Vascular effects of ACE inhibitors and statins in adolescents with type 1 diabetes: the Adolescent type 1 Diabetes cardio-renal Intervention Trial (AdDIT)

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ABSTRACT

An increased albumin-creatinine ratio (ACR) within the normal range can identify adolescents at higher risk of developing adverse cardio-renal outcomes as they progress into adulthood. Utilizing a parallel randomized controlled trial and observational cohort study, we characterized the progression of vascular phenotypes throughout this important period and investigated the effect of ACE inhibitors and statins in high-risk adolescents. Endothelial function (flow-mediated dilation; FMD and reactive hyperemia index; RHI) and arterial stiffness (carotid-femoral pulse wave velocity; PWV) were assessed in 158 high-risk participants recruited to a randomized, double-blind placebo-controlled 2x2 factorial trial (RCT) of ACE inhibitors and/or statins in adolescents with type 1 diabetes (AdDIT). Identical measures were also assessed in 215 lower-risk individuals recruited to a parallel observational study. In the RCT, high-risk patients randomized to ACE inhibitors had improved FMD after 2-4 years of follow-up (mean [95%CI]: 6.6%[6.0,7.2] vs. 5.3%[4.7,5.9]; p=0.005), whereas no effect was observed following statin use (6.2%[5.5,6.8] vs. 5.8%[5.1,6.4]; p=0.358). In the observational study, high-risk ACR patients showed evidence of endothelial dysfunction at the end of follow-up (FMD= 4.8%[3.8,5.9] vs. 6.3% [5.8,6.7] for high-risk vs. low-risk groups; p=0.015). Neither RHI nor PWV were affected by either treatment (p > 0.05 for both), but both were found to increase over the duration of follow-up (0.07[0.03,0.12]; p=0.001 and 0.5m/s[0.4,0.6]; p<0.001 for RHI and PWV, respectively). ACE inhibitors improve endothelial function in high-risk adolescents as they transition through puberty. The longer-term protective effects of this intervention at this early age remain to be determined.

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Keywords: type 1 diabetes, vascular disease, ACE inhibitors, statins, adolescence

INTRODUCTION

Cardiovascular disease (CVD) is the primary cause of premature morbidity and mortality in type 1 diabetes[1], with this effect particularly pronounced in those diagnosed at a younger age. The magnitude of this problem has recently been starkly highlighted by findings from the Swedish National Diabetes Register, where patients diagnosed with diabetes between 1-10 years of age were found to have a 10-times higher risk of future acute myocardial infarction compared to those diagnosed between the ages of 26-30 years, and over a 30-times higher risk than the general population[2]. These and other data[3] suggest that adolescence may be a particularly crucial time in the development of future CVD complications, and that effective intervention at this age may offer long-lasting benefits for cardio-renal health[4].

Accurate CVD risk stratification is essential in order to implement successful prevention strategies in youth with type 1 diabetes. We have previously demonstrated the ability of an increased albumin-creatinine ratio (ACR) within the normal range to identify adolescents with type 1 diabetes at increased risk for early cardio-renal complications[5–8]. In the Adolescent Type 1 Diabetes cardio-renal Intervention Trial (AdDIT), we conducted a double-blind, randomized, placebo-controlled trial (RCT) of ACE inhibitors and statins in 443 high-risk adolescents, accompanied by a parallel observational cohort of a similar number of untreated high- and low-risk individuals followed over the same time-frame[9]. In the observational cohort, we showed that adolescents with an ACR in the upper tertile of the observed range (i.e. high-risk) demonstrated a greater risk of developing a number of adverse phenotypes linked to future cardio-renal disease (increased carotid intima-media thickness [cIMT] and microalbuminuria [MA], respectively) as they transitioned through puberty[6]. In the RCT, we did not see changes in cIMT between treatment groups, but did demonstrate the ability of ACE inhibitors to reduce the progression to MA[10].

Previous studies from both ourselves and others have demonstrated evidence of numerous other adverse and physiologically-distinct structural and functional vascular changes in youth with type 1 diabetes. At a macrovascular level, evidence of vessel wall thickening (assessed via the ultrasound measurement of intima-media thickness; IMT)[8], arterial stiffening (assessed using pulse wave velocity; PWV)[11], and endothelial dysfunction (assessed by flow-mediated dilation; FMD)[8,12] have all been reported, suggesting an accelerated atherosclerotic disease process in the major conduit arteries which may predispose to future risk of CVD events. In addition, compromised microvascular function (assessed by microvascular reactive hyperemia; RHI) has also been observed[13], suggesting the presence of additional changes in resistance or micro-vessels which may be driven by different risk factors. No study to date, however, has assessed the development of these early markers of atherosclerotic disease over this potentially critical adolescent period, or whether these adverse changes can be prevented by ACE inhibitor or statin intervention.

In a subgroup of AdDIT individuals with additional vascular phenotyping, we now characterize the natural progression of a number of gold-standard non-invasive measures of macrovascular function (FMD), microvascular function (RHI), and arterial stiffness (PWV) across adolescence. Additionally, we investigate the impact that ACE inhibitors and statins have on these subclinical markers of atherosclerotic disease in high-risk individuals recruited to the RCT component of the trial.

METHODS

Data Availability

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

Study Design

AdDIT consisted of a double-blind, randomized, placebo-controlled trial and parallel observational study of adolescents with type 1 diabetes followed for between 2 and 4 years at 32 centers across 3 countries (UK, Canada, and Australia). The design and oversight of the trial have been reported in detail previously[9], and full inclusion and exclusion criteria can be found in the supplementary file. Full details on safety profiles and adverse events have also been published previously[10].

In brief, the factorial trial design evaluated an ACE inhibitor (Quinapril) in a daily variable dose of 5-10mg, a fixed dose of a statin (10mg Atorvastatin) and combinations of both interventions or placebo in 443 adolescents with type 1 diabetes deemed to be at higher risk of CVD and renal complications (as determined by an ACR in the upper tertile of the observed adjusted range). Participants were allocated to one of four treatment regimens (ACE Inhibitor-Placebo, Statin-Placebo, ACE Inhibitor-Statin, Placebo-Placebo) using a secure internet-based service (http://www.sealedenvelope.com) which included minimization of differences between arms for the following baseline characteristics: HbA1c (<7.5, 7.5-8.5, >8.5%), log ACR (1.2-1.7, >1.7), sex, age (11-13, >13 years), duration of disease (<5 years, ≥5 years), total cholesterol (≥ 4.46 or < 4.46 mmol/l), and country. In addition to the randomized trial, a parallel observational cohort of adolescents who were deemed to be at lower risk of complications (ACR in lower and middle tertiles) was also followed over the same period and compared to the untreated placebo-placebo high-risk RCT participants in order to track the natural progression of phenotypes over time (Figures 1 and S1).

