EXERCISE TRAINING IMPROVES VASCULAR FUNCTION IN ADOLESCENTS WITH TYPE 2 DIABETES

Louise H Naylor, PhD[1] Elizabeth A Davis, PhD, FRACP [2,3,4], Rachelle J Kalic BSc(Hons)[3,4], Niru Paramalingam, BSc(Hons)[3,4], Mary B Abraham, MD[2,4], Timothy W Jones, MD, FRACP[2,3,4], Daniel J Green, PhD[1,5]

[1]School of Sport Science, Exercise & Health, The University of Western Australia, Australia

[2]School of Paediatric and Child Health, The University of Western Australia, Australia

[3]Telethon Kids Institute, The University of Western Australia, Australia

[4]Department of Endocrinology and Diabetes, Princess Margaret Hospital, Australia

[5]Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, United Kingdom

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Corresponding author contact details:

Dr Louise Naylor

Address: School of Sport Science, Exercise & Health (M408)

The University of Western Australia, Crawley, Western Australia, 6009.

Phone: +61 8 6488 3887, *fax*: +61 8 6488 1039, *email*: Louise.Naylor@uwa.edu.au

Abstract

The impact of exercise training on vascular health in adolescents with type 2 diabetes has not been previously studied. We hypothesized that exercise training would improve micro and macro-vascular health in adolescents with type 2 diabetes. Thirteen adolescents (13-21yrs, 10F) with type 2 diabetes were recruited from Princess Margaret Hospital. Participants were randomized to receive either an exercise program along with standard clinical care (n=8) or standard care alone (n=5). Those in the intervention group received 12 weeks of gym-based, personalized and supervised exercise training. Those in the control group were instructed to maintain usual activity levels. Assessments were conducted at baseline and following week 12. The exercise group was also studied 12 weeks following the conclusion of their program. Assessments consisted of conduit artery endothelial function (flow mediated dilation, FMD) and micro-vascular function (cutaneous laser Doppler). Secondary outcomes included body composition (dual-energy X-ray absorptiometry, DXA), glycaemic control (whole body insulin sensitivity, M) assessed using the euglycaemic- hyperinsulinaemic clamp protocol, cardiorespiratory fitness ($\dot{V}O_{2peak}$) and muscular strength (1RM). Exercise training increased FMD (P<0.05), microvascular function (P<0.05), total lean mass (P<0.05) and muscle strength (P<0.001). There were no changes in cardiorespiratory fitness, body weight, BMI or M. In the control group, body weight (P<0.01), BMI (P<0.01) and total fat mass (P<0.05) increased. At week 24, improvements in vascular function were reversed. This study indicates that exercise training can improve both conduit and microvascular endothelial function and health, independent of changes in insulin sensitivity in adolescents with type 2 diabetes.

Abbreviation list

1RM- one-repetition maximum

CVC- cutaneous vascular conductance

DXA- dual-energy X-ray absorptiometry

FMD- flow mediated dilation

LDF- laser-Doppler flux

M- whole body insulin sensitivity

NO- nitric oxide

PU- perfusion units

$\dot{V}O_{2peak}$ - Peak oxygen consumption

Introduction

Type 2 diabetes in the young is an emerging paediatric health concern with profound societal implications (Riddoch, 1998). The prevalence of type 2 diabetes in adolescents increased approximately 10 fold between 1996 and 2002 (Hotu *et al.*, 2004), with a report from our group indicating a similar trend (Davis *et al.*, 2002). Insulin resistance, formerly rarely diagnosed prior to adulthood, is now estimated to be present in 20-25% of obese children and adolescents (Sinha *et al.*, 2002), with type 2 diabetes prevalent in >5% of this group.

Of particular concern in this high risk cohort is the rapid appearance and progression of vascular disease. We have previously published data indicating that early signs of vascular dysfunction are apparent in children and adolescents with obesity and type 2 diabetes, compared to both lean and BMI-matched counterparts (Naylor *et al.*, 2011). Adolescent onset type 2 diabetes is often aggressive, with earlier onset of complications than adult-onset type 2 diabetes or childhood type 1 diabetes. For example, in Japanese children with type 2 diabetes, incipient retinopathy was detected in 36% of the cases at the time of diagnosis and in 39% of the cases at 2 years follow-up, while microalbuminuria was observed in 39% at 2 years (Yokoyama *et al.*, 2000). Similarly, 22% of Pima Indian children with type 2 diabetes had microalbuminuria, and at follow-up (20-29 years) 60% had developed microalbuminuria and 17% macroalbuminuria (Krakoff *et al.*, 2003). Similarly, data from Australia indicate that young people with type 2 diabetes had significantly higher rates of microalbuminuria and hypertension, despite a shorter duration of diabetes and lower HbA1c than those with type 1 diabetes (Eppens *et al.*, 2006a). Indeed, an International Diabetes Federation consensus statement concluded that the "*onset of diabetes in childhood or adolescence heralds many years of disease and an increased risk that the full range of both micro- and macrovascular complications will occur when affected individuals are still relatively young*" (Alberti *et al.*,

2004). However, little is known about the most effective treatment approaches to reduce cardiovascular disease in these young patients (Kaufman, 2002; Copeland *et al.*, 2013), in whom surgical and medication strategies are not without risk and largely unproven in terms of long term efficacy.

