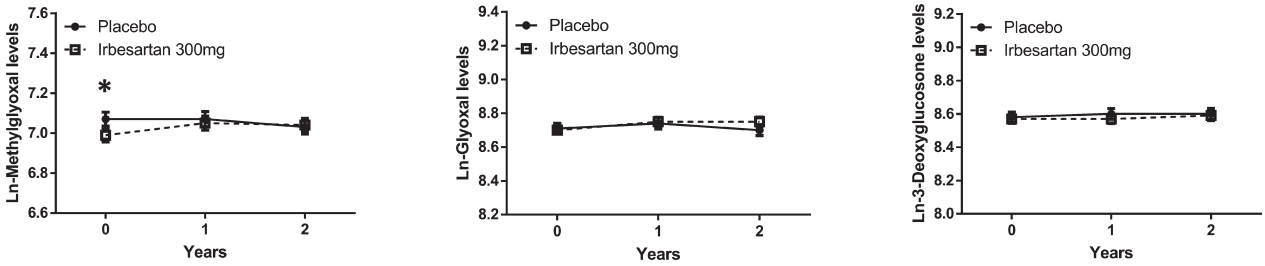

FIGURE 1 Two-way plots of plasma levels of Ln-transformed methylglyoxal, glyoxal and 3-deoxyglucosone at baseline, and 1- and 2-year follow-up for the irbesartan (IRB) 300 mg group and the placebo (PL) group. Data are presented as mean ± SE. *$P < 0.05$ vs. placebo. Differences between groups and over time were tested with repeated measures ANOVA, after Ln-transformation of the outcome variables

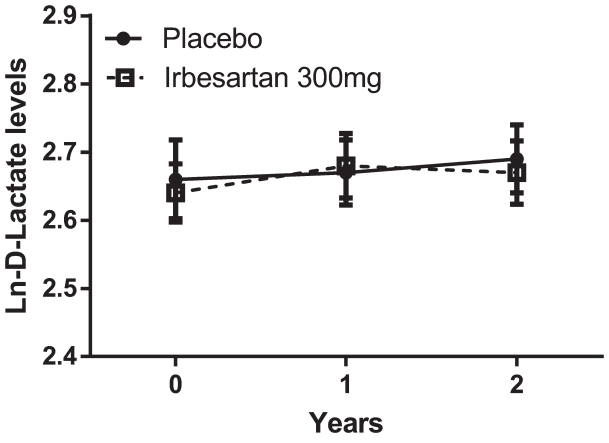

FIGURE 2 Two-way plot of plasma levels of D-lactate at baseline, and 1- and 2-year follow-up for the irbesartan (IRB) 300 mg group and the placebo (PL) group. Data are presented as mean ± SE. *$P < 0.05$ vs. placebo. Differences between groups and over time were tested with repeated measures ANOVA after Ln-transformation of the outcome variable

4 | DISCUSSION

The current analyses of the IRMA2 study showed that irbesartan at a concentration of 300 mg vs. placebo does not lower plasma levels of methylglyoxal or the other dicarbonyls glyoxal and 3-deoxyglucosone, D-lactate or the free plasma AGEs CML, CEL and MG-H1.

These results suggest that ARBs, or at least irbesartan, does not act as a potent agent to lower systemic dicarbonyl stress as reflected by changes in plasma dicarbonyl, D-lactate or free AGE levels, although the effectiveness of irbesartan has been demonstrated by its ability to reduce progression of albuminuria in the original trial. Because plasma dicarbonyl levels are not lower than in previous investigations we performed,[8,9,19] it seems unlikely that there was insufficient dicarbonyl stress in the current study to effectively detect a reduction by irbesartan. Our current finding is in line with prior analyses from our group in the IRMA2 subset, in which we found that the plasma protein-bound AGEs CML and CEL were not affected by irbesartan.[20] The current study expands upon this finding by direct measurements of dicarbonyls, as well as including free plasma AGEs and D-lactate as a potential marker of methylglyoxal detoxification by glyoxalase 1.

The IRMA2 study was performed in the clinical era prior to the newer glucose-lowering drugs such as sodium–glucose reuptake inhibitors (SGLT2i) and glucagon-like peptide 1 (GLP1) analogues. This is actually an advantage in terms of specifying the effects of ARBs on plasma dicarbonyls, because we can exclude an interaction of these newer compounds with plasma dicarbonyls, which could have masked the effects of irbesartan on plasma dicarbonyl levels. Adjustment for HbA_{1c} did not change our results, which makes it less likely that changes in glucose and thus dicarbonyls, covered the effect of ARBs on dicarbonyls.