The primary renal and cardiovascular outcomes of the AdDIT interventional study were area under the curve for albumin-creatinine ratio and carotid intima-media thickness, respectively, and findings relating to these outcomes have been published previously[10]. The current manuscript focuses on the previously unreported secondary cardiovascular outcomes listed in the trial protocol and statistical analysis plan. These consisted of measures of macrovascular endothelial function (flow-mediated dilation; FMD), microvascular endothelial function (reactive hyperemic index; RHI), and arterial stiffness (carotid-femoral pulse-wave velocity; PWV) measured in a subset of patients attending specialist vascular clinics in London, UK and Toronto, Canada.

The study conformed to the Declaration of Helsinki and was approved by the Cambridge University Hospitals Research Ethics Committee and other local ethics committees. Parents of participants provided written informed consent and study participants – not able to provide consent because of their age – were asked to provide their assent to the study procedures until they reached an appropriate age, when their further consent was sought.

Vascular Assessments

Following their initial and final study visits, participants attended a designated vascular assessment center for the measurement of FMD, RHI, and PWV. Prior to the initiation of the study, all sonographers were trained and accredited by the Vascular Physiology Unit, London; the central cardiovascular site for the study with extensive previous experience in successfully conducting large scale vascular phenotyping trials in both adults and children[14,15]. Reproducibility and variability data for each of the techniques can be found in the supplementary file.

FMD: Each participant underwent measurement of endothelial-dependent vascular responses of the right brachial artery by high-resolution ultrasound imaging (Aloka 5500, Hitachi Aloka, Tokyo, Japan; 7-MHz linear probe) Full details on procedures and reproducibility can be found in the supplementary file.

RHI: Endothelial peripheral arterial tonometry (EndoPAT, Itamar Medical, Israel) was carried out at the same time as FMD in order to assess reactive RHI as a marker of microvascular function. Full details of this method have been published elsewhere[16]. In short, a specialized latex fingertip probe was placed on the end of each index finger in order to measure the vasodilatory response in both arms following the 5min cuff occlusion. Integrated software then calculated the post-to-pre occlusion signal ratio in the occluded side, normalized to the control side and further corrected for baseline vascular tone. As per manufacturer's recommendation, this RHI was then natural-log transformed prior to analysis.

PWV: Pressure-pulse waveforms were recorded transcutaneously using a high-fidelity micromanometer (SphygmoCor MM3, AtCor Medical, NSW, Australia) from the carotid and femoral pulses using synchronous electrocardiography to provide an R-wave timing reference. Integral software (SphygmoCor version 7.1, AtCor Medical, NSW, Australia) processed the data to calculate the mean time difference between R waves and pressure waves on a beat-to-beat basis over 10 s. PWV was calculated using the mean time difference (in seconds) and arterial path length (in meters) between the 2 recording points.

Biochemical Assessments

HbA1c was assessed locally using Diabetes Control and Complications Trial–aligned methods, whereas all other biochemical measurements (total cholesterol, LDL, HDL, triglycerides, high sensitivity C-reactive protein [hsCRP], and urinary albumin creatinine ratio [ACR]) were performed in a central laboratory (WellChild Laboratory, Evelina Children's Hospital, London, U.K.) using standardized methods. Full details on techniques used and reproducibility of assays can be found in the supplementary file.

Efficacy Outcomes

The primary analysis was the effect of ACE inhibitors or statins (vs. placebo) on FMD, RHI and PWV between RCT participants at the end of the trial period. Secondary analysis was comparison of FMD, RHI and PWV between high-risk (placebo-placebo group from the RCT) and low-risk (parallel observational cohort) individuals over the same time-frame.

Power Calculations

For FMD, we estimated that an RCT with 160 patients (80 intervention/80 placebo) would provide 80% power to detect a true difference at follow-up of 1.6% (assuming a standard deviation of 3.0% for FMD and with the type I error probability of the null hypothesis being equal set at p = 0.01). For PWV, the same number of patients would have 80% power to detect a true difference of 0.4m/s (assuming standard deviation of 0.8 m/s), while for lnRHI it would be 0.15 (assuming a standard deviation of 0.3).

Statistical Analysis

Descriptive data are summarized as mean ± SD or median (IQR). For vascular outcomes in both the RCT and observational cohorts, multivariable linear regression models were used to estimate the mean effect (±95%CI) of each drug intervention (or high vs. low risk ACR groups in the observational cohort) while adjusting for the baseline covariates upon which participants were randomized. The final measure of each vascular phenotype was used as the dependent variable in each model, and covariates included age, sex, duration of disease, standardized ACR, total cholesterol, HbA1c, baseline phenotype, and country. Due to the 2x2 factorial trial design, each arm of the RCT was also adjusted for the other drug treatment (i.e. ACE inhibitor arm adjusted for statin use and vice versa). In addition, FMD was additionally adjusted for resting brachial diameter and PWV for mean arterial pressure due to the well-known association between these variables and their respective outcomes. As per recommended guidelines, FMD was reported as absolute change, relative change, and change normalized to peak shear stimulus[17]. Change in FMD was also calculated as difference between baseline and final visits. To assess changes in cardiovascular risk factors assessed at multiple timepoints during the study period, linear mixed models adjusted for age and sex were used to assess overtime differences in the intervention vs placebo and high- vs low-risk groups in the randomized controlled trial and observational cohort, respectively. Only participants with vascular phenotypes measured at both baseline and follow-up were included in analyses, with multiple imputation (20 imputed datasets) used to account for any missing covariates in these models. All analyses were carried out using SPSS v.25 (IBM, USA) and – as per the trial statistical analysis plan – the significance level for this secondary trial analysis were set at $p < 0.01$.

RESULTS

Full details of the characteristics of the study population can be found in Table 1. A total of 158 and 255 participants underwent additional vascular assessments in the RCT and observational cohorts, respectively, with a mean age at baseline of 14 years. Mean follow-up times for the RCT and observational participants included in this study were 3.4 ± 1.7 years and 3.6 ± 1.7 years respectively.

Vascular Assessments

FMD: Baseline FMD was similar in all four randomized arms of the RCT and in the observational cohort. In the RCT, high-risk patients randomized to ACE inhibitors using the factorial design had improved FMD at follow-up (6.6 [6.0, 7.2] % vs. 5.3 [4.7, 5.9] %; p = 0.005; Figure 2), These differences occurred as a result of improved FMD in the ACE inhibitor group between baseline and follow-up (mean increase of 1.0 [0.1, 1.9] %; p = 0.040), whereas no difference was seen in the placebo group (0.1 [-0.1, 1.1] %; p = 0.841). This improvement was not attributable to differences in post-ischemic shear rate, which was similar in all groups (Table 2). As a result, findings remained consistent following the normalization of FMD to shear stimulus (p = 0.003 for ACE inhibitor vs. placebo; Table 2). In contrast to ACE inhibitor use, no effect was observed following statin use (6.2 [5.5, 6.8] % vs. 5.8 [5.1, 6.4] %; p = 0.358; Figure 2). However, when comparing the untreated high-risk RCT participants with the observational cohort, high-risk participants showed evidence of endothelial dysfunction during follow-up when compared to the low-risk group (mean FMD [95%CI] = 4.8 [3.8, 5.9] % vs. 6.3 [5.8, 6.7] %; p = 0.015; Figure 2). Results when using stratified covariates rather than linear data were virtually identical to those presented above (Table S1).

