Effect of Metformin on Vascular Function in Children With Type 1 Diabetes: A 12-Month Randomized Controlled Trial

Jemma J. A. Anderson,[1,2] Jennifer J. Couper,[1,2] Lynne C. Giles,[3] Catherine E. Leggett,[1,4] Roger Gent,[5] Brian Coppin,[6] and Alexia S. Peña[1,2]

[1]Discipline of Paediatrics, Robinson Research Institute, University of Adelaide, North Adelaide, South Australia 5006, Australia; [2]Endocrinology and Diabetes Department, Women's and Children's Hospital, North Adelaide, South Australia 5006, Australia; [3]School of Public Health, Faculty of Health and Medical Sciences, University of Adelaide, North Adelaide, South Australia 5005, Australia; [4]Pharmacy, Women's and Children's Hospital, North Adelaide, South Australia 5006, Australia; [5]Medical Imaging, Women's and Children's Hospital, North Adelaide, South Australia 5006, Australia; and [6]Flinders Medical Centre, Bedford Park, South Australia 5042, Australia

Context: Children with type 1 diabetes have vascular dysfunction preceding atherosclerosis. Early interventions are needed to reduce cardiovascular disease.

Objective: To evaluate the effect of metformin on vascular function in children with type 1 diabetes.

Design: Twelve-month double-blind, randomized, placebo-controlled trial.

Setting: Tertiary pediatric diabetes clinic.

Participants: Ninety children (8 to 18 years of age), >50th percentile body mass index (BMI), with type 1 diabetes.

Intervention: Metformin (up to 1 g twice a day) or placebo.

Main Outcome Measure: Vascular function measured by brachial artery ultrasound [flow-mediated dilatation/glyceryl trinitrate–mediated dilatation (GTN)].

Results: Ninety participants were enrolled [41 boys, 13.6 (2.5) years of age, 45 per group], 10 discontinued intervention, and 1 was lost to follow-up. On metformin, GTN improved, independent of glycosylated hemoglobin (HbA1c), by 3.3 percentage units [95% confidence interval (CI) 0.3, 6.3, $P = 0.03$] and insulin dose reduced by 0.2 U/kg/d (95% CI 0.1, 0.3, $P = 0.001$) during 12 months, with effects from 3 months. Metformin had a beneficial effect on HbA1c at 3 months ($P = 0.001$) and difference in adjusted HbA1c between groups during 12 months was 1.0%; 95% CI 0.4, 1.5 (10.9 mmol/mol; 95% CI 4.4, 16.4), $P = 0.001$. There were no effects on carotid/aortic intima media thickness, BMI, lipids, blood pressure, or other cardiovascular risk factors. Median (95% CI) adherence, evaluated by electronic monitoring, was 75.5% (65.7, 81.5), without group differences. More gastrointestinal side effects were reported on metformin (incidence rate ratio 1.65, 95% CI 1.08, 2.52, $P = 0.02$), with no difference in hypoglycemia or diabetic ketoacidosis.

Conclusions: Metformin improved vascular smooth muscle function and HbA1c, and lowered insulin dose in type 1 diabetes children. These benefits and good safety profile warrant further consideration of its use. (***J Clin Endocrinol Metab*** **102: 4448–4456, 2017)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 2 April 2017. Accepted 3 October 2017.
First Published Online 9 October 2017

Abbreviations: BMI, body mass index; CI, confidence interval; DXA, dual-energy x-ray absorptiometry; FMD, flow-mediated dilatation; GTN, glyceryl trinitrate–mediated dilatation; HbA1c, glycosylated hemoglobin; IMT, intima media thickness; MEMS, medication event monitoring system; SD, standard deviation.

Cardiovascular disease is the leading cause of mortality in type 1 diabetes (1), and glycemic control is the major modifiable risk factor for prevention of vascular complications. However, individuals with type 1 diabetes and excellent glycemic control still have three times the risk for cardiovascular causes of death as matched controls (2), so that many patients need additional strategies to improve cardiovascular health.

Vascular dysfunction is a critical event in the development of cardiovascular disease and is detectable years before cardiovascular disease develops (3). Vascular function can be assessed by ultrasound measurement of brachial artery responses to increase in flow [flow-mediated dilatation (FMD)] and to glyceryl trinitrate [glyceryl trinitrate–mediated dilatation (GTN)]. FMD increases in artery diameter are dependent on endothelium nitric oxide release (endothelium-dependent response). Glyceryl trinitrate is a nitric oxide donor that increases the artery diameter independent of the endothelium and, therefore, assesses vascular smooth muscle response (4). Vascular function, measured by FMD and GTN, correlates with coronary atherosclerosis on angiography (5, 6) and with cardiovascular risk factors (6–8).

We and others have shown that vascular function is impaired and intima media thickness (IMT) is increased in children at increased risk of atherosclerosis, including children with type 1 diabetes (4, 9–12). Importantly, these early vascular changes in function and structure are potentially reversible.

Metformin reduces cardiovascular events and improves body composition and glycemic control in adults with type 2 diabetes (13, 14). In adults with type 1 diabetes, metformin has inconsistently improved glycosylated hemoglobin (HbA1c), body mass index (BMI), and insulin dose (15, 16). In one pilot study in adults with type 1 diabetes, metformin improved endothelial function (17). In children with type 1 diabetes, metformin can reduce HbA1c, insulin dose, and BMI (18–20), but there are no data on its effect on vascular health. Metformin stimulates nitric oxide synthesis *in vitro* in endothelium and smooth muscle (21). Therefore, we aimed to determine the effect of metformin on vascular health in children with type 1 diabetes and above average weight. We hypothesized that metformin would improve vascular function, independent of other benefits on cardiovascular risk factors.

Research Design and Methods

Study design and setting

This parallel, randomized, placebo-controlled trial was conducted at a single site at Women's and Children's Hospital in Adelaide (SA, Australia). It was approved by two recruitment sites (Women's and Children's Hospital HREC 2327/12/13 and Flinders Medical Centre HREC 443.12) and prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12611000148976). Participants were recruited from August 2011 and the trial was completed in June 2015. Written informed consent was obtained from all participants' parents, and written informed assent was obtained from all participants. Assessments were done at baseline, 3, 6, and 12 months. The study protocol was published (22) and is summarized later.