Exercise is a cornerstone of diabetes prevention and management in adults (Colberg *et al*., 2010; American Diabetes Association, 2011) and has been demonstrated to improve endothelial function in both resistance (forearm plethysmography and infusions of acetylcholine, ACh) and conduit vessels (flow-mediated dilation, FMD) in adults with type 2 diabetes (Maiorana *et al.*, 2001). Similarly, Colberg et al. (Colberg *et al.*, 2002) assessed skin blood flow responses before and after an exercise intervention in adults with type 2 diabetes and reported that exercise training enhanced cutaneous microvascular function, assessed using laser Doppler. Such studies have not, however, been performed in children or adolescents and this is highly relevant since interventions that modify vascular function and health in the early, pre-clinical stages of atherosclerotic disease (Ross, 1993) hold the potential to contribute to prolonged retardation of lesion development and clinical progression. From the experimental viewpoint, type 2 diabetes in children also offers a model to improve our understanding of primary prevention, independent of the influence of aging and comorbidities that are common in adults.

To date, no studies have assessed whether exercise interventions can induce similar improvements in function as those seen in adults. Of the few studies carried out in adolescents, mixed results have been reported following intensive lifestyle modifications (Reinehr *et al.*, 2008; TODAY study group, 2012; Copeland *et al.*, 2013). In addition, few

studies have investigated the effects of exercise training on aspects of the insulin resistance syndrome in obese children (Ferguson *et al.*, 1999; Bell *et al.*, 2007) and studies are altogether lacking in type 2 diabetic youth. We hypothesized that exercise training would enhance conduit and microvessel endothelial function, glycaemic control, body composition and cardiorespiratory fitness in adolescents with type 2 diabetes, compared to those who only received standard care.

Materials and Methods

Ethical Approval

All study procedures were approved by the Human Research Ethics Committee of Princess Margaret Hospital for Children and the University of Western Australia and the study conformed to the *Declaration of Helsinki*. All participants provided written consent and, for those participants <18yrs, their parents also gave written informed consent.

Subject Characteristics

Thirteen participants (13 to 21 years) diagnosed with type 2 diabetes, who were free from pre-existing type 1 diabetes or diagnosed cardiovascular disease, were recruited to this study from the Department of Endocrinology and Diabetes at Princess Margaret Hospital for Children. The diagnosis of type 2 diabetes was made on clinical and biochemical grounds, according to ISPAD guidelines (Zeitler *et al.*, 2014).

Study Design

All measures were collected prior to and following a 12-week intervention period. After baseline assessment, block randomization was used to allocate participants to receive an exercise intervention along with standard clinical care or to act as controls who received

standard clinical care alone (education about lifestyle changes with or without metformin and/or insulin). Additionally, to determine whether changes induced by the training program were maintained, measures were repeated in the exercise group following a further 12 week period after the formal training program ceased. The use of block randomization ensured exercise in small groups (maximum 4 per group), allowing for direct supervision and individualization of programs to all participants.

Exercise Intervention

Participants attended 3, one-hour, exercise-training sessions per week for a period of 12 weeks. An experienced Advanced Exercise Physiologist (AEP) closely supervised all exercise sessions to ensure compliance with the prescribed exercise programs. All sessions used a combined aerobic (65-85% of HRmax) and resistance (55-70% MVC) training regimen, with HRmax determined from the $\dot{V}O_2$max assessment, and MVC via strength assessments (see below). Training volume was gradually and progressively increased. Regular stretching and core strengthening exercises were included in the sessions to minimize the risk of injury.

Experimental Measures

Experimental measures included the assessment of conduit artery endothelial function using the flow mediated dilation (FMD) technique and micro-vascular assessment via cutaneous laser Doppler using a combined pharmacological and heating interventions. Glycaemic control was assessed via the gold standard hyperinsulinaemic euglycaemic clamp (clamp), whilst body composition was assessment using dual-energy X-ray absorptiometry (DXA). Cardiorespiratory fitness ($\dot{V}O_{2peak}$) was evaluated using a graded exercise test and muscular strength using one-repetition maximum (1RM) tests. All assessments were conducted in a

quiet, temperature controlled environment and all studies were conducted at the same time of the day to eliminate possible circadian variations (Jones, 2012). All post-intervention assessments were performed at least 72 hours post-exercise to ensure that acute exercise did not impact upon the results.