RHI: Neither ACE inhibitors nor statins had any effect on RHI in the RCT (p > 0.05 for all; Figure 3), and no differences were observed between high- and low-risk ACR participants in the observational study. Results when using stratified covariates rather than linear data were virtually identical to those presented above (Table S1). All groups were therefore combined for whole-cohort analysis, demonstrating a significant increase in RHI over the duration of follow-up (mean increase = 0.07 [0.03, 0.12]; p = 0.001; Figure 3).

PWV: Similar to RHI, neither ACE inhibitors nor statins had any effect on PWV in the RCT ($p > 0.05$ for all; Figure 3), and no differences were observed between high- and low-risk ACR patients in the observational study. Results when using stratified covariates rather than linear data were virtually identical to those presented above (Table S1). Combining groups for whole-cohort analysis also demonstrated a significant increase in PWV over the duration of follow-up (mean increase = 0.5 m/s [0.4, 0.6]; $p < 0.001$; Figure 3).

Cardiovascular Risk Factors

Cardiovascular risk factors were unchanged with ACE inhibitor use, with the exception of a small elevation in total cholesterol. In contrast, statin use resulted in significant reductions in total cholesterol, LDL-c, and triglycerides (Table 3).

DISCUSSION

This combined RCT and parallel longitudinal observational study is the first to investigate the effect of ACE inhibitors and statins on the progression of endothelial dysfunction and arterial stiffness in adolescents with type 1 diabetes. Our findings demonstrate that ACE inhibitors improve endothelial function in high-risk adolescents with type 1 diabetes as they transition through puberty, and may therefore offer long-term cardio-renal benefit during this potentially critical time-period for the development of CVD. In contrast, neither drug had any impact on reactive hyperemic index or arterial stiffness, which increased in all study participants throughout the study period.

Both our group and others have demonstrated that the pathogenesis of CVD likely begins soon after diabetes diagnosis, with numerous well-established markers of subclinical disease already present in children with type 1 diabetes when compared to their healthy peers[4,8]. Accumulating evidence also suggests that puberty may be a particularly critical time for further progression of these early vascular complications; with hormonal changes, suboptimal glycemic control, and increasing exposure to other CV risk factors such as high blood pressure and lipid levels combining to adversely affect arterial structure and function throughout the adolescent years[3]. Early identification of high-risk individuals and timely preventive interventions at this young age may therefore have the potential to offer long-term cardio-renal benefits in this group[4]. Hard clinical endpoints (cardiovascular events or death) are rare in this age group, however, making robust trials assessing drug efficacy difficult to conduct. This is compounded by traditionally poor adherence rates in adolescent diabetes management as young people transition through puberty into adulthood. Here, we present a secondary analysis of a double-blind RCT with high adherence rates (75-80%), in tandem with the natural adolescent progression of a number of subclinical markers of atherosclerosis with well-established predictive power for future CVD events.

One of the earliest detectable indicators of arterial damage is endothelial dysfunction – an established predictor of future adverse CVD outcomes which may occur in both major conduit arteries and/or microvascular beds. Previous data from both this cohort and other studies has shown conduit artery endothelial dysfunction (as measured by FMD) to already be evident in children and adolescents with type 1 diabetes in comparison to healthy controls[8,12]. In the observational arm of the current study, we expand upon these findings to show that endothelial function is further impaired in high-risk individuals with type 1 diabetes at the end of adolescence when compared to lower-risk individuals. Perhaps more importantly, in the RCT we demonstrate the ability of ACE inhibitors to improve FMD over this same time frame, providing evidence of an additional vascular benefit alongside the reduced incidence of microalbuminuria previously reported from this trial[10]. Interestingly, microalbuminuria has long been considered to be associated with a generalized systemic endotheliopathy in type 1 diabetes[18], raising the possibility that the vascular and renal improvements observed following ACE inhibition in AdDIT may be linked by an underlying improvement in endothelial function. The mechanistic basis for this improvement remains to be determined, but agrees with previous research in which quinapril (but not other classes of ACE inhibitors) was found to improve FMD in a diverse range of patient groups with pre-existing endothelial dysfunction[19–21]. Due to the potent antihypertensive effects of ACE inhibitors and the well-recognized relationship between elevated blood pressure and endothelial dysfunction, it is plausible that these improvements are at least in part mediated through a reduction in blood pressure. However, it should be noted that blood pressure values in this young cohort were within normal range at baseline and remained unchanged throughout the trial[10], and alternative pleiotropic actions of ACE inhibition may also warrant consideration. ACE inhibitors have been shown in previous studies to also exhibit anti-inflammatory effects, and improvements in FMD in patients taking quinapril have previously been shown to occur in line with accompanying reductions in the inflammatory biomarkers tumour necrosis factor alpha (TNFα) and C-reactive protein (CRP)[19]. While no change in CRP was observed following ACE inhibition in the current study[10], the possibility exists that this biomarker

– a non-specific downstream acute phase protein rather than causal risk factor for CVD – may not adequately capture the upstream inflammatory risk pathways relevant to endothelial dysfunction at this age. In support of this, previous work from our group in a subset of this cohort has shown endothelial dysfunction to be related to inflammation only when using a cohort-specific inflammatory risk score composed of numerous pro-inflammatory cytokines known to be elevated in this patient group, but not when using traditional biomarkers lying upstream of CRP such as TNFα and IL-6[22]. In addition, the lack of improvement in FMD in the statin arm in the current trial – despite a trend for reduced CRP – suggests that different inflammatory biomarkers may be necessary at this age to track the relationship between diabetes-related inflammation and early vascular damage, although this clearly warrants further investigation.

