LIVERPOOL JOHN MOORES UNIVERSITY

LJMU Research Online

Schreuder, TH, Green, DJ, Nyakayiru, J, Hopman, MT and Thijssen, DHJ

Time-course of vascular adaptations during 8 weeks of exercise training in subjects with type 2 diabetes and middle-aged controls

http://researchonline.ljmu.ac.uk/id/eprint/2691/

Article

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Schreuder, TH, Green, DJ, Nyakayiru, J, Hopman, MT and Thijssen, DHJ (2015) Time-course of vascular adaptations during 8 weeks of exercise training in subjects with type 2 diabetes and middle-aged controls. European Journal of Applied Physiology, 115 (1). pp. 187-196. ISSN 1439-

LJMU has developed **LJMU Research Online** for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact researchonline@ljmu.ac.uk

http://researchonline.ljmu.ac.uk/

TIME-COURSE OF VASCULAR ADAPTATIONS DURING 8 WEEKS OF EXERCISE TRAINING IN SUBJECTS WITH TYPE 2 DIABETES AND MIDDLE-AGED CONTROLS

TIM H.A. SCHREUDER[1], DANIEL J. GREEN[2,3], JEAN NYAKAYIRU[1], MARIA T.E. HOPMAN[1], DICK H.J. THIJSSEN[1,3]

[1]*Department of Physiology*,

Radboud University Nijmegen Medical Centre, the Netherlands

[2]*School of Sport Science, Exercise and Health,*

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

[3]*Research Institute for Sport and Exercise Sciences, Liverpool John Moores University,*

Tom Reilly Building, Byrom Street, Liverpool L3 3AF, United Kingdom

Running title: Time-course of vascular adaptations in type 2 diabetes

Tables: 2

Figures: 1

Word count text: 3895

Word count abstract: 191

Author for correspondence:

Dick H.J. Thijssen, Department of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands. Tel: +31243614222, Email: D.Thijssen@fysiol.umcn.nl

ABSTRACT

PURPOSE Exercise training in healthy volunteers rapidly improves vascular function, preceding structural remodelling. No study examined the time-course of such adaptations in subjects with *a priori* endothelial dysfunction. METHODS We examined brachial artery endothelial and smooth muscle function using flow-mediated dilation (FMD) and glyceryl trinitrate (GTN) administration in 13 type 2 diabetes patients (59±6 years) and 10 healthy subjects (58±7 years) before, during (2-weekly) and after an 8-week training program. Arterial structure was assessed via peak blood flow and artery diameter. RESULTS Training increased peak oxygen uptake (P=0.03), comparable between groups (P=0.276). We observed a similar impact of training on brachial artery vasomotor function across the training period in diabetes patients and controls (FMD/GTN-ratio), with a higher FMD/GTN-ratio at 2, 6 and 8 weeks (P=0.036). Artery diameter, peak blood flow or peak diameter had not changed after training. CONCLUSION Training leads to rapid improvement in brachial artery vascular function in diabetes patients and controls. In contrast to previous observations in healthy young subjects, the increase in function was preserved after 8 weeks of training in middle-aged diabetes patients and controls, suggesting a different time-course in vascular adaptations in subjects with endothelial dysfunction.

KEYWORDS: cardiovascular risk; exercise training; metabolic disease; time course

ABBREVIATIONS

ANOVA	analysis of variance
BMI	body mass index
bpm	beats per minute
eNOS	endothelial nitric oxide synthase
FMD	flow mediated dilation
GTN	glyceryl trinitrate
HDL	high density lipoprotein
HOMA-IR	homeostasis model assessment for insulin resistance
HR	heart rate
LDL	low density lipoprotein
LSD	least significant differences
NO	nitric oxide
rpm	rotations per minute
RQ	respiratory quotient
T2DM	type 2 diabetes mellitus
W	watt

INTRODUCTION

Regular exercise training has strong and independent cardioprotective effects in asymptomatic subjects and in those at increased cardiovascular risk (Blair and Morris 2009), an impact that can only partly be explained by changes in traditional cardiovascular risk factors (Mora et al. 2007). One explanation for this 'risk factor gap' invokes direct effects of exercise on the vasculature (Green et al. 2008; Joyner and Green 2009). Insight into adaptations in vascular function will therefore contribute to a better understanding of the cardioprotective effects of exercise training.