Assessment of conduit artery endothelial function: Flow-mediated dilation (FMD)

Participants arrived at the cardiovascular laboratory following at least 6 hours of fasting, having abstained from alcohol and/or caffeine and exercise for 72 hours prior to testing. After a 20-minute rest period, the brachial artery diameter response to FMD was assessed, using a 12L5v linear array transducer, attached to a high resolution ultrasound machine (t3200; Terason, Burlington, MA). Detailed descriptions of this technique are provided elsewhere (Woodman *et al.*, 2001; Thijssen *et al.*, 2011). Briefly, participants lay supine with their arms extended at ~80° from their torso. A rapid inflation/deflation pneumatic cuff (AG 101 Hokanson, Bellevue USA) was placed around the arm immediately distal to the olecranon process. When an optimal B-mode image was obtained, images were collected using an insonation angle (always <60°), which did not vary during each study or within individuals across the intervention. Baseline images were recorded for 1 minute, before the forearm cuff was inflated to 220mmHg for 5-minutes. Recording resumed 30sec prior to cuff deflation and continued for 5-minute post-deflation. Flow mediated dilation is presented as the relative (%) rise from the preceding baseline diameter.

Assessment of microvascular function: Microdialysis and laser-Doppler flowmetry

The microdialysis technique involving prolonged non-painful local heating, as described by Black *et al*. (Black *et al.*, 2008), was adopted for this study and performed during euglycaemic hyperinsulinaemic clamps (see below for further details) to ensure that potential

variance in glycaemic control did not influence the results. After lying on the bed comfortably with the 2 catheters inserted on the right arm for the glycaemic clamps, two very fine microdialysis fibres (Linear 30, CMA Microdialysis Ltd, Stockholm, Sweden), containing 10mm long 6-kDa membranes, were placed in the dermal layer of the skin following initial placement of a 21-gauge needle. The needles were then removed and the embedded fibres perfused with Ringer's (site 1) or the nitric oxide (NO) blocker N^G-monomethyl-L-arginine (LNMMA, 10mMol; site 2) at a rate of 5µl/min using a microinfusion pump (Model 11 plus, Harvard Apparatus, MA, USA). LNMMA induces vasoconstriction of the microcirculation by blocking NO synthase and production. Skin perfusion was measured over both microdialysis sites using a Perimed System 5000 with integrated laser Doppler probes each consisting of a 7 laser array (Model 413, Periflux 5001 System, Perimed AB, Sweden), above each microdialysis fibre. The laser-Doppler probe signals were continuously monitored via a software chart recorder (LabChart 7). At each designated study time-point (5 minute intervals), skin blood flow was assessed by averaging laser-Doppler flux (LDF), measured in perfusion units (PU), over a stable 2 minute period. These data were subsequently converted to cutaneous vascular conductance (CVC), calculated as PU ÷ MAP (mmHg), where MAP (mean arterial pressure) was derived from contemporaneous automated blood pressure (Critikon DINAMAP Vital Signs Monitor 8100) measures.

Following the equilibration period involving Ringer's or LNMMA infusions, skin blood flow at 33ºC was recorded for a 20-minute period. Both probes were then heated gradually, using local heating disks surrounding the Doppler probes and overlaying the microdialysis sites, from 33ºC-42ºC at a rate of 0.5ºC every 5 minutes to a temperature of 42ºC (90mins), as described by Black *et al.* (Black *et al.*, 2008). Both sites were continuously heated at 42º for

the remainder of the study. At the end of the study, sodium nitroprusside (56mM) was infused through both sites to stimulate a maximal skin blood flow response (Cracowski *et al.*, 2006). For details of the study design pertaining to assessment of NO-mediated microvascular function, refer to Figure 1. By comparing the magnitude of CVC% increase with heating in the presence of LNMMA at site 2 to the magnitude of increase with heating in the Ringers' solution (site 1), we were able to identify the magnitude of the NO contribution to the local heating response.

Assessment of insulin sensitivity

Participants attended the research unit for the clamp study, having fasted from 10pm of the previous night. They were advised to omit metformin in the 24 hours preceding the study and insulin on the morning of the study. They were also advised to avoid exercise for 72 hours prior to testing to account for the effect of acute exercise on glycaemic control.