Study participants

Participants were eligible for the study if they were diagnosed with type 1 diabetes at least 6 months prior, had an insulin requirement >0.5 U insulin/kg/d, aged 8 to 18 years, and a BMI >50th percentile for age and sex (Centers for Disease Control and Prevention 2000 standardized reference charts; http://wwwn.cdc.gov/epiinfo/). They were excluded if they had a severe hypoglycemic episode in 6 months prior to recruitment, more than two episodes of diabetes ketoacidosis in the previous 12 months, serious comorbidities, contraindication to metformin therapy, or were already on metformin, statins, multivitamins, or antihypertensives. Participants were recruited and enrolled by a single investigator (J.J.A.A.) and assigned a code in sequence (1 to 90). Participants were allocated in a 1:1 ratio to metformin or placebo by the pharmacist using the randomization list generated by a statistician external to the study using statistical software S-PLUS version 8.1. Medication bottles were identical between groups aside from batch number, which the pharmacist used to allocate participants to treatment group (labeled as A or B). Participants, their care providers, and investigators were blinded to treatment group (22).

Intervention

Participants received up to 1 g twice a day according to weight (≥60 kg, 1 g twice per day; <60 kg, 500 mg twice per day). The dose was increased during 2 to 6 weeks up to the full dose, as tolerated. Metformin and placebo tablets (Generic Health, Melbourne, VIC, Australia) were identical in appearance and ingredients aside from the active ingredient, metformin hydrochloride. All participants received standardized type 1 diabetes dietary advice for 60 minutes from the one dietitian at baseline and 3 months (22).

Vascular function outcomes: FMD (primary outcome measure) and GTN (secondary outcome measure)

FMD and GTN were assessed at each visit, as we have previously described (4, 9, 12, 22, 23). Experienced and blinded sonographers (trained by R.G.) performed B mode ultrasound examinations with a 17 to 5 MHz linear array transducer (iU22; Phillips, Bothel, WA). In brief, brachial arterial diameter was measured in four scans: (1) baseline artery diameter scan; (2) reactive hyperemia was induced by occluding arterial blood flow using a sphygmomanometer inflated to 250 mm Hg for 4 minutes; FMD scan was recorded 45 to 75 seconds after cuff deflation; (3) recontrol scan 10 to 15 minutes later; and (4) GTN scan, taken 4 minutes after sublingual administration of GTN spray (400 μg, Nitrolingual pumpspray, G. Pohl-Boskamp, Hohenlockstedt, Germany). For each scan, measurements were made using ultrasonic calipers by observers blinded to intervention type over four consecutive cardiac cycles, incident with R wave on electrocardiogram (*i.e.*, at end-diastole).

Measurements were averaged and expressed as percentages of the baseline artery diameter scan. Our coefficient of variation between 20 controls was 3.9% for FMD and 4.0% for GTN (9, 22).

Secondary and other outcomes

HbA1c, insulin dose, BMI, body composition, waist circumference, mean of three consecutive blood pressures, fasting lipid profile, high-sensitivity C-reactive protein, adiponectin, leptin, and early morning urinary albumin/creatinine ratio were evaluated at all visits, as previously described (22). Estimated insulin sensitivity was calculated using a validated equation as follows: $4.06154 - 0.01317 \times$ waist (cm) $- 1.09615 \times$ insulin dose (U/kg/d) + $0.02027 \times$ adiponectin (μg/mL) $- 0.27168 \times$ triglycerides (mmol/L) $- 0.00733 \times$ diastolic blood pressure (mm Hg) (24). This surrogate marker of insulin sensitivity has been shown to correlate well with measured glucose infusion rate in euglycemic hyperinsulinemic clamps in adolescents and adults with type 1 diabetes (24).

Mean/maximum carotid IMT, aortic IMT, and total body dual-energy x-ray absorptiometry (DXA) scan were assessed at baseline and 12 months as previously described (11, 12, 22).

Safety outcomes and adherence assessment

Participants received fortnightly phone calls (J.J.A.A.) for the first 3 monthly and monthly phone calls thereafter to complete a side effects questionnaire, titrate medication, and adjust insulin doses. The single blinded investigator (J.J.A.A.) adjusted insulin during the commencement of the study medication, as the dose was increased according to the protocol, and then throughout the study participants were able to phone J.J.A.A. for adjustments and were requested to do so if there was any increased frequency of hypoglycemia. Additionally, all participants continued to see their standard health professionals (pediatric endocrinologist and diabetes educator) at 3 monthly intervals at diabetes clinics according to routine care and aiming for a target HbA1c of <7.5%.

The questionnaire included gastrointestinal and other side effects, as well as hypoglycemic events (defined as moderate if the participant required assistance and severe if the participant collapsed or had a seizure). Side effects were also recorded at study visits. Any hospital admissions were reported within 24 hours to an independent safety-monitoring committee. Events were reported regardless of whether they were considered related to medication. Lactate, liver, and renal function tests, vitamin B12, and folate status were measured at each visit (22). Study medication adherence was assessed at each visit by pill count and medication event monitoring system (MEMS) cap download (Aardex Group, Sion, Switzerland).

Statistical analysis

Demographic measures were summarized by mean and standard deviation (SD) or median and interquartile range for continuous variables according to normality. Distributions of all variables were assessed for normality using histograms and Kolmogorov–Smirnov tests. Variables with skewed distributions were log transformed, as appropriate, and the transformed variables were analyzed; geometric means were then reported for these transformed variables. Statistical significance was set at $P < 0.05$ (two-sided) with no adjustment for multiple comparisons. All analyses followed a prespecified analysis plan and used Stata version 14.1 (StataCorp, College Station, TX).

Analyses were performed (L.C.G.) on an intention-to-treat basis and blinded to which treatment group was metformin (*i.e.*, analyses were conducted comparing groups A and B). Once the analyses were completed, unblinding of groups occurred (A corresponded to placebo and B to metformin). Continuous outcomes were analyzed with linear mixed effects models, including treatment group, time, and their interaction in the models. Treatment effects were expressed as differences in means and 95% confidence interval (CI). Analyses were adjusted for age and HbA1c at each time point as well as sex.

A random subject effect was included in each statistical model to allow for correlation between observations on the same subject at different time points. Multiple imputation was performed separately by treatment group using chained equations to create 100 complete datasets (25). Sensitivity analyses with available data and different imputation models were performed to assess stability of results. The relative risk and associated 95% CI of any side effects for metformin vs placebo was calculated. Similarly, incidence rate ratios and 95% CI were calculated to compare total count of each side effect per participant between treatment groups.

Although no studies have looked at the effect of metformin on vascular function (FMD or GTN) in type 1 diabetes in this age group, our previous work found improvement in FMD of 3.1% (SD 4.3) in children with type 1 diabetes receiving folic acid during 8 weeks (23). Assuming equivalent improvement with metformin from baseline to 12 months, we estimated a total sample size of 90 participants would have 90% statistical power (with two-tailed α level of 0.05) to detect an absolute mean difference in FMD of 3.1% (SD 4.3) in comparison of metformin and placebo groups, allowing for 10% attrition.