Previous studies suggest that improvement in vascular function after dynamic exercise training is not universal (Green et al. 2004; Green et al. 2011), especially in healthy volunteers. The presence of time-dependent adaptations in vascular function in response to exercise training may partly explain this observation. Originally based on findings in animals (Laughlin 1995), human studies have also demonstrated that short-term exercise training enhances conduit artery function in subjects with cardiovascular risk factors or disease (Green et al. 2004; Maiorana et al. 2001; Watts et al. 2004), whilst prolonged training induces structural changes (i.e. increased diameter) (Brown 2003; Prior et al. 2003). Studies involving 2-weekly measurements across an 8-week period of large (i.e. cycling/running exercise) (Birk et al. 2012; Tinken et al. 2008) or small muscle group (i.e. handgrip exercise) (Tinken et al. 2010) training in healthy young volunteers have consistently demonstrated that 2 weeks of exercise training is sufficient to significantly enhance vascular function. This initial rapid increase in vascular function is often normalised after 6-8 weeks of training. These observations support the idea that exercise training leads to time-dependent adaptation in conduit artery function, which is superseded by arterial remodelling.

Studies performed in patients at increased cardiovascular risk, such as those with type 2 diabetes, have demonstrated that exercise training improves vascular function (Maiorana et al. 2001; Okada et al. 2010), but the presence of time-dependent adaptations in this group has not, to our knowledge, been addressed. Given that *a priori* endothelial dysfunction is evident in subjects with type 2 diabetes, a different time-dependent adaptation in vascular function may be apparent compared to that observed in healthy young subjects. The purpose of the present study was therefore to assess brachial artery function at 2-weekly intervals across an 8-week exercise training program (Ginsberg and MacCallum 2009; Janka 1996; Kannel 2002; Marks and Raskin 2000) in patients with type 2 diabetes and controls. We hypothesize that, in keeping with findings in healthy young subjects, a rapid increase in vascular function would be present during the initial weeks of training, followed by a normalisation after 6-8 weeks.

METHODS

Subjects

We recruited 13 male patients from the community with type 2 diabetes (59±6 years) and 10 middle-aged apparently healthy men (58±7 years) as controls. Type 2 diabetes patients had been diagnosed for at least 2 years. Exclusion criteria for both type 2 diabetes and controls included overt coronary artery disease, smoking, type I diabetes mellitus, age <40 or >65, and diabetes-related manifestations of vascular disease. We also excluded subjects who performed regular physical exercise. The study procedures were approved by the medical ethical committee of the Radboud University Nijmegen Medical Centre and adhered to the Declaration of Helsinki. All subjects gave written informed consent before participation in this study.

Experimental design

First, subjects reported to the laboratory for pre-training assessment of brachial artery function and structure, subject characteristics and physical fitness. Subsequently, all subjects underwent an 8-week exercise-training program. Vascular assessments were repeated at 2-weekly intervals to examine the time-course of adaptation in these parameters across the 8-weeks of exercise training. Physical fitness and subject characteristics were examined before and after the 8-week exercise training program only.

Measurements: *Vascular function*

All subjects refrained from alcohol, caffeine, and vigorous physical exercise for at least 24 hours prior to testing. The morning of the test, subjects were instructed not to take any medication. All tests were performed in laboratory conditions with constant temperature (20°C for physical fitness testing, 22°C for vascular testing) and humidity (35%). We performed all tests between 8 AM and 4 PM. To control for diurnal variation in FMD, all measurements within subjects were performed at the same time of day (Jones et al.).

Brachial artery endothelium-dependent dilation. Measurement of brachial artery endothelium-dependent dilation (using flow-mediated dilation FMD) was performed by an experienced vascular sonographer. A 10 MHz multifrequency linear array probe attached to a high resolution ultrasound machine (T3000; Terason, Burlington, MA, USA) was used to image the brachial artery in the distal 1/3rd of the upper arm. When an optimal image was obtained, the probe was held stable and ultrasound parameters were set to optimize the longitudinal, B-mode images of lumen–arterial wall interface.