Two intravenous catheters (BD Insyte I.V. Catheter, 22GA 1.00IN; 0.9 × 25 mm) were inserted into superficial veins of the right arm. The first catheter, in the antecubital fossa, was used for infusion of insulin and glucose, whilst the second catheter, in a superficial digital vein, was used to measure blood glucose to guide the rate of glucose infusion. Following insertion of catheters, target plasma glucose levels at each stage of the clamp were achieved by titrating the rate of infusion of a 20% dextrose solution whilst maintaining constant rate of insulin (A 1:1 dilution of 60U Humalog in 60mls of 0.9% saline solution) at 60 mU m^{-2} min^{-1}. Blood samples were taken every 5-10 minutes and analyzed at the bed-side using a YSI2300 glucose analyzer (YSI2300. Yellow Springs Instrument, OH, USA). Blood glucose concentrations were stabilized at 5.5 (±0.1) $mmol.L^{-1}$ over a period of 90 min. Following this stabilization period, all participants were clamped at euglycaemia (~5.5 mmol L^{-1}) using the

hyperinsulinaemic euglycaemic clamp technique first described by De Fronzo *et al.* (DeFronzo *et al.*, 1979), for 60mins for assessment of insulin sensitivity.

Assessment of body composition

On arrival to the laboratory, height and weight were measured and BMI and BMI z-scores calculated. Whole body dual emission x-ray absorptiometry (DXA) assessment (Lunar Prodigy, GE Medical Systems, Madison, WI, USA) was used to determine body composition. Specifically, total fat mass, total lean body mass and body fat percentage were assessed. Regional components of body composition were determined off-line using dedicated software.

Assessment of aerobic fitness

Graded exercise tests were performed on a bicycle ergometer (Monarck). The test consisted of 3 min exercise epochs, increasing until volitional exhaustion. Expired air was analyzed for O_2 and CO_2 concentrations (Ametek Gas Analyzers, Applied Electrochemistry, SOC S-3A/1 and COV CD-3A, Pittsburgh, PA) and ventilation (VE) was recorded at 15 s intervals using a turbine ventilometer (Morgan, 225A, Kent, England). Prior to and following each test, the ventilometer and gas analyzers were calibrated according to manufacturer's instructions using a 1L syringe and gases of known concentration (BOC Gases, Chatswood, Australia). Peak oxygen consumption ($\dot{V}O_{2peak}$) was determined by summing the four highest consecutive 15s $\dot{V}O_2$ values in each workload. Heart rate was continually monitored using a Polar Heart Rate Monitor (Polar F1, Finland) throughout the test and recorded in the last 10s of each workload.

Assessment of muscular strength

Maximal upper and lower body strength was determined using upper body exercises (bench press, pectoral strength, bicep curl) and squat exercises according to a 1RM protocol. Participants were briefed on correct form for each exercise and performed familiarization lifts. A warm-up of 10 repetitions at 50% of their predicted 1RM was given followed by 5 repetitions at 70%, 3 repetitions at 80% and 1 repetition at 90% of predicted 1RM. Participants were then given 3 attempts to determine their actual 1RM. A recovery of 5 minutes was given between efforts.

Statistics

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, Illinois). All data are reported as mean ± SE, unless stated otherwise. Statistical significance was assumed at $P < 0.05$. Two-way ANOVA was used to determine the effect of exercise training on all outcome measures. Post hoc *t*-tests were conducted to further interrogate statistically significant ANOVA results. One-way ANOVA was used to determine whether the impact of training persisted beyond the formal training period.

Results

There were no differences in terms of subject characteristics between the groups at baseline, indicating that the groups were similar at entry (Table 1). There were no differences between the exercise and control groups in resting HR (79 ± 5 vs 71 ± 3 bpm, P = 0.26), resting SBP (128 ± 4 vs 121 ± 4 mmHg, P = 0.18), resting DBP (86 ± 3 vs 81 ± 4 mmHg, P = 0.30) or HbA1c (8.8 ± 1.0 vs 6.6 ± 0.8%, P = 0.11). Four participants in the exercise group, and 3 in the control group were prescribed metformin, whilst two participants in the exercise group were using a combination of metformin and insulin. The remaining participants were not

prescribed any medication for their diabetes. Three participants, two from the exercise, and one from the control group, opted not to participate in the glycaemic clamp or microvascular assessments.

Impact of exercise training on body weight and composition

There was no increase in body weight and BMI following the 12 week intervention period in participants who received the exercise program, but a significant increase in weight and BMI was observed in the control group during this period. Body composition (DXA) measures revealed a significant effect on total fat mass (time*group interaction effect via 2 way ANOVA, $P<0.05$), with a mean reduction of total fat mass by 1.10kg in the exercise group and an increase of 1.96kg in the control group. This was accompanied by a slight, although not statistically significant, change in fat free mass (+1.35kg in the exercise group, compared to +0.23kg increase in control group, Figure 2).