Results

Of 428 consecutive clinic patients, 215 were ineligible, 123 declined, and 90 participants were randomized to metformin or placebo. One participant was lost to follow-up; 10 discontinued intervention (Fig. 1). Mean (SD) age of participants was 13.6 (3.5) years, and 54% (n = 49) were female. Median (interquartile range) HbA1c was 8.7% (8.1 to 9.9)/72 mmol/mol (65 to 85) and mean (SD) BMI *z* score was 0.89 (0.57). Fifty-four participants had normal BMI (BMI 50% to 84%), 25 were overweight (BMI 85% to 95%), and 11 were obese (BMI > 95%). Eighty-eight (97%) participants were white, one was African, and one was Asian. Twenty-one of 45 (47%) placebo and 22 of 45 (49%) metformin participants were <60 kg. There were no statistically significant differences between groups at baseline in each measured variable with the exception of diastolic blood pressure (Table 1).

Median (95% CI) adherence, evaluated by MEMS and pill count, was 75.5% (65.7, 81.5) and 76.6% (69.7, 80.5), respectively, during 12 months, with no differences between metformin and placebo groups. Adherence was highest at 3 months [MEMS median 88.9% (77.0, 91.6); pill count 87.1% (78.2, 90.5)] and decreased over time (time effect $P < 0.001$ for MEMS and pill count). All

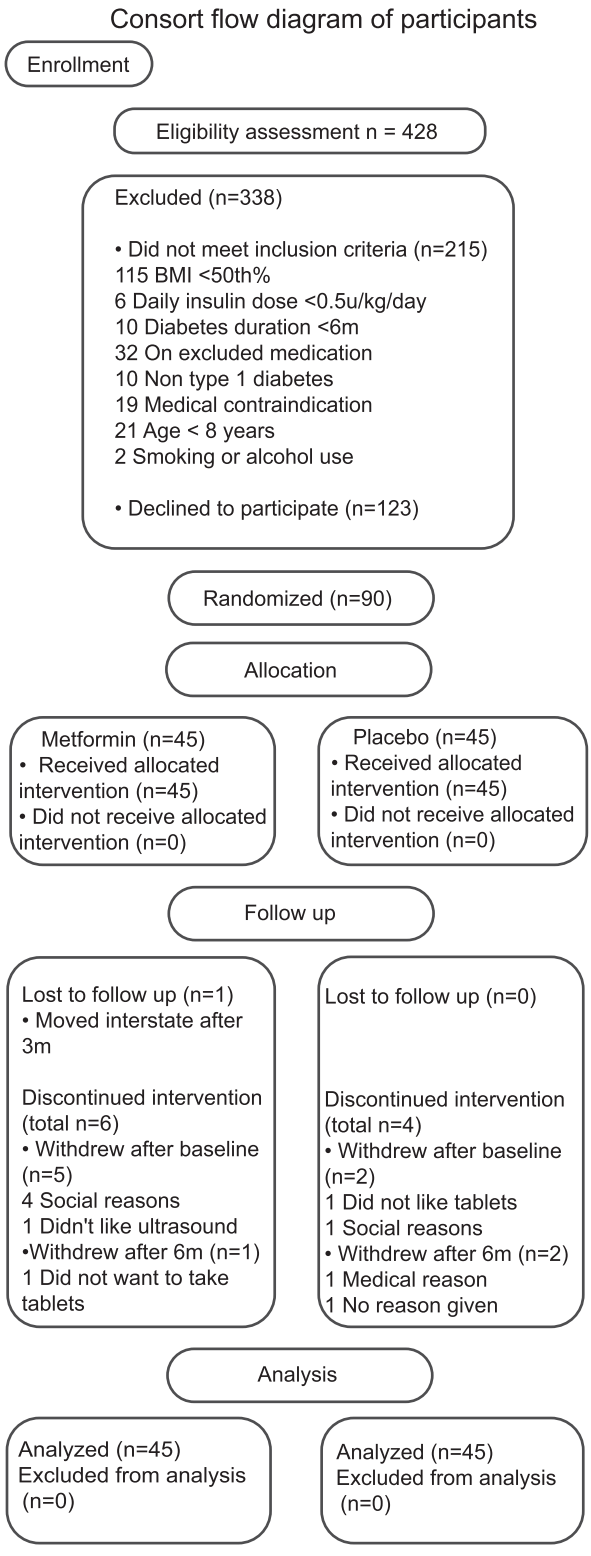

Figure 1. Consort flow diagram of participants.

participants tolerated the full dose according to the weight criteria, described previously, except for three participants <60 kg receiving metformin (two tolerated 250 mg twice per day and one tolerated 250 mg per day) and three participants >60 kg who tolerated 250 mg to 1.5 g per day. Side effects in these six patients included nausea (five of six), reduced appetite (one of six), vomiting (four of six), and diarrhea (two of six). All side effects resolved on the reduced metformin dose.

Cardiovascular health and risk factors

Vascular smooth muscle function (GTN) improved, independent of HbA1c, by 3.3 percentage units (95% CI 0.3, 6.3, $P = 0.03$) during the 12-month intervention in the metformin group compared with placebo (Table 2). The improvement in GTN was also independent of baseline pubertal status in the participants (coefficient 3.4 percentage units; 95% CI 0.4, 6.4, $P = 0.03$). Sensitivity analyses with available data and different imputation models showed the same results. GTN adjusted for covariates (age, sex, and HbA1c) was highest in the metformin group at 3 months compared with placebo [25.0 percentage units (95% CI 22.9, 27.2) vs 21.7 percentage units (95% CI 19.6, 23.8), $P = 0.03$].

There was no significant effect of metformin on other measures of vascular health and cardiovascular risk factors, including FMD, blood pressure, BMI, waist and hip circumference, fat mass, lipid profile, high-sensitivity C-reactive protein, adiponectin, adiponectin/leptin ratio, and urine albumin creatinine ratio during 12 months. There was a significant decrease in leptin in the metformin group (Table 2). Inclusion of study medication dose/kg/d and adherence, as measured by tablet count or MEMS made no significant difference in the results for FMD or GTN (data not shown).

There was no significant effect of metformin on mean/maximum aortic IMT (−0.02 mm; 95% CI −0.08, 0.03, $P = 0.4$/−0.04 mm; 95% CI −0.1, 0.02, $P = 0.2$), mean/maximum carotid IMT (−0.01 mm; 95% CI −0.04, 0.01, $P = 0.3$/−0.01 mm; 95% CI −0.04, 0.02, $P = 0.5$), DXA fat (−0.8 percentage; 95% CI −4.4, 2.7, $P = 0.7$) or DXA fat mass (−1.7 kg; 95% CI −5.1, 1.8, $P = 0.3$) during 12 months. There was a difference in DXA lean mass at 12 months in the metformin group of 2.9 kg (95% CI −5.4, −0.3, $P = 0.03$) compared with placebo.