In addition to macrovascular function, we also set out to assess early changes in microvascular endothelial dysfunction using peripheral arterial tonometry (EndoPAT). In contrast to FMD, no evidence of microvascular dysfunction was observed in the high- vs. low-risk risk patient groups during the trial, and no benefit was observed following either ACE inhibitor or statin treatment. Instead, increases in the reactive hyperemic index (RHI) measured by the EndoPAT device suggested a potential improvement of microvascular function throughout the trial. These findings agree with a number of studies in healthy children and adolescents published since the design of AdDIT, in which RHI measured by EndoPAT has been found to have strong positive relationships with both stature and pubertal development as children transition through the adolescent phase. These results suggest a powerful effect of body growth and development on the RHI measure as body mass increases and the microcirculatory circulation matures[23,24], a hypotheses supported in the current study by the observation that adjusting RHI for body mass normalized RHI to baseline values (data not shown). Furthermore, no difference over time (or effect of drugs) was noted when using the magnitude of Doppler-measured hyperemic flow as a marker of microvascular function, suggesting that the benefit of ACE inhibitors at this age may primarily be confined to the conduit vessels.

Our final vascular measure – carotid-femoral pulse wave velocity (PWV) – is a non-invasive measure of arterial stiffness, a powerful predictor of future CVD in adult populations[25], and has previously been shown to be adversely affected in youth with type 1 diabetes[11,26]. In the current study, PWV was observed to increase by ~ 0.5 m/s in all arms of the study throughout the follow-up period, and was unaffected by either ACE inhibitor or statin use. PWV is tightly correlated to age, body size, and blood pressure; and the magnitude of increase observed in our current cohort agrees with reference values published elsewhere for healthy individuals transitioning through adolescence[27,28]. While these increases may simply represent natural growth and maturation, a previous finding of increased aortic intima-media thickening in the AdDIT cohort suggests that the aorta may indeed be a site of early vascular damage in children with type 1 diabetes[8]. Unfortunately, AdDIT did not recruit an accompanying healthy control population for comparison, and it is therefore not possible to discern whether the presence of type 1 diabetes results in accelerated stiffening of the major arteries at this age. Mechanistically, while poor glycemic control, endothelial dysfunction, elevated cholesterol, oxidative stress, and inflammation have all been implicated in the acceleration of arterial stiffness, one of the primary drivers at this early age is most likely blood pressure – resulting in a functional stiffening of the vessel due to increased distending pressure rather than structural stiffening *per se.* The overall lack of blood pressure reduction with either drug treatment in the current trial may therefore offer a potential explanation for their lack of effect at this age. Whether higher doses of ACE inhibitors would offer a protective effect at this age remains to be determined.

The major strength of this study is that it is the first double-blind randomized placebo-controlled trial to assess the effects of ACE inhibitors and statins on a number of well-established surrogate markers of subclinical atherosclerosis in high-risk adolescents with type 1 diabetes. The relatively short duration of the trial is a potential limitation to this study, and longer-term follow-up is therefore needed to assess whether intervention at this potentially important phase of CVD development has longer-term benefits to cardiovascular health. That said, the demonstrated effect on endothelial function is considerably longer than previous reported interventions to improve endothelial function in this age group[29,30]. The use of surrogate subclinical markers as outcomes is also a potentially limiting factor, but is unavoidable in young cohorts such as this when clinical events are rare. The surrogate markers chosen here, therefore, represent a range of physiologically distinct structural and functional changes with proven predictive value for future CV events, and were carried out by a group with significant experience and previous success of performing these measures in large trials and cohorts of both adults and children[14,15,31]. As the outcomes reported in this paper are secondary trial analyses, a more conservative p-value of 0.01 was set for statistical significance. While the statistical analysis plan for AdDIT stated that covariates in all analyses would be stratified prior to inclusion in statistical models, this technique may potentially reduce statistical power[32] and raises the risk of a type II error in this smaller sub-group of participants. We therefore included covariates in all models for main analysis as linear data, with stratified versions presented in the supplementary file for comparison. As can be seen from these comparisons, effect estimates were virtually identical between the two approaches, providing further reassurance on findings.

PERSPECTIVES

In a unique and robust randomized clinical trial involving a population at high-risk for future vascular complications, we provide compelling evidence of an improvement in endothelial function following short-term treatment with ACE inhibitors during adolescence. Together with prior evidence of a reduced progression to microalbuminuria in this same patient group[10], these findings are likely to inform future clinical strategies by focusing efforts towards the early identification and treatment of subclinical changes which may underlie an increased risk of CVD in type 1 diabetes. Furthermore, these findings may ultimately prove to be 'practice-changing' in this patient group in the longer-term if ongoing follow-up of this cohort provides evidence of persistent vascular benefit into adulthood.

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DISCLOSURES

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SUPPLEMENTAL MATERIALS

Expanded Methods / Online Table S1 / Online Figure S1

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NOVELTY AND SIGNIFICANCE

What is new?

- Treatment with ACE inhibitors, but not statins, improves macrovascular endothelial function in adolescents with type 1 diabetes.
- Neither drug, however, was found to improve aortic stiffness or microvascular function at this age.

What is relevant?

- A diagnosis of type 1 diabetes prior to puberty confers a significantly increased risk of cardiovascular disease in later life compared to a diagnosis in early adulthood, suggesting that adolescence may be a particularly critical time for the early development of cardiovascular complications.
- Until now, the effect of commonly-prescribed blood pressure and cholesterol lowering drugs (ACE inhibitors and statins) on early markers of subclinical atherosclerosis had never been investigated in this high-risk population.

Summary

ACE inhibitors may offer vascular benefit in high-risk adolescents with type 1 diabetes by improving endothelial function during the transition through adolescence. The longer-term protective effects of these interventions at this early age remains to be determined.

TABLE LEGENDS

Table 1: Baseline characteristics for RCT and observational cohorts

Data are mean ± SD or median (IQR). The terms 'ACEi Arm' and 'Statins Arm' represent factorial arms of the randomized clinical trial, whereas 'high' and 'low' risk groups in the observational study represent individuals recruited to the placebo-placebo arm of the RCT and untreated individuals from the parallel longitudinal cohort, respectively. Further details of groupings can be seen in Figure S1. Asterisk denotes significant difference from corresponding placebo/low-risk group ($p < 0.05$).

Table 2: Absolute, relative, and shear-normalized FMD per trial arm at end of study

Data are presented as mean and 95% CI. Results are from multivariable linear regression models adjusted for age, sex, duration of disease, standardized ACR, total cholesterol, HbA1c, and country. Relative change in FMD additionally adjusted for resting brachial artery diameter.

Table 3: Changes in cardiovascular risk factors per trial arm

Results are from linear mixed models, adjusted for age and sex. Data are reported as β-Estimate and 95% CI. β-Estimates are equal to the mean difference between the intervention and placebo groups in the RCT and between the high-risk and low-risk groups in the observational cohort.