Impact of exercise training on muscular strength and cardiorespiratory fitness

Combined upper and lower limb strength increased significantly as a result of the exercise training (time*group interaction effect via 2 way ANOVA, $P<0.005$). Post-hoc analysis revealed that the combined strength increased by 31kg in the exercise group ($P<0.001$) with no changes in combined strength in the control group (Table 1). Exercise training induced significant increases in lower limb strength (+23kg, $P<0.001$) and pectoral strength (+14kg, $P<0.05$) measures. No significant changes in any measures of muscular strength were observed in the control group following the 12 week intervention period.

Cardiorespiratory fitness ($\dot{V}O_{2peak}$) was not significantly altered following the intervention (time*group interaction effect via ANOVA P=0.07). In agreement with this result, there was

no significant change in max HR (P= 0.886), maximal power (P = 0.980) or time to exhaustion (P = 0.812) following the intervention. Although statistical significance was not achieved, exercise training did attenuate the decrease in cardiorespiratory levels observed in the control group (-2.5 $ml.kg^{-1}.min^{-1}$, Table 1).

Impact of exercise training on vascular endothelial function

Following exercise training, ANOVA indicated that there was a significant time*group interaction effect in conduit artery endothelial function (FMD, P<0.05). Post hoc analysis revealed a significant increase of FMD (+2.2 ± 1.1%, P <0.05) following exercise training, whilst no changes were evident in the control group (Figure 1). Brachial artery diameter did not change in either group following the intervention period (Table 1, P = 0.77, ANOVA). There was a significant time*group interaction effect in NO-mediated microvascular function ($CVC\%_{max}$, ANOVA time*group interaction effect, P<0.05), with a significant improvement following exercise training (P< 0.01, Figure 4). The NO contribution to the response to localised heating was improved following the exercise intervention (Figure 5, time*group interaction effect via 2 way ANOVA, *P*<0.05). No significant differences were observed in the control group (Figure 5, P =0.40, paired t-test).

Impact of exercise training on glycaemic control

Whole body insulin sensitivity (M) did not significantly change in either group (Table 1), although the average decrease observed in the control group was attenuated by the exercise training intervention (Table 1).

Impact of detraining

Following the completion of the intervention period, those in the exercise training group were followed for a further 12 weeks to assess persistence of the training. During this time, the exercise training sessions were stopped and participants were instructed to return to their normal activity levels. One individual from the exercise group was lost to follow-up due to changes in medication (commencement of insulin). This participant did not receive the clamp or microvascular assessments at baseline and week 12.

Compared to values at entry into the study, no changes in either body weight or BMI were evident following the detraining period (Table 2). Total fat mass was stable following detraining, whilst the increase in fat free mass with training was largely maintained (Table 2). Increases in lower limb and upper body strength were maintained following training, whereas $\dot{V}O_2$peak did not change (Table 2).

The improvement in conduit artery FMD following exercise training were not sustained following detraining. Similarly, improvements noted following the training program in stimulated microvascular endothelial function returned to baseline levels following the detraining period (11.4 ± 0.8 $CVC\%_{max}$).

No significant changes were observed in relation to whole body insulin sensitivity (M) following the detraining period (Table 2, one way ANOVA, P = 0.6).

Discussion

In our cohort of adolescents with type 2 diabetes, a 12 week structured exercise training program had beneficial impacts on measures of conduit and micro-vessel endothelial

function, body composition and strength. Our findings complement previous reports that exercise training can improve vascular function in adults with type 2 diabetes (Maiorana *et al.*, 2001) and in obese, non-diabetic children (Watts *et al.*, 2004b) and adolescents (Watts *et al.*, 2004a). However this is the first study to report that arterial function can be improved as a result of exercise training in adolescents with type 2 diabetes. This is particularly important in this clinical population, who are at highly elevated risk of developing vascular disease (Eppens *et al.*, 2006b; Naylor *et al.*, 2011).