For assessment of FMD, subjects rested in the supine position for a period of at least 20

minutes to facilitate stable baseline measurement of artery diameter and blood flow. To examine brachial artery FMD, the arm was extended and positioned at an angle of ~80 degrees from the torso. A rapid inflation and deflation pneumatic cuff was positioned on the forearm of the imaged limb, immediately distal to the olecranon process, to provide a stimulus of forearm ischaemia. Continuous Doppler velocity assessment was obtained using the lowest possible insonation angle (consistently <60 degrees), which did not vary during or between measurements. The forearm cuff was inflated to 220 mmHg for 5 min. Diameter and flow recordings resumed 30 s prior to cuff deflation and continued for 3 min thereafter. Time to peak was calculated from the point of cuff deflation to the maximum post-deflation diameter. Calculation of FMD and time to peak were therefore observer-independent and based on standardized algorithms applied to data, which had undergone automated edge-detection and wall-tracking.

Brachial artery endothelium-independent dilation. Following a rest period of at least 15 minutes to allow brachial artery diameter and flow to return to baseline levels, a 1-min baseline recording of diameter and flow was taken. Subsequently, brachial artery endothelium-independent vasodilation was examined after administration of a single spray of sublingual GTN (400 µg), a nitric oxide donor. This was followed by 10 min continuous recording of brachial artery diameter and blood flow.

Brachial artery peak blood flow. After a further rest period (>15-min), a 1 min baseline recording of brachial artery diameter and blood flow was performed. Brachial artery dilation was then examined after a 5-min period of ischaemia. During this ischaemic period, consisting of 1 min ischaemia, followed by 3-min isotonic handgrip exercise and a final 1 min of ischaemia, the cuff remained inflated at 220 mmHg. Handgrip exercise involved 1

contraction every 2 s of a 3-kg load. The peak hyperemic forearm blood flow in response to this stimulus in humans provides an index of resistance artery size or remodeling whilst the brachial dilator response provides a surrogate for maximal dilator capacity (Naylor et al. 2005). We resumed diameter and flow recordings 30 s prior to cuff deflation and continued for 3 min thereafter.

Measurements: *Physical fitness and subject characteristics*

Subject characteristics. During the first visit all subjects completed a questionnaire concerning their medical history and medication use. We measured height, weight and resting blood pressure after a 5-minute seated rest using a manual sphygmomanometer. A venous blood sample was taken for assessment of fasting glucose, insulin, total cholesterol, HDL, LDL, triglycerides. From the glucose and insulin levels we calculated the HOMA-IR index as a valid measure of insulin resistance (Matthews et al. 1985).

Physical fitness. On a subsequent day, subjects performed an incremental cycle exercise test to examine maximal workload and peak oxygen consumption. Data from this test were used to determine maximal heart rate, which was used to calculate workload during exercise training. Each subject performed an incremental maximal exercise test on a cycle ergometer (Lode, Excalibur, Groningen, the Netherlands) before and after training. The test started at a power output of 10 W and power output increased by 10 W/min until voluntary exhaustion. Subjects were instructed to maintain a cadence of between 60 and 80 rpm during the test. We continuously recorded oxygen consumption (VO_2, in mlO_2/kg/min), ventilation (Ve, in l/min), respiratory quotient (RQ) (Oxycon IV, Jaeger, Germany) and heart rate (HR, in bpm). Furthermore, we measured blood lactate levels (mmol/l) using Accutrend® Lactate (Roche Diagnostics GmbH, type 3012522, Mannheim, Germany) before and 2 min after finishing the

maximal exercise test. For a test to be classified as successful, at least 3 out of the following 4 criteria had to be met: clinical signs of exhaustion of the participant, respiratory quotient ≥1.10, finishing within 10 beats of the maximum predicted heart rate (=220-age), and flattening of VO_2 uptake curve (≤110mL increase during the last minute) (Balady et al. 2010). Cardiac rhythm via ECG was assessed at rest (before the test), and continuously throughout the maximal exercise test. All subjects were screened by a physician before commencing the test, and all maximal exercise tests were supervised by a physician. An automated external defibrillator was present in the room where the test took place, and the supervisors of the tests were trained in its use.