FIGURE LEGENDS

Figure 1: Flowchart of participant recruitment

Figure 2: FMD in ACE inhibitor and statin arms of RCT and in observational cohort

Data are presented as mean and 95% CI. Results are from multivariable linear regression models adjusted for age, sex, duration of disease, standardized ACR, total cholesterol, HbA1c, other drug treatment, and country. In addition, FMD was additionally adjusted for resting brachial artery diameter

Figure 3: RHI and PWV in ACE inhibitor and statin arms of RCT, observational cohort, and with all participants combined

Data are presented as mean and 95% CI. Results are from multivariable linear regression models adjusted for age, sex, duration of disease, standardized ACR, total cholesterol, HbA1c, other drug treatment, and country. PWV additionally adjusted for mean arterial pressure.

Table 1

Variables	Randomized Controlled Trial				Observational Cohort	
	ACEi Arm		Statins Arm			
	ACEi	Placebo	Statins	Placebo	High Risk	Low Risk
N	83	75	79	79	40	215
Sex (male, %)	55.0%	62.0%	60%	56%	55%	56%
Age (yr)	13.9 ± 1.7	13.8 ± 1.6	13.9 ± 1.8	13.8 ± 1.5	13.8 ± 1.5	13.9 ± 1.6
Age at diagnosis (yr)	8.8 ± 3.4	8.5 ± 3.6	8.5 ± 3.8	8.7 ± 3.3	8.7 ± 3.1*	6.7 ± 3.4
T1D duration (yr)	5.4 ± 3.5	5.1 ± 3.2	5.4 ± 3.3	5.1 ± 3.5	5.0 ± 3.0*	7.2 ± 3.4
HbA1c (%)	8.2 ± 1.3	8.5 ± 1.5	8.2 ± 1.5	8.4 ± 1.3	8.7 ± 1.4	8.4 ± 1.2
Height (cm)	163 ± 11	163 ± 10	164 ± 11	162 ± 9	162 ± 9	162 ± 10
Weight (kg)	56.1 (46.8, 60.0)	52.8 (44.1, 62.8)	53.1 (45.6, 63.6)	54.0 (44.9, 60.0)	54.0 (44.6, 61.0)	57.2 (49.7, 66.2)
BMI (kg/m^2)	20.0 (18.6, 22.9)	19.6 (17.5, 22.5)	19.8 (18.0, 23.1)	20.2 (18.0, 22.6)	20.6 (17.8, 22.7)	21.2 (19.2, 23.9)
Waist Circumference (cm)	72.0 (67.0, 77.5)	71.2 (66.0, 78.4)	72. 0 (66.0, 79.0)	72.0 (66.2, 77.0)	71.8 (66.8, 77.3)	72.0 (67.3, 79.6)
Systolic BP (mmHg)	114 ± 11	113 ± 11	113 ± 12	115 ± 10	116 ± 11	115 ± 11
Diastolic BP (mmHg)	66 ± 8*	63 ± 8	65 ± 8	65 ± 8	65 ± 8	67 ± 7
Total Cholesterol (mmol/L)	4.3 ± 1.0	4.1 ± 1.0	4.2 ± 1.1	4.3 ± 0.9	4.0 ± 1.1*	4.4 ± 0.9
LDL (mmol/L)	2.4 ± 0.7	2.2 ± 0.6	2.3 ± 0.7	2.3 ± 0.6	2.2 ± 0.4	2.3 ± 0.7
HDL (mmol/L)	1.6 ± 0.4	1.6 ± 0.4	1.6 ± 0.4	1.5 ± 0.3	1.5 ± 0.3	1.6 ± 0.4
Triglycerides (mmol/L)	0.7 (0.6, 1.1)	0.8 (0.6, 1.1)	0.8 (0.6, 1.1)	0.8 (0.6, 1.2)	0.8 (0.6, 1.3)	0.8 (0.6, 1.1)
Glucose (mmol/L)	10.1 ± 4.2	10.0 ± 4.5	10.3 ± 4.7	9.8 ± 4.0	9.9 ± 4.2	9.5 ± 3.2
hsCRP (mg/L)	0.4 (0.2, 1.2)	0.4 (0.2, 1.0)	0.4 (0.2, 1.1)	0.5 (0.2, 1.0)	0.6 (0.2, 1.0)	0.5 (0.2, 1.1)