Previously, Maiorana et al. (2001) demonstrated improved endothelial function in both resistance and conduit vessels following 8 weeks of supervised exercise training in 16 adults with type 2 diabetes. Endothelial function of the resistance vessels was assessed using forearm plethysmography with concomitant intra-brachial infusions of acetylcholine, ACh, whilst FMD was employed to assess conduit artery endothelial function. Of note, smooth muscle mediated vasodilation (analysed via the use of SNP and GTN for resistance and conduit arteries respectively) were unchanged following the training program. Cohen et al. (Cohen *et al.*, 2008) also reported that a 14-month progressive resistance training program improved microvessel endothelial function in adults with type 2 diabetes. In this study, endothelial function was assessed in the skin microcirculation using laser Doppler flow following iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP). To the contrary, Middlebrooke and colleagues (Middlebrook *et al.*, 2006) reported that 6 months of aerobic training did not improve endothelial function in adult skin microvessels (age 62.9±7.6 yrs) of type 2 diabetics with good glycaemic control (HbA1c 6.8±0.9%). In that study, microvessel function was assessed using the maximum skin hyperaemia to local heating and endothelial and non-endothelial responsiveness following the iontophoretic application of acetylcholine (ACh) and sodium nitroprusside (SNP). It is also worthwhile

noting that, similar to our findings, no improvements were seen in $\dot{V}O_2max$ following the exercise intervention. Exercise training studies have also shown improvement in plasma biomarkers of inflammation and endothelial function in older adults with type 2 diabetes (Zoppini *et al.*, 2006). In this cohort, the authors showed improvements unrelated to changes in body weight, waist circumference, blood pressure, HbA1c, plasma triglyceride or LDL cholesterol concentrations, in keeping with our findings. The discrepancies noted above may, at least in part, be attributed to the variance in training programs and patient populations, however, overall they provide support of our current findings in an adolescent cohort.

In addition, the present study reports a significant improvement in the NO contribution to microvascular function. This may have some clinical relevance, given evidence indicating that cutaneous microvessel dysfunction is correlated with coronary endothelial dysfunction (Shamim-Uzzaman *et al.* 2002; Khan *et al.* 2008) and cardiovascular risk factors (Khan *et al.* 1999; Carberry *et al.* 1992; Rizzoni *et al.* 2003). The improvements in NO-mediated function observed in this study therefore support the use of exercise training to prevent future microvascular disease in this population of young individuals.

Paediatric populations are distinctly different in terms of physiology from their adult counterparts. For example, puberty induces increased resistance to the action of insulin, resulting in relative hyperinsulinaemia (Moran *et al.*, 1999), and after puberty basal and stimulated insulin responses decline. In the presence of normal pancreatic-cell function, puberty-related insulin resistance is compensated by increased insulin secretion. It is therefore important to emphasize that these findings are the first in young people with type 2 diabetes to demonstrate that vascular function measures, collected by distinct techniques and at different levels of the arterial tree, consistently increased following exercise training.

In our study exercise training also produced modest benefits in terms of body composition. Notably, these improvements were not reflected in terms of changes in either total body weight, or BMI. This is similar to findings reported by Watts et al. (Watts *et al.*, 2004a), where there were no changes in either total weight or BMI as a result of exercise training, but significant improvements in adiposity and muscle mass observed following an 8 week exercise training intervention in 19 obese adolescents (14.3 ± 1.5 yrs, mean BMI 34.4 ± 0.8). Collectively, these findings emphasize the importance of comprehensive assessment of body composition, rather than reliance on body weight or BMI as primary outcome measures in exercise intervention studies.

We did not observe significant changes in insulin sensitivity in this study. It is well established that exercise training is an effective strategy to *prevent* diabetes (Diabetes Prevention Program Group 2002) and we have previously reported that a similar training protocol induces improvements in insulin sensitivity in obese, non-diabetic adolescents (Bell *et al.*, 2007). However, there is very little published data regarding the impact of physical activity and exercise training in adolescents with established type 2 diabetes. In accordance with our finding, the TODAY study group (2012) reported that in 10-17 year olds with type 2 diabetes, the addition of a lifestyle intervention to medication did not produce superior benefits compared to treatment by medication alone. Failure to achieve durable glycaemic control (HBA1c >8%) was reported in 46.6% of those in the metformin plus lifestyle intervention, compared to 51.7% of those who only received metformin. The sole use of a lifestyle intervention was unsuccessfully in controlling glucose metabolism in German and Austrian children and adolescents with established type 2 diabetes (Reinehr *et al.*, 2008). These studies differ importantly from the findings of the diabetes prevention study, in which

adults showed nearly twice the benefit of metformin as a result of lifestyle modification involving exercise (Diabetes Prevention Program Research Group, 2002). Apart from revealing a possible distinction between studies of children and adults with diabetes, these studies also emphasize the importance of promoting a healthy lifestyle and regular exercise in obese young people before they develop type 2 diabetes, when improvements in glycaemic control may be more difficult to achieve via exercise training. Notwithstanding the absence of change in insulin sensitivity, it is important to reiterate that beneficial adaptations were apparent in both conduit and microvascular function as a consequence of exercise training in our study.