Exercise training intervention

Exercise training was performed over an 8-week training period with subjects visiting our facility 3 times per week. Each session was supervised by one of our researchers and consisted of a warm-up, followed by a circuit of resistance exercises (leg press, calf raise, leg curl, leg extension, lower back, abdominal crunch, 3 series of 12 repetitions each, with 1 minute of rest between sets within each exercise) interspersed with aerobic activities (e.g. cycling, running) (Maiorana *et al.*, 2002; Watts *et al.*, 2004). The total protocol was as follows: 5 minutes warming-up (cycling), 5 minutes of cycling, leg curl, leg extension, 5 minutes of running, lower back, abdominal crunch, 5 minutes of cycling, leg press, calf raise, 5 minutes of running. Total duration of each session was approximately 60 minutes. A heart rate monitor (Polar Electro Oy, Kempele, Finland) was used to continuously monitor heart rate during the aerobic exercise and heart rate was maintained at 70-75% of heart rate reserve. The intensity level for each of the resistance exercises was set at a level which enables the participant to complete the three series of 12 repetitions. Intensity was increased each week under the guidance of the trainers. If a participant missed a supervised exercise session, an

extra session was planned in the same or following week, so that each participant performed a total of 24 supervised sessions (100% compliance). This type of training is demonstrated to improve vascular function and structure in healthy subjects (Black et al. 2008; Tinken et al. 2008) as well as in those with cardiovascular risk or disease (Green 2009; Maiorana et al. 2002; Maiorana et al. 2011).

Data Analysis

Post-test analysis of brachial artery diameter and velocity was performed using custom-designed edge-detection and wall-tracking software, which is independent of investigator bias (Woodman et al. 2001). The echo-Doppler signal was real-time encoded and stored as a digital file. Subsequent software analysis of these data was performed at 30 Hz using an icon-based graphic programming language and toolkit (LabView 6.02; National Instruments, Austin, TX). The program allows users to identify a region of interest on the clearest portion of the vascular wall. It then identifies, via the intensity of the brightness of the walls versus the lumen of the vessel, the walls of the artery. Regions of interest were selected for diameter and blood velocity on the duplex images. From this synchronized diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity) was calculated at 30 Hz. Baseline diameter, flow and shear rate were calculated as the mean of data acquired across the 1 minute preceding the cuff inflation period.

FMD was calculated using the baseline and peak diameter following cuff deflation. Peak diameter was automatically detected according to an algorithm, which identified the maximum bracket of data subsequent to performance of a moving window smoothing function. This smoothing routine calculates the median value from 100 consecutive samples, before the window shifts to the next bracket of data, which shares 20% overlap with the

preceding bracket. The maximum value of all the calculated median values is then automatically detected and chosen to represent the peak of the post-deflation artery diameter curve. FMD was calculated as the percentage rise of this peak diameter from the preceding baseline diameter. We have shown that reproducibility of diameter measurements using this semi-automated software is significantly better than manual methods, reduces observer error significantly, and possesses an intra-observer coefficient of variance of 6.7% (Woodman et al. 2001). We also present the FMD/GTN-ratio as this ratio corrects the FMD for potential differences between and within subjects in the endothelium-independent dilation (i.e. GTN%). This is of special importance given recent observations of differences between (clinical) groups with and without atherosclerosis (Maruhashi et al. ; Raitakari et al. 2001).

Statistical analysis

Statistical analyses were performed using SPSS 20.0 (SPSS, Chicago (Illinois), USA) software. According to Woodman *et al.* our sample size is sufficient to detect clinically relevant differences in our primary outcome measures (Woodman et al. 2001). All data are reported as mean ± SD unless stated otherwise, and statistical significance was assumed at $P<0.05$. Unpaired Student's *t*-tests were used to compare baseline values between groups, and checked our data for normality. A two-way repeated measures ANOVA was used to examine changes in our primary outcome parameter FMD across the exercise-training period ('training'; 0, 2, 4, 6, *versus* 8 weeks), and whether the magnitude of exercise training-mediated adaptations differ between groups ('group'; T2DM *versus* control). A similar statistical approach was used to examine changes in diameter, peak blood flow/diameter, physical fitness, and glucose homeostasis. When a significant main- or interaction-effect was found, post-hoc comparisons were performed to identify which time-points significantly differ from pre-training. Post-hoc analysis was performed using the least significant difference

(LSD) method for pair-wise multiple comparisons when a significant main effect was observed (Perneger 1998). According to a recent study by Atkinson *et al.*, inadequate scaling for FMD would be present if the upper confidence limit of the regression slope of the relationship between logarithmically transformed base diameter and peak diameter is less than one (Atkinson et al. 2013). In such an event, FMD% is not an appropriate measure to estimate endothelial function. We checked our data for this phenomenon, and found that in the case of our FMD data it was not appropriate to perform the allometric modelling solution proposed by Atkinson *et al* (Atkinson et al. 2013).