Glycemic control and insulin dose

There was a significant benefit in adjusted (age, sex) HbA1c at 3 months for the metformin group (8.4%; 95% CI 8.0, 8.8) (68 mmol/mol; 95% CI 64, 73) vs placebo group (9.3%; 95% CI 9.0, 9.7) (78 mmol/mol; 95% CI 75, 83) ($P = 0.001$), and this was primarily responsible for the overall benefit of metformin compared with placebo during the study period. The adjusted overall difference in HbA1c between the groups during the intervention was 1.0% (95% CI 0.4, 1.5) 10.9 mmol/mol (95% CI 4.4, 16.4), $P = 0.001$ (Table 2) during 12 months. HbA1c was significantly related to study medication dose, measured in mg/kg/d, but the magnitude of the dose effect was very

Table 1. Baseline Characteristics

Variable	Placebo (n = 45)	Metformin (n = 45)
Age, y	13.3 ± 2.6	14.0 ± 2.5
Sex (M/F)	20/25	21/24
Diabetes duration, y	5.8 ± 4.1	5.2 ± 3.6
Puberty, n (pre/mid/post)	14/9/22	11/7/27
Insulin regimen (MDI/CSII)	23/22	22/23
Insulin dose, U/kg/d	0.85 ± 0.21	0.82 ± 0.22
BMI *z* score	0.9 ± 0.5	0.9 ± 0.6
Weight *z* score	1.1 ± 0.7	0.9 ± 0.7
Waist circumference, cm	71 ± 1	72 ± 9
Hip circumference, cm	89 ± 1	89 ± 9
Waist/hip ratio	0.8 ± 0.1	0.8 ± 0.1
DXA total lean mass, kg	39 ± 11	39 ± 9
DXA fat, %	31 ± 10	31 ± 10
BIA fat, %	27.8 ± 7.1	27.1 ± 6.8
BIA fat mass, kg	17.1 ± 8.1	16.5 ± 6.7
BIA fat free mass, kg	42.5 ± 11.6	43.2 ± 10.0
Systolic blood pressure, mm Hg	112 ± 8	112 ± 9
Diastolic blood pressure, mm Hg[a]	62 ± 5	64 ± 6
Total cholesterol, mmol/L	4.4 ± 0.8	4.4 ± 0.7
Triglycerides, mmol/L	0.8 ± 0.3	0.8 ± 0.3
HDL cholesterol, mmol/L	1.6 ± 0.3	1.6 ± 0.4
LDL cholesterol, mmol/L	2. 5 ± 0.7	2. 5 ± 0.5
Glucose, mmol/L	12 ± 5	10 ± 4
HbA1c, %[b]	8.8 (8.2–9.9)	8.4 (7.8–9.7)
HbA1c, mmol/mol[b]	73 (66–85)	68 (62–83)
hsCRP, mg/L	2.7 ± 7.1	2.3 ± 5.4
Alanine transaminase, IU/L	14.4 ± 3.9	15.2 ± 5.5
Urea, mmo/L	4.8 ± 1.1	4.3 ± 1.1
Creatinine, μmol/L	48.8 ± 10.8	51.8 ± 11.6
Lactate, mmol/L	1.2 ± 0.7	1.1 ± 0.4
Homocysteine, μmol/L	5.6 ± 1.4	6.2 ± 1.9
Adiponectin, μg/mL	13.8 ± 8.8	12.2 ± 6.1
Leptin, ng/mL	12.6 ± 1.7	11.7 ± 1.6
Adiponectin/leptin ratio, μg/ng	2.3 ± 3.7	2.0 ± 2.2
Vitamin B12, pmol/L	477 ± 181	414 ± 168
Urine albumin/creatinine ratio, mg/mmol	1.0 ± 1.8	0.9 ± 1.6
Mean aortic IMT, mm	0.5 ± 0.1	0.5 ± 0.1
Mean carotid IMT, mm	0.4 ± 0.1	0.4 ± 0.1
Maximum aortic IMT, mm	0.6 ± 0.1	0.6 ± 0.1
Maximum carotid IMT, mm	0.5 ± 0.1	0.5 ± 0.1
FMD, percentage units	6.3 ± 4.5	6.1 ± 4.5
GTN–mediated dilatation, percentage units	23.7 ± 6.7	25.3 ± 6.2
Brachial artery diameter, cm	0.3 ± 0.04	0.3 ± 0.04

Data are means ± SD.

Abbreviations: BIA, bioelectrical impedance analysis; CSII, continuous subcutaneous insulin infusion; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MDI, multiple daily injection.

[a] $P = 0.03$.

[b] Median (interquartile range).

small (0.08%; 95% CI 0.04, 0.12) (0.9 mmol/mol; 95% CI 0.4, 1.3) ($P < 0.001$).

Total daily insulin dose was reduced by 0.2 U/kg/d during 12 months (95% CI 0.1, 0.3, $P = 0.001$) in the metformin group compared with placebo (Table 2). The adjusted insulin doses remained the same in each group from 3 to 12 months [metformin, 0.7 (95% CI 0.4, −1.0); placebo, 0.9 (95% CI 0.6, −1.2) U/kg/d, $P = 0.001$]. Estimated insulin sensitivity as calculated previously remained 0.2 U higher during 12 months (95% CI 0.06, 0.34, $P = 0.005$) in the metformin group compared with placebo.

Those who discontinued intervention (n = 11, metformin = 7, placebo = 4) vs those who continued intervention for 12 months (n = 79) had higher baseline (mean ± SD) HbA1c (10.3% ± 2.2% vs 8.8% ± 1.2%, 89 ± 24 vs 73 ± 13.1 mmol/mol, $P = 0.001$), total cholesterol (4.8 ± 1.0 vs 4.1 ± 1.0 mmol/L, $P = 0.01$), low-density lipoprotein cholesterol (2.9 ± 0.7 vs 2.4 ± 0.5, $P = 0.01$) and total daily insulin dose (1.0 ± 0.2 vs 0.8 ± 0.2 U/kg/d, $P = 0.003$). There were no statistically significant differences in other baseline variables, including vascular function: FMD (6.3 ± 4.5 vs 6.1 ± 4.5 percentage units, $P = 0.9$) and GTN (23.7 ± 6.7 vs 25.3 ± 6.2 percentage units, $P = 0.2$).