Whilst improvements in insulin sensitivity were not significant in the exercise group, there was a non-significant decrease in the control group, accompanied by a significant increase in fat mass. This implies that, whilst exercise training did not reveal significant improvements in insulin sensitivity in our modest study sample, it may attenuate the natural progression of type 2 diabetes and its complications in this adolescent cohort. There is no doubt the small sample size in this mechanistic experiment limits our ability to draw definitive conclusions regarding the impact of diabetes and exercise training on glycaemic control, but the trends that have emerged should encourage larger funded trials of these outcomes.

It is also interesting to note that, while we observed significant strength gains in our cohort, no changes were seen in terms of cardiorespiratory fitness following the exercise intervention. Whilst this may seem unexpected, we are not the first group to report this. Recent data even suggest that individuals with a first degree relative with type 2 diabetes have a lower response in terms of fitness gain, compared to those without a family history (Ekman *et al.*, 2015).

Finally, we also assessed detraining data. These data indicate that the favorable changes in body composition and strength induced by the exercise training can be maintained beyond the duration of the training program. However, vascular function declined following the cessation of training, suggesting that a continuation of the stimulus to vascular adaptation, likely to be shear stress (Joyner & Green, 2009; Tinken *et al.*, 2009), may be necessary to maintain gains in arterial health.

To monitor compliance, all exercise training sessions were supervised by clinical exercise physiologists and personalized programs were designed for each participant. Another strength of the study is the inclusion of a control group of subjects who undertook routine clinical care, which allowed us to determine the natural progression of type 2 diabetes in adolescents. Limitations of this study include our small sample size. Although the study was adequately powered to detect the magnitude of observed changes in the primary outcome measures (conduit and microvessel function) (Woodman *et al.*, 2001), the comparison between the exercise intervention group and control group should be interpreted with caution due to differences between the groups at baseline. It is worthwhile noting that the participants in our study possessed poor metabolic control (average HbA1c of all participants at entry = 8%), and that although the difference in HbA1c at entry was not statistically significant, differences were noted between the groups. Nonetheless, the control group deteriorated across the intervention period, whilst the trained group, who exhibited worse physiological function and health at baseline, demonstrated exercise training mediated improvements. For example, despite the control group being leaner and having a lower body weight at baseline, there were significant increases in weight, which occurred alongside a reduction in insulin sensitivity (M), indicating a decline in health over the 12 week intervention period.

In conclusion, we present data in a sample of subjects suggesting that exercise training interventions can improve both conduit and microvascular endothelial function and health in adolescents with type 2 diabetes. Detraining data imply that continuous training is likely to be necessary to maintain these clinically important benefits. We conclude that personalised and intensive exercise training may be effective in ameliorating the detrimental clinical impacts of type 2 diabetes and may decrease the future risk of cardiovascular complications in this very high risk cohort.

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Competing interests.

The authors have nothing to disclose.

Author contributions

The flow mediated dilation studies, body composition, muscular strength and aerobic fitness assessments were conducted in the exercise physiology laboratories at the School of Sport Science, Exercise and Health at the University of Western Australia. Microvascular function assessments were conducted at the Children's Clinical Research Facility, Princess Margaret Hospital. The exercise interventions were conducted at the School of Sport Science, Exercise and Health at the University of Western Australia, or in local training facilities near the participant's home.

Each author has approved the submission of this version and the contributions of each author is as follows:

LN: Substantial contributions to the conception and design of the work, acquisition, analysis, and interpretation of data for the work. She also produced the first draft of the manuscript.

ED: Substantial contributions to the conception and design of the project, input into the interpretation of data and revising the manuscript critically for important intellectual content.

RK: Substantial contributions to the acquisition, and analysis of data, assistance with drafting the work

NP: Substantial contributions to the acquisition, analysis, and interpretation of data, and revising the manuscript critically for important intellectual content.

MA: Substantial contributions to the acquisition, and analysis of data, and revising the manuscript critically for important intellectual content.

TJ: Substantial contributions to the conception and design of the project, input into the interpretation of data and revising the manuscript critically for important intellectual content.