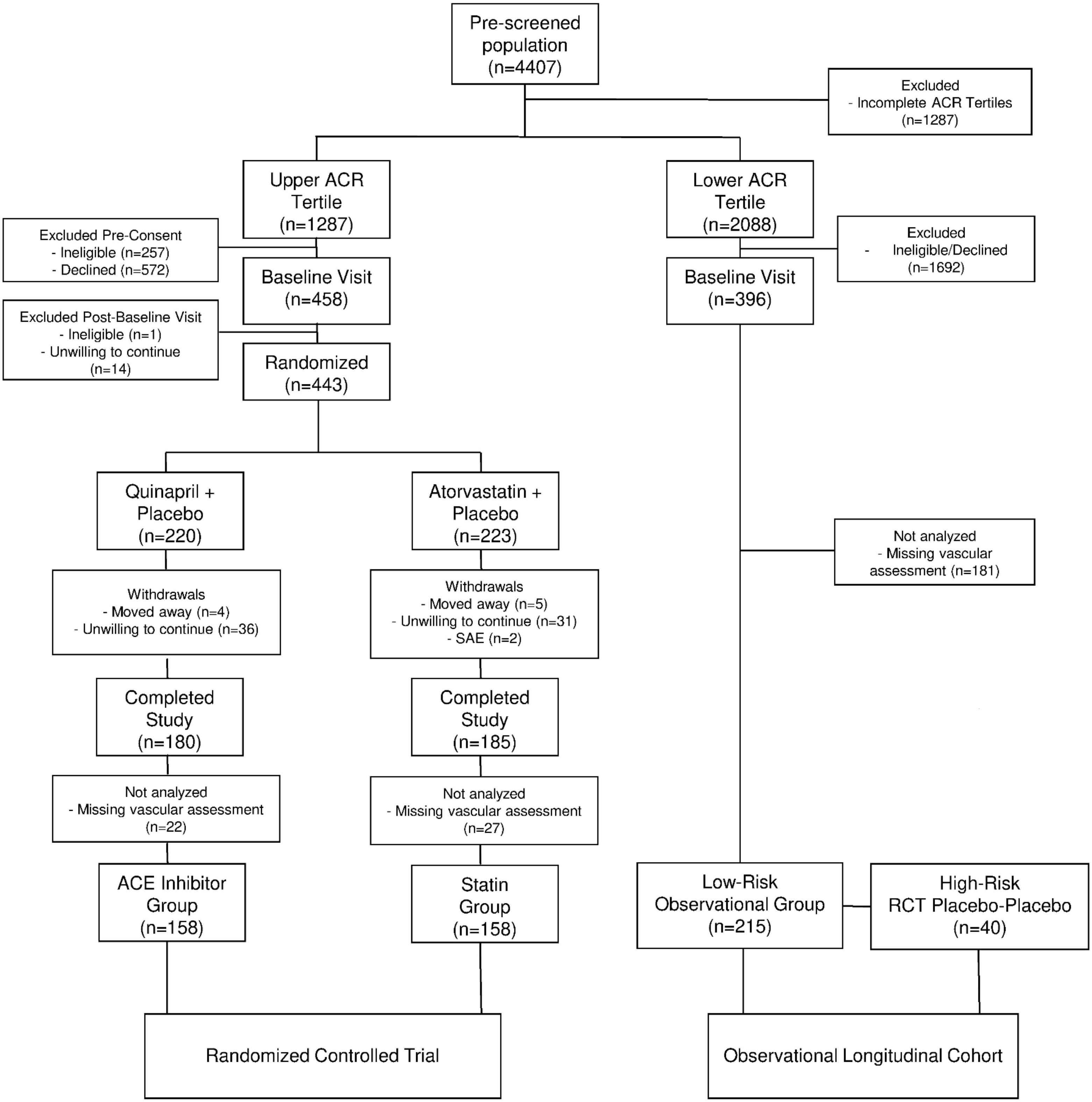
Table 2

	Randomized Controlled Trial								Observational Study			
Variables	**ACEi vs. Placebo Mean (95%CI)**				**Statin vs. Placebo Mean (95%CI)**				**High-Risk vs. Low-Risk Mean (95%CI)**			
	ACEi	**Placebo**	**Difference**	**p**	**Statin**	**Placebo**	**Difference**	**p**	**High**	**Low**	**Difference**	**p**
Baseline Diameter (mm)	3.34 (3.25, 3.43)	3.35 (3.26, 3.45)	-0.01 (-0.14, 0.12)	0.871	3.37 (3.28, 3.47)	3.32 (3.23, 3.41)	0.05 (-0.08, 0.18)	0.430	3.33 (3.20, 3,47)	3.32 (3.27, 3.38)	0.01 (-0.13, 0.16)	0.859
Absolute FMD Difference (mm)	0.21 (0.19, 0.23)	0.17 (0.15, 0.19)	0.04 (0.01, 0.07)	0.011	0.20 (0.18, 0.22)	0.19 (0.17, 0.21)	0.01 (-0.02, 0.04)	0.572	0.16 (0.12, 0.19)	0.21 (0.19, 0.22)	-0.05 (-0.09, - 0.01)	0.011
Relative FMD Difference (%)	6.6 (6.0, 7.2)	5.3 (4.7, 5.9)	1.3 (0.4, 2.2)	0.005	6.2 (5.5, 6.8)	5.8 (5.1, 6.4)	0.4 (-0.5, 1.3)	0.358	4.8 (3.8, 5.9)	6.3 (5.8, 6.7)	-1.4 (-2.5, -0.3)	0.015
Peak Shear Stimulus (s^{-1})	173 (159, 187)	176 (161, 190)	-3 (-18, 23)	0.801	177 (163, 191)	171 (157, 185)	6 (-14, 26)	0.572	164 (144, 183)	178 (169, 186)	-14 (-36, 7)	0.196
Relative FMD (%) normalized to shear	0.04 (0.03, 0.05)	0.03 (0.02, 0.04)	0.01 (0.00, 0.02)	0.003	0.04 (0.03, 0.04)	0.04 (0.03, 0.04)	0.00 (-0.01, 0.01)	0.653	0.03 (0.03, 0.04)	0.04 (0.04, 0.04)	-0.01 (-0.01, 0.00)	0.102

Table 3:

Variables	Randomized Controlled Trial						Observational Cohort		
	β-Estimate (95% CI)			β-Estimate (95% CI)			β-Estimate (95% CI)		
	ACEi vs Placebo	p-value		Statins vs Placebo	p-value		High Risk vs Low risk	p-value	
N	158			158			255		
BMI z-score	0.04 (-0.23, 0.31)	0.754		-0.10 (-0.37, 0.7)	0.464		-0.08 (-0.36, 0.20)	0.566	
Waist Circumference (cm)	-0.05 (-2.69, 2.58)	0.970		-1.03 (-3.65,1.59)	0.440		3.75 (0.04, 7.09)	0.03	
Systolic BP (mmHg)	0.76 (-1.63, 3.14)	0.531		-1.19 (-3.5, 1.19)	0.325		1.75 (-0.96, 4.46)	0.205	
Diastolic BP (mmHg)	0.43 (-1.14, 2.00)	0.587		-0.08 (-1.64, 1.49)	0.924		-0.27 (-2.03, 1.50)	0.766	
HbA1c (%)	0.015 (-0.37, 0.40)	0.940		-0.13 (-0.51, 0.26)	0.519		0.23 (-0.18, 0.64)	0.267	
Total Cholesterol (mmol/L)	0.24 0.008, 0.47)	0.042		-0.50 (-0.72, -0.28)	<0.001		-0.32 (-0.58, 0.06)	0.02	
LDL (mmol/L)	0.12, (-0.09, 0.33)	0.256		-0.47 (-0.67, -0.28)	< 0.001		-0.19 (-0.41, 0.02)	0.084	
HDL (mmol/L)	0.07 (-0.21, 0.16)	0.128		0.03 (-0.06, 0.12)	0.573		-0.04 (-0.15, -0.06)	0.406	
Triglycerides (mmol/L)	-0.001 (-0.13, 0.12)	0.908		-0.16 (-0.27, -0.04)	0.009		0.07 (-0.07, 0.21)	0.333	
hsCRP (mg/L)	0.02 (-0.58, 0.62)	0.949		-0.42 (-1.02, 0.17)	0.162		0.44 (-0.32, 1.21)	0.256	