Safety data

A total of 133 side effects were reported with more side effects in the metformin group compared with placebo [80 vs 53, relative risk 1.51 (95% CI 1.05, 2.18), $P = 0.02$]. Gastrointestinal side effects were more common in the metformin group compared with placebo (Table 3). There were no significant differences in nongastrointestinal side effects, moderate hypoglycemic events, or diabetes ketoacidosis between groups (Table 3). There were no episodes of severe hypoglycemia or lactic acidosis during 12 months.

Vitamin B12 levels were significantly lower overall in the metformin group compared with placebo but were still within reported reference range (140 to 700 pmol/L) with no change in homocysteine levels (Table 2). There was no significant difference in lactate, liver function, and renal function tests between metformin and placebo groups during 12 months (Table 2).

Conclusions

We report that metformin improved vascular smooth muscle function during 12 months in above average weight children with type 1 diabetes. The effect was modest and independent of the improvement in HbA1c on metformin. Benefits for both vascular smooth function and HbA1c were greatest at 3 months, when the participants' adherence was at its highest. These benefits were seen in participants who were prepubertal or pubertal at baseline. There was no significant benefit of metformin on measures of vascular structure, nor on traditional cardiovascular risk factors.

Table 2. Primary, Secondary, and Safety Outcomes

Variable	Adjusted Mean (SE)						Adjusted Overall Treatment Effect (95% CI)[a]	*P*
	3 Months		6 Months		12 Months			
	Placebo	Metformin	Placebo	Metformin	Placebo	Metformin		
Primary outcome measure								
FMD, percentage units	5.5 (0.6)	6.5 (0.6)	5.7 (0.5)	6.0 (0.6)	6.8 (0.5)	5.9 (0.5)	1.1 (−0.7, 2.8)	0.2
Secondary outcome measures								
GTN, percentage units	21.7 (1.1)	25.0 (1.1)	22.1 (1.0)	24.5 (1.1)	22.9 (1.1)	25.1 (1.2)	3.3 (0.3, 6.3)	0.03
Brachial artery diameter, cm	0.28 (0.01)	0.29 (0.01)	0.28 (0.01)	0.29 (0.01)	0.28 (0.01)	0.29 (0.01)	−0.005 (−0.02, 0.01)	0.5
Insulin dose, U/kg/d	0.9 (0.03)	0.7 (0.03)	0.9 (0.03)	0.7 (0.03)	0.9 (0.03)	0.7 (0.03)	−0.2 (−0.3, −0.1)	0.001
HbA1c, %[b]	9.3 (0.2)	8.4 (0.2)	9.3 (0.2)	8.7 (0.2)	9.5 (0.2)	9.0 (0.3)	−1.0 (−1.5, −0.4)	0.001
HbA1c, mmol/mol[b]	78 (17.5)	68 (9.8)	78 (17.5)	72 (13.1)	80 (18.6)	75 (16.4)	−10.9 (−16.4, −4.4)	0.001
Glucose, mmol/L	11.5 (0.7)	11.1 (0.7)	11.1 (0.6)	11.5 (0.7)	11.0 (0.6)	11.3 (0.7)	−0.4 (−2.4, 1.6)	0.7
hsCRP, mg/L[c]	1.3 (0.1)	1.0 (0.1)	1.3 (0.1)	1.0 (0.1)	1.8 (0.1)	1.2 (0.1)	−0.1 (−0.3, 0.1)	0.3
LDL, mmol/L	2.5 (0.1)	2.3 (0.1)	2.5 (0.1)	2.4 (0.1)	2.3 (0.1)	2.2 (0.1)	−0.2 (−0.4, 0.1)	0.2
HDL, mmol/L	1.6 (0.1)	1.5 (0.1)	1.6 (0.1)	1.5 (0.1)	1.6 (0.1)	1.6 (0.1)	−0.1 (−0.2, 0.1)	0.4
Total cholesterol, mmol/L	4.4 (0.1)	4.2 (0.1)	4.4 (0.1)	4.3 (0.1)	4.3 (0.1)	4.2 (0.1)	−0.2 (−0.6, 0.2)	0.3
Triglycerides, mmol/L	0.8 (0.1)	0.8 (0.1)	0.9 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	−0.01 (−0.2, 0.2)	0.9
Adiponectin, μg/mL	11.3 (1.0)	10.1 (1.1)	11.4 (1.0)	8.8 (1.0)	11.2 (1.0)	9.2 (1.0)	−1.2 (−4.1, 1.6)	0.4
Leptin, ng/mL[c]	13.2 (0.1)	8.6 (0.1)	8.4 (0.1)	13.3 (0.1)	12.7 (0.1)	9.5 (0.1)	−0.3 (−0.5, −0.1)	0.02
Adiponectin/leptin ratio	2.4 (0.7)	2.6 (0.8)	2.5 (0.7)	2.1 (0.7)	2.1 (0.8)	1.9 (0.8)	0.2 (−1.9, 2.3)	0.9
Urea, mmol/L	4.7 (0.2)	4.7 (0.2)	4.5 (0.2)	4.7 (0.2)	4.6 (0.2)	4.5 (0.2)	0.04 (−0.4, 0.5)	0.9
Creatinine, μmol/L	52.6 (1.2)	52.6 (1.2)	52.1 (1.2)	51.7 (1.2)	52.0 (1.2)	52.4 (1.3)	0.02 (−3.4, 3.4)	1.0
ALT, IU/L	14.7 (0.9)	14.5 (1.0)	15.8 (0.9)	14.9 (1.0)	14.2 (0.9)	14.0 (0.9)	−0.2 (−2.9, 2.5)	0.9
Homocysteine, μmol/L	6.0 (0.2)	6.5 (0.3)	6.1 (0.3)	6.3 (0.3)	6.3 (0.3)	7.1 (0.3)	0.5 (−0.3, 1.1)	0.2
Urine ACR, mg/mmol	0.9 (0.4)	0.9 (0.4)	0.8 (0.3)	1.0 (0.3)	0.6 (1.1)	1.5 (0.3)	0.5 (−0.5, 1.5)	1.0
Waist, cm[b]	73.4 (1.2)	71.0 (1.2)	73.0 (1.1)	70.4 (1.2)	73.2 (1.1)	70.2 (1.2)	−2.4 (−5.8, 1.0)	0.2
Hip, cm[b]	92.4 (1.0)	90.5 (1.0)	92.8 (1.0)	90.6 (1.0)	92.6 (1.1)	90.0 (1.2)	−1.9 (−4.6, 0.8)	0.2
Waist/hip ratio[b]	0.8 (0.01)	0.8 (0.01)	0.8 (0.01)	0.8 (0.01)	0.8 (0.01)	0.8 (0.01)	−0.01 (−0.04, 0.01)	0.4
BIA fat, %[b]	28.0 (0.8)	27.0 (0.9)	28.0 (0.8)	26.3 (0.9)	27.8 (0.9)	26.5 (0.9)	−1.0 (−3.3, 1.4)	0.4
BIA fat free mass, kg[b]	46.3 (1.0)	43.8 (1.0)	46.1 (0.9)	43.9 (1.0)	46.0 (1.0)	43.2 (1.0)	−2.5 (−5.2, 0.2)	0.07
Systolic BP, mm Hg[b]	113.1 (1.1)	110.6 (1.1)	111.2 (1.1)	111.2 (1.1)	112.4 (1.1)	110.0 (1.1)	−2.5 (−5.6, 0.6)	0.1
Diastolic BP, mm Hg[b]	62.5 (0.7)	62.4 (0.7)	62.0 (0.7)	62.8 (0.7)	62.2 (0.7)	62.7 (0.8)	−0.1 (−2.0, 1.7)	0.9
Safety outcome measures								
Lactate, mmol/L	1.3 (0.1)	1.4 (0.1)	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)	0.1 (−0.2, 0.4)	0.5
Vitamin B12, pmol/L	494 (21)	407 (22)	489 (24)	377 (25)	451 (33)	347 (34)	−87.3 (−148.4, −26.2)	0.01