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Table 1. Baseline and week 12 data

	Exercise group		Control Group		P-values
	Pre	Post	Pre	Post	Exercise vs Control Pre
Gender	6F, 2M		4F, 1M		
Duration of Diabetes (months)	38.6 ± 6.9		17.2 ± 7.5		0.14
Age (years)	17.3 ± 0.8		15.3 ± 0.8		0.11
Anthropometric Data					
Height (cm)	167.3 ± 4.3	167.7 ± 4.1	166.3 ± 3.8	166.8 ± 3.6	0.88
Weight (kg)	100.1 ± 9.6	100.1 ± 9.7	84.1 ± 9.4	86.5 ± 9.8**	0.29
Total fat (kg)	43.65 ± 6.59	42.74 ± 6.18	34.50 ± 4.79	36.46 ± 4.76*	0.34
Total Lean (kg)	52.02 ± 3.40	53.36 ± 4.01**	45.55 ± 4.82	45.78 ± 4.94	0.28
% Fat	44.1 ± 2.8	43.2 ± 2.9	42.7 ± 2.4	44.0 ± 2.2	0.74
BMI $kg.m^{-2}$	36.1 ± 3.9	36.0 ± 3.8	30.0 ± 2.2	30.7 ± 2.4**	0.27
BMIz-score	1.9 ± 0.2	1. 9 ± 0.2	1.8 ± 0.3	1.8 ± 0.3	0.72
Glycaemic control					
HbA1c (%)	8.8 ± 1.0	9.2 ± 1.0	6.6 ± 0.2	6.5 ± 0.2	0.11
M (lbm; $mg.kg^{-1}.min^{-1}$)†	4.7 ± 1.7	5.0 ± 1.5	5.7 ± 0.8	5.1 ± 1.2	0.66
Fitness and strength data					
$\dot{V}O_2$peak ($ml.kg^{-1}.min^{-1}$)	25.7 ± 2.4	26.4 ± 3.5	29.9 ± 2.7	27.4 ± 2.7	0.28
Total Strength (kg)	60.2 ± 7.4	91.6 ± 8.7**	60.2 ± 8.4	66.6 ± 9.8	0.99

Vascular data					
Brachial artery diameter (cm)	3.38 ± 0.18	3.49 ± 0.19	3.03 ± 0.21	2.97 ± 0.20	0.19
Max diameter post ischaemia (cm)	3.65 ± 0.19	3.86 ± 0.25	3.27 ± 0.24	3.20 ± 0.20	0.244
FMD (%)	7.62 ± 1.2	9.82 ± 1.0*	7.84 ± 1.0	7.35 ± 1.1	0.91
Time to peak diameter (s)	68.4 ± 5.9	57.3 ± 11.7	57.7 ± 7.8	56.8 ± 11.2	0.29

**** (P<0.05) compared to pre, ** compared to pre (P<0.01),***
† n = 6 in the exercise group and n= 4 in the control group for clamp data

Table 2. Impact of detraining (n=7)

	Pre-Training	Post-Training	Detraining
Anthropometric Data			
Weight (kg)	97.7 ± 10.7	97.9 ± 11.5	97.6 ± 15.4
Total fat (kg)	42.17 ± 7.41	40.21 ± 7.08	40.43 ± 6.63
Total Lean (kg)	51.07 ± 3.78	53.45 ± 4.9	53.35 ± 5.26
% Fat	43.6 ± 3.2	41. 9 ± 3.2	42.3 ± 2.8
BMI ($kg.m^{-2}$)	34.5 ± 4.1	34.5 ± 4.4	34.3 ± 4.3
BMI z-score	1.8 ± 0.2	1.8 ± 0.2	1.8 ± 0.2
Glycaemic control			
HbA1c (%)	8.5 ± 1.1	8.7 ± 1.7	8.8 ± 1.2
M (lbm; $mg.kg^{-1}.min^{-1}$)†	4.7 ± 1.7	5.0 ± 1.5	4.0 ± 1.7
Fitness and strength data			
$\dot{V}O_2$peak ($ml.kg^{-1}.min^{-1}$)	25.8 ± 2.9	27.8 ± 4.5	26.8 ± 3.4
Total Strength (kg)	36.4 ± 5.4	60.0 ± 7.2**	57.9 ± 8.4*
Upper Limb Strength (kg)	26.3 ± 3.1	35.4 ± 4.3*	33.8 ± 4.2*
Lower Limb Strength (kg)	62.7 ± 8.1	95.4 ± 9.7**	91.7 ± 11.2*
Vascular data			
Brachial artery diameter (cm)	3.49 ± 0.14	3.60 ± 0.21	3.34 ± 0.21
Max diameter post ischaemia (cm)	3.77 ± 1.68	3.99 ± 2.40	3.61 ± 2.47
FMD (%)	8.07 ± 1.25	10.26 ± 0.97	8.21 ± 1.14
Time to peak diameter (s)	70.1 ± 6.40	57.4 ± 13.5	40.33 ± 15.24
Microvessel endothelial function (%CVCmax)†	13.7 ± 3.5	27.8 ± 3.4*	11.4 ± 0.8

*****P<0.05 compared to baseline data, *P<0.01 compared to baseline data***
†n = 6 for these measures