RESULTS

Subject characteristics

Prior to the 8-week intervention, subjects with type 2 diabetes demonstrated a significantly higher weight and BMI and lower cholesterol and LDL compared with controls (Table 1). In addition, type 2 diabetes possessed significantly higher glucose, insulin, and HOMA-IR compared with controls (Table 1). No significant differences between groups were found for age, height, systolic and diastolic blood pressure, HDL and triglycerides. The 8-week exercise training intervention did not alter these characteristics in type 2 diabetes patients or in middle-aged men (Table 1). Medication use is described in table 3.

Physical fitness

Before training, a significantly lower peak oxygen uptake and peak workload were observed in type 2 diabetes patients compared to controls (Table 1). Exercise training resulted in a significant increase in maximal oxygen uptake and maximal load, with the magnitude of increase comparable between groups (Table 1). For both groups, maximal heart rate and peak

lactate were similar before and after the exercise training program (Table 1).

Vascular function

At baseline, we observed no significant differences between groups in brachial artery diameter, FMD%, peak blood flow, peak diameter, GTN% and FMD/GTN-ratio (all comparisons P>0.05, Table 2). We found no effect of exercise training on brachial artery diameter, FMD%, shear rate area-under-the-curve or GTN% in type 2 diabetes patients or controls. However, when the FMD was corrected for the endothelium-independent dilation, the FMD/GTN-ratio, a time-dependent increase across the exercise training program was present in both groups (Table 2, Figure 1). Post-hoc analysis revealed that FMD/GTN-ratio was significantly higher after 2, 6, and 8 weeks of training compared with baseline. Brachial artery dilator capacity, peak diameter, and peak blood flow did not change by exercise training in type 2 diabetes patients or controls (Table 2, Figure 1).

DISCUSSION

The purpose of the present study was to examine whether an 8-week exercise training program induces time-dependent adaptation in conduit artery function in type 2 diabetes mellitus patients and controls. In keeping with previous studies in healthy young volunteers, exercise training lead to a rapid functional increase in both groups. Whilst continued exercise training has been associated with normalisation of endothelial function after 6-8 weeks in young healthy subjects (Tinken et al. 2008; Tinken et al. 2010; Birk et al. 2012), we observed preservation of the increase in brachial artery endothelial function after 6-8 weeks in both groups in this study. This suggests the presence of a distinct time-course in vascular adaptations to exercise training in middle-aged type 2 diabetes patients and older subjects,

compared with young healthy individuals. The preserved improvement in brachial artery endothelial function may have clinical relevance.

We found an 8% increase in peak oxygen uptake after the exercise-training program in type 2 diabetes patients, indicating that the training intervention was successful in improving physical fitness. Although we did not include a non-exercising control group, previous experiments have shown that physical fitness was not altered in subjects that did not perform exercise training (Honkola et al. 1997; Maiorana et al. 2001). The magnitude of improvement in physical fitness after 8 weeks is in agreement with studies that used an exercise training program of similar duration and intensity (Dunstan et al. 1997; Honkola et al. 1997; Maiorana et al. 2002). Healthy subjects also demonstrated a similar magnitude of benefit in physical fitness and workload. Despite these effects of exercise training on physical fitness, we found no effect on subject characteristics and traditional cardiovascular risk factors. Although somewhat counterintuitive, this finding is in agreement with others who have also demonstrated no consistent change in traditional cardiovascular risk factors after exercise training (Maiorana et al. 2002; Green et al. 2003; Lehmann et al. 1995; Zierath and Wallberg-Henriksson 1992). It is also broadly consistent with the notion that exercise benefits, in terms of vascular function and cardiovascular risk, are largely due to factors other than modification of traditional risk factors (Green et al. 2003; Joyner and Green 2009; Mora et al. 2007).