Abbreviations: ACR, albumin/creatinine ratio; ALT, alanine aminotransferase; BIA, bioelectric impedance analysis; BP, blood pressure; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; SE, standard error.

[a] Adjusted for age, sex, and HbA1c, unless otherwise indicated.

[b] Adjusted for age and sex only.

[c] Geometric means.

The study contributes important original findings to our knowledge of metformin use in children with type 1 diabetes. First, it has shown an effect on vascular function. Second, children studied were above average weight but most were not overweight, unlike other metformin studies in adolescents (18, 26). Therefore, our results can be applied to patients of a wider weight range and different pubertal status. Third, the study was of longer duration than previous studies in childhood and as such was well placed to demonstrate a good safety profile during 12 months. Finally, adherence was measured objectively using prospective electronic monitoring.

The significance of vascular smooth muscle function as measured by GTN is as follows: it relates to preclinical carotid atherosclerosis in children (27) and in adults (28) with accelerated atherosclerosis. It is impaired in adults with coronary artery disease (6). It can be impaired independently of endothelial dysfunction (7) and in some studies it is a better predictor than FMD of cardiovascular events and coronary artery calcification (29, 30). Furthermore, the improvement in vascular function, independent of HbA1c, is consistent with metformin's direct effect *in vitro* on smooth muscle by stimulating nitric oxide synthesis via activation of AMP kinase in smooth muscle (21), which is reflected by improvement in GTN rather than FMD. The effect on GTN alone rather than both GTN and FMD does suggest a more modest benefit of metformin on vascular function.

The HbA1c improvement with a fall in insulin dose, and an increase in estimated insulin sensitivity during 12 months in the metformin group, provides a significant clinical benefit. Note that overall benefit of metformin

Table 3. Summary of Side Effects

	Metformin (n = 45)	Placebo (n = 45)	Total (n = 90)	RR/IRR (95% CI)	*P*
Gastrointestinal side effects					
No. affected, n (%)	22 (49)	14 (31)	36 (40)	1.57[a] (0.93–2.66)	0.09
No. per participant					
Median (IQR)	0 (0-2)	0 (0-1)	0 (0-1)	1.65[b] (1.08–2.52)	0.02
Nongastrointestinal side effects					
No. affected, n (%)	10 (22)	10 (22)	20 (22)	1.00[a] (0.46–2.17)	1.00
No. per participant					
Median (IQR)	0 (0,0)	0 (0,0)	0 (0,0)	1.17[b] (0.54–2.52)	0.7
Moderate hypoglycemic events					
No. affected, n (%)	4 (9)	2 (4)	6 (7)	2.00[a] (0.39–10.38)	0.4
No. per participant					
Median (IQR)	0 (0,0)	0 (0,0)	0 (0,0)	4.00[b] (0.85–18.84)	0.08
Diabetic ketoacidosis					
No. affected, n (%)	2 (4)	2 (4)	4 (4)	1.00[a] (0.15–6.79)	1.00
No. per participant					
Median (IQR)	0 (0,0)	0 (0,0)	0 (0,0)	1.00[b] (0.14–7.10)	1.00

Abbreviations: IQR, interquartile range; IRR, incidence rate ratio; RR, risk ratio.

[a]Risk ratios reported for comparison of participants with side effects between metformin and placebo.

[b]Incidence rate ratios for comparison of number of events per participant between metformin and placebo.

was primarily explained by the improvement in HbA1c at 3 months, which then waned over time. Most children with type 1 diabetes in Australia do not achieve target HbA1c levels (31, 32), and approximately a third of children with type 1 diabetes in Australia are overweight (32). Therefore, the combination of improved metabolic control and a lower insulin dose requirement is particularly relevant to this group. A similar improvement in HbA1c from a similar baseline level was not sustained beyond 13 weeks in a recent randomized controlled trial of metformin in adolescents with type 1 diabetes (18). There were several differences in this United States study in comparison with our study that may explain this: the adolescents were all overweight/obese, had a higher insulin dose, were older and mostly postpubertal, and had a longer duration of type 1 diabetes. Additionally, these adolescents were required to be adherent with blood glucose monitoring prior to study entry, and HbA1c level >10% was an exclusion criteria. Additionally, the study duration was 6 months and was conducted in 26 centers, with a higher number of clinicians adjusting insulin doses, whereas our study was conducted in one center with only four pediatric endocrinologists, in addition to the primary investigator, adjusting insulin doses throughout the study to aid consistency.

Metformin was well tolerated in our study, as expected from previous systematic reviews in children (33, 34). The mild fall in vitamin B12 detected on metformin, without homocysteine changes, would not normally be regarded as clinically significant, unless baseline levels of vitamin B12 were already low. No participant experienced severe hypoglycemia, and moderate hypoglycemia was not significantly increased in the metformin group. In addition to weekly phone calls, participants had 24-hour access to one of four clinicians if they experienced an increase in mild hypoglycemic events, and insulin dose was adjusted accordingly. This consistency in care may explain why we had no severe hypoglycemia unlike some other studies (18, 20, 26).

We did not demonstrate any reduction in BMI, body fat percentage, waist circumference, or adiponectin/leptin ratio during 12 months. This is in contrast to other studies of metformin in overweight/obese children with type 1 diabetes. The difference may be explained by our inclusion criteria threshold of BMI > 50th percentile rather than overweight/obese. Meta-analysis supports this difference in study findings whereby BMI is reduced in overweight/obese, but not in above average weight, populations (33). The loss in lean body mass in the metformin group was an unexpected finding of uncertain clinical significance.