Figure 1: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(n=1287)
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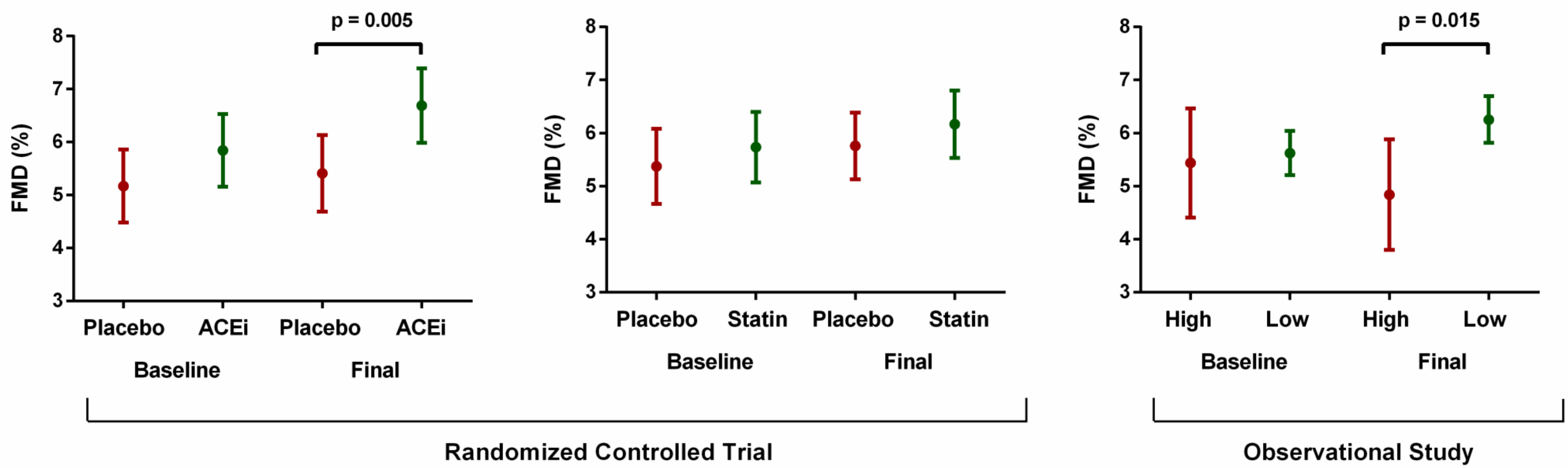
Lower ACR Tertile (n=2088)
Excluded Pre-Consent
- Ineligible (n=257)
- Declined (n=572)
Excluded
- Ineligible/Declined
(n=1692)
Baseline Visit (n=458)
Baseline Visit (n=396)
Excluded Post-Baseline Visit
- Ineligible (n=1)
- Unwilling to continue
(n=14)
Randomized (n=443)
Quinapril + Placebo (n=220)
Atorvastatin + Placebo (n=223)
Not analyzed
- Missing vascular
assessment (n=181)
Withdrawals
- Moved away (n=4)
- Unwilling to continue (n=36)
Withdrawals
- Moved away (n=5)
- Unwilling to continue (n=31)
- SAE (n=2)
Completed Study (n=180)
Completed Study (n=185)
Not analyzed
- Missing vascular assessment
(n=22)
Not analyzed
- Missing vascular assessment
(n=27)
ACE Inhibitor Group (n=158)
Statin Group (n=158)
Low-Risk Observational Group (n=215)
High-Risk RCT Placebo-Placebo (n=40)
Randomized Controlled Trial
Observational Longitudinal Cohort

Figure 2:



p = 0.005
8
7
6
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FMD (%)
Placebo
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FMD (%)
Placebo
Statin
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Baseline
Final
p = 0.015
8
7
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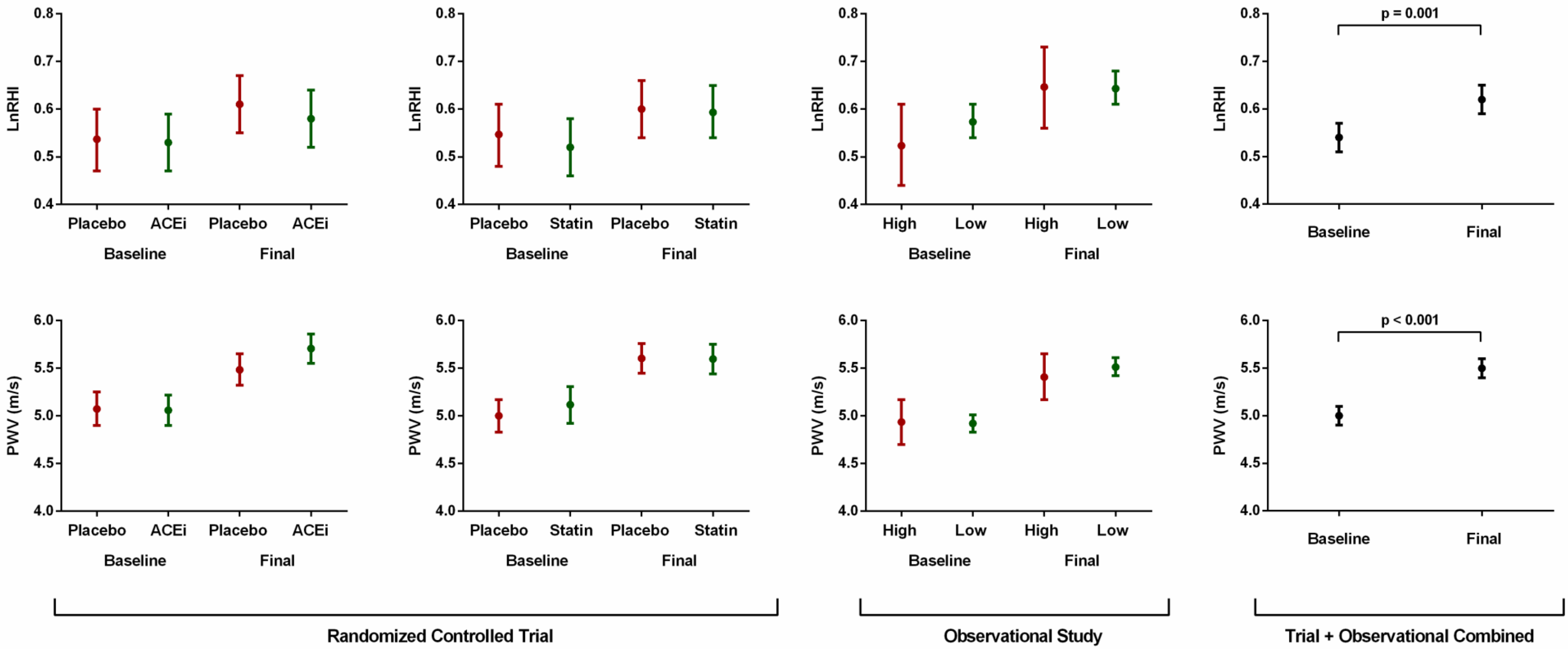
3
FMD (%)
High
Low
High
Low
Baseline
Final
Randomized Controlled Trial
Observational Study

Figure 3:


0.8
0.7
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0.5
0.4
LnRHI
Placebo
ACEi
Placebo
ACEi
Baseline
Final
Placebo
Statin
Placebo
Statin
Baseline
Final
High
Low
High
Low
Baseline
Final
p = 0.001
Baseline
Final
6.0
5.5
5.0
4.5
4.0
PWV (m/s)
Placebo
ACEi
Placebo
ACEi
Baseline
Final
Placebo
Statin
Placebo
Statin
Baseline
Final
High
Low
High
Low
Baseline
Final
p < 0.001
Baseline
Final
Randomized Controlled Trial
Observational Study
Trial + Observational Combined