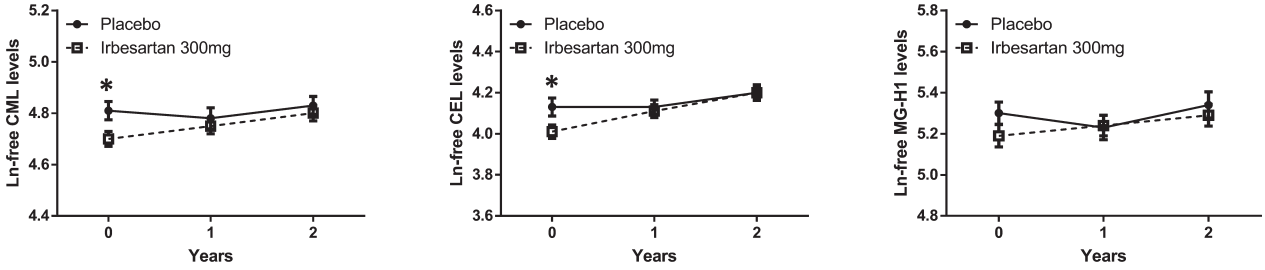

FIGURE 3 Two-way plots of plasma levels of free N^{ε}-carboxymethyllysine (CML), N^{ε}-carboxyethyllysine (CEL) and methylglyoxal-derived hydroimidazolone (MG-H1) at baseline, and 1- and 2-year follow-up for the irbesartan (IRB) 300 mg group and the placebo (PL) group. Data are presented as mean ± SE. *$P < 0.05$ vs. placebo. Differences between groups and over time were tested with repeated measures ANOVA after Ln-transformation of the outcome variable

Previous experimental studies have mainly focused on in-depth analyses of the influence of ARBs on methylglyoxal and glyoxalase expression in retinal and renal tissues[15,21,22] and it has been demonstrated that ARBs modulate methylglyoxal levels in specific microvascular beds.[15] This is likely achieved by blocking angiotensin II signalling and related down-regulation of glyoxalase 1. In this study, however, we did not find an effect of irbesartan on plasma levels of methylglyoxal. Because we do not have data about tissue methylglyoxal levels, we cannot rule out the possibility that there is an effect of irbesartan on methylglyoxal levels in specific tissues that are not reflected by changes in plasma glycation levels. This was indeed suggested by a rodent study that showed decreased CML levels in diabetic kidneys by the ARB valsartan, while overall plasma fluorescence as a reflection of glycation was not altered.[22] Therefore, we cannot disprove earlier pre-clinical studies based on our current investigation. Additionally, it might be that the beneficial effects of ARBs are restricted to experimental models of diabetes.

This study also has the limitation that we have only data about plasma levels of dicarbonyls and not urinary levels; we cannot exclude an effect of ARBs on urinary dicarbonyls. In fact, it has been demonstrated that irbesartan significantly decreased urinary excretion of the dicarbonyls-derived AGEs MG-H1 and glyoxal-derived hydroimidasolone (G-H1). As urinary free MG-H1 and G-H1 are thought to originate mainly from proteolysis of methylglyoxal and glyoxal-modified proteins,[23] these changes may indicate decreased dicarbonyl stress in renal tissues with irbesartan. This is in accordance with the effect of angiotensin blockade in regulation of glyoxalase 1 *in vitro*.[15] However, because of the potent effect of glucose-lowering treatment on plasma methylglyoxal levels in type 2 diabetes,[19,24] it is unlikely that ARBs constitute a main mitigating treatment to reduce dicarbonyl stress in diabetes.

4.1 | Conclusion

In conclusion, irbesartan did not lower plasma dicarbonyls and it is therefore less likely that ARBs attenuate renal and cardiovascular risk through attenuation of dicarbonyl stress. In addition, there is still an unmet need for compounds to effectively lower systemic dicarbonyl stress in order to reduce the burden of CKD and CVD in diabetes. It is not known whether more recent reno-protective agents like SGLT2 inhibitors and GLP1 receptor agonists have an effect on dicarbonyl stress. SGLT2 inhibitors in particular have demonstrated renal benefits and although initially introduced for their glucose-lowering effect, this seems not to explain the renal and cardiovascular benefits.

COMPETING INTERESTS

FP reports having received research grants from AstraZeneca, Novo Nordisk and Novartis and lecture fees from Novartis, Eli Lilly, MSD, AstraZeneca, Sanofi, Novo Nordisk and Boehringer Ingelheim and having served as a consultant for Astra Zeneca, Bayer, Amgen, Novo Nordisk and MSD. PR has received consultancy and/or speaking fees (to his institution) from Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Eli Lilly, Merck, Novo Nordisk, Sanofi Aventis, and Vifor. Research grants from AbbVie, AstraZeneca and Novo Nordisk.

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AUTHOR CONTRIBUTIONS

MP and NMJH analysed the data and wrote the manuscript. JLJMS measured dicarbonyls, wrote and edited the manuscript. MvW measured dicarbonyls and edited the manuscript. FP, MvG, PR, HHP and CDAS edited the manuscript. CGS is the principal investigator of the study, wrote and edited the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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