The strengths of our study are that it was conducted at a single center with the same experienced sonographer performing ultrasound studies for assessment of vascular function and conferring high fidelity on all measures. Adherence was accurately assessed with two objective measures (MEMS caps and pill count), and there was a high study retention rate during 12 months. Limitations were that the duration of 12 months only provided the opportunity to realistically detect change in vascular function. Change in vascular structure likely requires several years of follow-up in type 1 diabetes (35–37), and the study was powered to detect significant change in vascular dysfunction, not other vascular structural markers. Additionally, we relied on participant reports of hypoglycemia, so accurate assessment of severe and moderate episodes, but not mild episodes, of hypoglycemia could be made.

In summary, the demonstrated benefit of metformin on vascular function, HbA1c, insulin dose, and estimated insulin sensitivity during 12 months in children with type 1 diabetes who are above average weight, as well as the good safety profile, warrants ongoing consideration of its use in this population.

Acknowledgments

The data in this study were the subject of a poster presented at: Endocrine Society Annual Meeting; 1–4 April 2016; Boston, MA. The authors thank Drs. Jan Fairchild and Elaine Tham (Endocrinology and Diabetes Department, Women's and Children's Hospital, North Adelaide, SA, Australia) and Drs. Felix Tan and David Everett (Flinders Medical Centre, Bedford Park, SA, Australia) for clinical care of study participants, as well as Dr. Oana Maftei (Endocrinology and Diabetes Department, Women's and Children's Hospital, North Adelaide, SA, Australia) and Melissa LaForgia (Medical Imaging, Women's and Children's Hospital, North Adelaide, SA, Australia) for assistance with analysis of vascular health measures. The authors thank all participants in the study for their involvement.

Financial Support: This study was funded by Diabetes Australia Research Trust, Women's and Children's Foundation research project grants and Australasian Paediatric Endocrine Care grants. J.J.A.A. held an M.S. McLeod Research Foundation fellowship. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Clinical Trial Information: Australian New Zealand Clinical Trials Registry no. ACTRN12611000148976 (registered 8 February 2011).

Author Contributions: J.J.C., C.E.L., A.S.P., and J.J.A.A. had full access to all of the data in the study and take responsibility for the integrity of the contents of the article, the data, and the accuracy of the data analysis. A.S.P., J.J.C., R.G., and J.J.A.A. conceived and designed the study. A.S.P., J.J.C., J.J.A.A., R.G., C.E.L., B.C., and L.C.G. acquired, analyzed, and interpreted the data. L.C.G. provided statistical analysis; A.S.P., J.J.C., C.E.L., and J.J.A.A. drafted the manuscript. All authors critically reviewed the manuscript for important intellectual content and approved the final version for publication. A.S.P., J.J.C., and J.J.A.A. obtained funding for this study.

Correspondence and Reprint Requests: Jemma J. A. Anderson, MBBS, Discipline of Paediatrics, Women's and Children's Hospital, 72 King William Road, North Adelaide, South Australia 5006, Australia. E-mail: jemma.anderson@adelaide.edu.au.

Disclosure Summary: The authors have nothing to disclose.