Supplementary Material

Vascular effects of ACE inhibitors and statins in adolescents with type 1 diabetes: the Adolescent type 1 Diabetes cardio-renal Intervention Trial (AdDIT)

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Short Title: ACE inhibitors and statins in type 1 diabetes

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SUPPLEMENTARY METHODS

Inclusion and Exclusion Criteria

Inclusion criteria were: 1) age 10-16 years; 2) type 1 diabetes diagnosed for more than 1 year or C-peptide negative; 3) centralized assessment of ACR based on six early morning urines in the upper tertile (trial cohort) or lower and middle tertiles (observational cohort), after adjustment for age, sex, and duration of disease. Exclusion criteria were 1) other types of diabetes; 2) severe hyperlipidemia and family history data to support diagnosis of familial hypercholesterolemia; 3) established hypertension unrelated to diabetic nephropathy; 4) prior exposure to the investigational products (ACE inhibitors and statins); 5) other co-morbidities considered unsuitable by the investigator (excluding treated hypothyroidism and celiac disease); 6) proliferative retinopathy. Specific exclusion criteria for the trial cohort were: 1) pregnancy or unwillingness to comply with contraceptive advice and regular pregnancy testing throughout the trial; and 2) breast feeding.

Vascular Assessments

Participants were requested arrive at the laboratory at least 2hrs after their last insulin dose. Upon arrival, a blood glucose measurement was self-recorded, and participants were left to rest for 10min in a supine position in a temperature-controlled room (24°C). A straight non-branching segment of the brachial artery above the antecubital fossa was then selected and scanned longitudinally. Brachial artery diameter was recorded (baseline) for 1 min, following which a pneumatic cuff was inflated to 300 mmHg on the forearm for 5 min. After rapid deflation of the cuff, the segment of brachial artery was recorded continuously for another 5 min. End-diastolic images at 3-second intervals were assessed, and changes in brachial artery diameter were measured offline by an automatic edge detection system (Brachial Tools, Medical Imaging Applications, Coralville, Iowa). As per recommended guidelines[21], FMD was expressed as the absolute difference between maximal and resting vessel diameters, as a percentage change of resting diameter, and as FMD/peak shear stimulus. Delta FMD was also calculated as final measure – baseline measure. Shear stress was calculated as (8*mean blood velocity)/vessel diameter, with blood velocity calculated using the velocity-time integral of the pulse-wave Doppler signal. To minimise variability during analysis, all FMD scans were analysed at the central London cardiovascular site by a trained sonographer.

Vascular Assessments Reproducibility

For FMD, intra-session intra-observer variability showed a mean difference in FMD of 0.1% with a COV of 14%, whereas inter-observer variability showed a mean difference of 0.7% with a COV of 20%. Intersession test-retest variability showed a mean difference of 1.2% with a COV of 24%. For PWV, intra-session intra-observer variability showed a mean difference in PWV of 0.1m/s with a COV of 3%, whereas inter-observer variability showed a mean difference of 1.1m/s with a COV of 13%. Inter-session test-retest variability showed a mean difference of 0.2m/s with a COV of 5%. For FMD analysis at the central vascular site, mean difference in intra-observer variability was 0.2% with a COV of 9%. Reproducibility data for EndoPAT – a user-independent automated device – have been published previously[1].

Biochemical Assessments and Reproducibility

Urine albumin was measured using nephelometric immunoassay according to the manufacturer's instructions (BN Prospec; Siemens). Urine albumin concentrations below the limit of quantitation of nephelometry, typically 2.1 mg/L, were measured using ELISA. Between-batch imprecision was 3.7% at 4.16 mg/L (n = 51), 2.9% at 19.0 mg/L (n = 55), and 2.9% at 144 mg/L (n = 54). Between-batch imprecision on the ELISA at ,2.1 mg/L was,15%. Urine creatinine was measured using a chromatographic stable isotope dilution electrospray mass spectrometry–mass spectrometry (MSMS) method on an AB SCIEX API5000. Between-batch imprecision (n = 48) was 2.6% at 6.89 mmol/L and 3.3% at 17.4 mmol/L. Plasma creatinine was measured using a reference stable isotope dilution electrospray MSMS. Between-batch imprecision (n = 30) was 2.8% at 66.1 mmol/L and 2.5% at 333.3 mmol/L. Cystatin C was measured by particle-enhanced nephelometric immunoassay according to the manufacturer's instructions (BN Prospec). Between-batch imprecision (n = 38) for cystatin C was 3.5% at 0.87 mg/L and 3.6% at 4.64 mg/L. Plasma ADMA was measured using a chromatographic stable isotope dilution fragmentation-specific electrospray MSMS. Between-batch imprecision (n = 30) for ADMA was 2.5% at 401 nmol/L, 2.7% at 917 nmol/L, and 2.7% at 2,413 nmol/L. hs-CRP was measured by particle-enhanced nephelometric immunoassay according to the manufacturer's instructions (BN Prospec). Between-batch imprecision (n = 38) was 5.8% at 0.89 mg/L and 3.6% at 4.73 mg/L. Total cholesterol (second-generation formulation), HDL cholesterol (third-generation formulation), LDL cholesterol, and triglycerides were measured colorimetrically on a COBAS INTEGRA 400 plus according to the manufacturer's instructions. Between-batch imprecision for total cholesterol (n = 35) was 2.6% at 4.71 mmol/L and 2.1% at 8.62 mmol/L, for HDL cholesterol (n = 35) was 3.1% at 0.86 mmol/L and 3.9% at 1.49 mmol/L, for LDL cholesterol (n = 36) was 3.1% at 3.07 mmol/L and 2.5% at 4.92 mmol/L, and for triglycerides (n = 35) was 2.9% at 1.47 mmol/L and 2.8% at 4.82 mmol/L.

SUPPLEMENTARY TABLE

Table S1: Main analyses performed with covariates stratified at SAP-determined cut-offs

	Randomised Controlled Trial				Observational Study	
Variable	**ACEi vs. Placebo**		**Statin vs. Placebo**		**High vs. Low Risk**	
	Mean Difference (95% CI)	**p-value**	**Mean Difference (95% CI)**	**p-value**	**Mean Difference (95% CI)**	**p-value**
FMD (%)	1.1 (0.2, 2.1)	0.016	0.3 (-0.7, 1.2)	0.573	-1.0 (-2.2, 0.1)	0.079
LnRHI	0.05 (-0.04, 0.14)	0.302	-0.02 (-0.11, 0.07)	0.658	0.11 (-0.05, 0.28)	0.102
PWV (m/s)	0.2 (-0.1, 0.4)	0.102	0.0 (-0.2, 0.3)	0.744	0.1 (-0.2, 0.3)	0.715

SUPPLEMENTARY FIGURE

Figure S1: Structure of RCT and observational trials