References

1. Secrest AM, Becker DJ, Kelsey SF, Laporte RE, Orchard TJ. Cause-specific mortality trends in a large population-based cohort with long-standing childhood-onset type 1 diabetes. *Diabetes*. 2010;**59**(12):3216–3222.
2. Lind M, Svensson AM, Kosiborod M, Gudbjörnsdottir S, Pivodic A, Wedel H, Dahlqvist S, Clements M, Rosengren A. Glycemic control and excess mortality in type 1 diabetes. *N Engl J Med*. 2014;**371**(21):1972–1982.
3. Cohen R. Dysfunction of vascular endothelium in diabetes mellitus. *Circulation*. 1993;**87**:V67–V76.
4. Peña AS, Wiltshire E, MacKenzie K, Gent R, Piotto L, Hirte C, Couper J. Vascular endothelial and smooth muscle function relates to body mass index and glucose in obese and nonobese children. *J Clin Endocrinol Metab*. 2006;**91**(11):4467–4471.
5. Neunteufl T, Katzenschlager R, Hassan A, Klaar U, Schwarzacher S, Glogar D, Bauer P, Weidinger F. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis*. 1997;**129**(1):111–118.
6. Raitakari OT, Seale JP, Celermajer DS. Impaired vascular responses to nitroglycerin in subjects with coronary atherosclerosis. *Am J Cardiol*. 2001;**87**(2):217–219.
7. Adams MR, Robinson J, McCredie R, Seale JP, Sorensen KE, Deanfield JE, Celermajer DS. Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. *J Am Coll Cardiol*. 1998;**32**(1):123–127.
8. Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, Kajikawa M, Matsumoto T, Hidaka T, Kihara Y, Chayama K, Noma K, Nakashima A, Goto C, Higashi Y. Nitroglycerine-induced vasodilation for assessment of vascular function: a comparison with flow-mediated vasodilation. *Arterioscler Thromb Vasc Biol*. 2013;**33**(6):1401–1408.
9. Wiltshire EJ, Gent R, Hirte C, Pena A, Thomas DW, Couper JJ. Endothelial dysfunction relates to folate status in children and adolescents with type 1 diabetes. *Diabetes*. 2002;**51**(7):2282–2286.
10. Järvisalo MJ, Raitakari M, Toikka JO, Putto-Laurila A, Rontu R, Laine S, Lehtimäki T, Rönnemaa T, Viikari J, Raitakari OT. Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. *Circulation*. 2004;**109**(14):1750–1755.
11. Maftei O, Pena AS, Sullivan T, Jones TW, Donaghue KC, Cameron FJ, Davis E, Cotterill A, Craig ME, Gent R, Dalton N, Daneman D, Dunger D, Deanfield J, Couper JJ; AdDIT Study Group. Early atherosclerosis relates to urinary albumin excretion and cardiovascular risk factors in adolescents with type 1 diabetes: Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AdDIT). *Diabetes Care*. 2014;**37**(11):3069–3075.
12. Harrington J, Peña AS, Gent R, Hirte C, Couper J. Aortic intima media thickness is an early marker of atherosclerosis in children with type 1 diabetes mellitus. *J Pediatr*. 2010;**156**(2):237–241.
13. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet*. 1998;**352**(9131):854–865.
14. Lee A, Morley JE. Metformin decreases food consumption and induces weight loss in subjects with obesity with type II non-insulin-dependent diabetes. *Obes Res*. 1998;**6**(1):47–53.
15. Liu C, Wu D, Zheng X, Li P, Li L. Efficacy and safety of metformin for patients with type 1 diabetes mellitus: a meta-analysis. *Diabetes Technol Ther*. 2015;**17**(2):142–148.
16. Vella S, Buetow L, Royle P, Livingstone S, Colhoun HM, Petrie JR. The use of metformin in type 1 diabetes: a systematic review of efficacy. *Diabetologia*. 2010;**53**(5):809–820.
17. Pitocco D, Zaccardi F, Tarzia P, Milo M, Scavone G, Rizzo P, Pagliaccia F, Nerla R, Di Franco A, Manto A, Rocca B, Lanza GA, Crea F, Ghirlanda G. Metformin improves endothelial function in type 1 diabetic subjects: a pilot, placebo-controlled randomized study. *Diabetes Obes Metab*. 2013;**15**(5):427–431.
18. Libman IM, Miller KM, DiMeglio LA, Bethin KE, Katz ML, Shah A, Simmons JH, Haller MJ, Raman S, Tamborlane WV, Coffey JK, Saenz AM, Beck RW, Nadeau KJ; T1D Exchange Clinic Network Metformin RCT Study Group. 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.
19. Nadeau KJ, Chow K, Alam S, Lindquist K, Campbell S, McFann K, Klingensmith G, Walravens P. Effects of low dose metformin in adolescents with type I diabetes mellitus: a randomized, double-blinded placebo-controlled study. *Pediatr Diabetes*. 2015;**16**(3): 196–203.
20. Hamilton J, Cummings E, Zdravkovic V, Finegood D, Daneman D. Metformin as an adjunct therapy in adolescents with type 1 diabetes and insulin resistance: a randomized controlled trial. *Diabetes Care*. 2003;**26**(1):138–143.
21. Davis BJ, Xie Z, Viollet B, Zou MH. Activation of the AMP-activated kinase by antidiabetes drug metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase. *Diabetes*. 2006; **55**(2):496–505.
22. Anderson J, Peña AS, Sullivan T, Gent R, D'Arcy B, Olds T, Coppin B, Couper J. Does metformin improve vascular health in children with type 1 diabetes? Protocol for a one year, double blind, randomised, placebo controlled trial. *BMC Pediatr*. 2013;**13**(1): 108.
23. Peña AS, Wiltshire E, Gent R, Hirte C, Couper J. Folic acid improves endothelial function in children and adolescents with type 1 diabetes. *J Pediatr*. 2004;**144**(4):500–504.
24. Duca LM, Maahs DM, Schauer IE, Bergman BC, Nadeau KJ, Bjornstad P, Rewers M, Snell-Bergeon JK. Development and validation of a method to estimate insulin sensitivity in patients with and without type 1 diabetes. *J Clin Endocrinol Metab*. 2016; **101**(2):686–695.
25. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, Wood AM, Carpenter JR. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ*. 2009;**338**(jun29 1):b2393.
26. Nwosu BU, Maranda L, Cullen K, Greenman L, Fleshman J, McShea N, Barton BA, Lee MM. A randomized, double-blind, placebo-controlled trial of adjunctive metformin therapy in overweight/obese youth with type 1 diabetes. *PLoS One*. 2015; **10**(9):e0137525.
27. Järvisalo MJ, Lehtimäki T, Raitakari OT. Determinants of arterial nitrate-mediated dilatation in children: role of oxidized low-density lipoprotein, endothelial function, and carotid intima-media thickness. *Circulation*. 2004;**109**(23):2885–2889.
28. Kawano N, Emoto M, Mori K, Yamazaki Y, Urata H, Tsuchikura S, Motoyama K, Morioka T, Fukumoto S, Shoji T, Koyama H, Okuno Y, Nishizawa Y, Inaba M. Association of endothelial and vascular smooth muscle dysfunction with cardiovascular risk factors, vascular complications, and subclinical carotid atherosclerosis in type 2 diabetic patients. *J Atheroscler Thromb*. 2012; **19**(3):276–284.
29. Kullo IJ, Malik AR, Bielak LF, Sheedy PF II, Turner ST, Peyser PA. 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.
30. Akamatsu D, Sato A, Goto H, Watanabe T, Hashimoto M, Shimizu T, Sugawara H, Sato H, Nakano Y, Miura T, Zukeran T, Serizawa F, Hamada Y, Tsuchida K, Tsuji I, Satomi S. Nitroglycerin-mediated vasodilatation of the brachial artery may predict long-term cardiovascular events irrespective of the presence of atherosclerotic disease. *J Atheroscler Thromb*. 2010;**17**(12):1266–1274.
31. Cameron F, Cotterill A, Couper J, Craig M, Davis E, Donaghue K, Jones T, King B, Sheil B. Short report: care for children and adolescents with diabetes in Australia and New Zealand: have we achieved the defined goals? *J Paediatr Child Health*. 2013;**49**(4):E258–E262.
32. Phelan H, Clapin H, Bruns L, Cameron FJ, Cotterill AM, Couper JJ, Davis EA, Donaghue KC, Jefferies CA, King BR, Sinnott RO, Tham EB, Wales JK, Jones TW, Craig ME. The Australasian Diabetes Data Network: first national audit of children and adolescents with type 1 diabetes. *Med J Aust*. 2017;**206**(3):121–125.
33. Liu W, Yang XJ. The effect of metformin on adolescents with type 1 diabetes: a systematic review and meta-analysis of randomized controlled trials. *Int J Endocrinol*. 2016;**2016**:3854071.
34. McDonagh MS, Selph S, Ozpinar A, Foley C. Systematic review of the benefits and risks of metformin in treating obesity in children aged 18 years and younger. *JAMA Pediatr*. 2014;**168**(2):178–184.
35. Shah AS, Dabelea D, Fino NF, Dolan LM, Wadwa RP, D'Agostino R, Jr, Hamman R, Marcovina S, Daniels SR, Urbina EM. Predictors of increased carotid intima-media thickness in youth with type 1 diabetes: the SEARCH CVD study. *Diabetes Care*. 2016;**39**(3):418–425.
36. Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group. Effect of intensive diabetes treatment on carotid artery wall thickness in the epidemiology of diabetes interventions and complications. *Diabetes*. 1999;**48**(2):383–390.
37. Nathan DM, Lachin J, Cleary P, Orchard T, Brillon DJ, Backlund JY, O'Leary DH, Genuth S; Diabetes Control and Complications TrialEpidemiology of Diabetes Interventions and Complications Research Group. Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N Engl J Med*. 2003; **348**(23):2294–2303.