An important question in our study relates to the presence of time-dependent adaptations in vascular function across 8-weeks of exercise training in subjects with *a priori* endothelial dysfunction. Although we did not directly compare our results to a young control group, the pre-training values of brachial artery FMD in type 2 diabetes and controls (3.4-3.9%) are lower than typically reported in studies examining healthy young men (Tinken et al. 2008;

Tinken et al. 2010; Birk et al. 2012). Regarding the impact of exercise training on endothelial function in type 2 diabetes and controls, we did not find a significant change in brachial artery FMD across the 8-week exercise training. This observation contrasts with previous studies that report an increase in FMD after training in type 2 diabetes (Colberg et al. 2002; Maiorana et al. 2001). However, a recent study by Barone Gibbs *et al.* demonstrated no effect of exercise on FMD in type 2 diabetes, despite marked improvements in fitness, body composition, and glycaemic control (Barone Gibbs et al. 2012). When we corrected the brachial artery responses for underlying changes in endothelium-independent dilation, exercise training resulted in a significant increase in FMD/GTN-ratio in both groups. This measure is believed to reflect compound vascular function by correcting FMD for differences and/or changes in endothelium-independent dilation. We detected time-dependent effects of exercise training on the FMD/GTN-ratio in both type 2 diabetes and age-matched controls. In agreement with studies of healthy young volunteers (Birk et al. 2012; Tinken et al. 2008; Tinken et al. 2010), two weeks of exercise training in type 2 diabetes and age-matched controls significantly enhanced endothelial function. Whilst continuing exercise training in young healthy controls was associated with return to baseline levels after 6-8 weeks (Birk et al. 2012; Tinken et al. 2008; Tinken et al. 2010), the present study revealed sustained elevation in FMD/GTN-ratios with prolonged training. This suggests that, somewhat in contrast with younger healthy subjects, exercise training leads to improvement in endothelial function that remains elevated in subjects with *a priori* endothelial dysfunction.

The notion that exercise training can lead to rapid initial changes in function is supported by previous human and animal data. For example, Sun *et al.* found that daily exercise bouts for 2-4 weeks enhanced endothelial nitric oxide synthase (eNOS) in rat endothelial cells (Sun et al. 1994) and Sessa *et al.* found that mRNA-levels of the calcium-dependent eNOS were

rapidly upregulated in exercised dogs (Sessa et al. 1994). Upregulation of eNOS in response to episodic changes in shear stress induced by repeated exercise bouts seems a sensible hypothesis to explain the initial improvements in vascular function we observed (Niebauer and Cooke 1996). In previous human and animal experiments, the normalization of function in the longer term has been attributed structural adaptation (Laughlin 1995; Tinken et al. 2010), which is also believed to be endothelium and NO-mediated (Tronc et al. 1996; Langille and O'Donnell 1986) and acts to supersede the functional response. The current results differ from this paradigm in that function remained elevated in older healthy subjects and type 2 diabetes and there was no suggestion of arterial remodelling. Therefore, a longer, higher intensity, or a different type of training such as interval exercise training program may be necessary to induce improvements in vascular structure in these clinical groups. Furthermore, we cannot fully rule out the possibility that the structural remodelling was negatively influenced by the resistance training, as the transfers between different exercises in the circuit may have decreased the overall workload. Previous studies have also observed that FMD is elevated after prolonged (3 months) exercise training in type 2 diabetes patients (Okada et al. 2010) and sedentary middle-aged subjects (Babbitt et al. 2013). Furthermore, subjects with established coronary heart disease (Ades et al. 2011) show no larger increase in vascular function after prolonged exercise training than studies adopting a 4-8 week training protocol, providing some support for our findings that the initial improvement in vascular function remains when continuing exercise training. There are limited animal data on the interaction between arterial function and structure in response to prolonged exercise training, but our findings suggest that adaptations may differ in groups with antecedent endothelial dysfunction. This may be due to differences in the impact of oxidative stress or inflammation, but future studies examining the impact of (short-and long-term) exercise training (in those with endothelial dysfunction) on a molecular level will be required to shed further light on

this observation.

Another explanation for our consistent improvement in vascular function across the training program may relate to time-dependent changes in NO-sensitivity of the smooth muscle cells, rather than changes in the endothelium *per se*. Relatively little is known about the impact of exercise training on smooth muscle cell sensitivity to vasodilators, such as NO, in humans. In animals, studies have been inconclusive. A recent study in rats indicated that, although 8-12 weeks of exercise training increased eNOS function, it did not alter vascular sensitivity to NO (McAllister and Price 2010). However, evidence also exists that exercise training changes response of coronary smooth muscle cells to vasoactive substances and enhances myogenic reactivity in animals (Laughlin and McAllister 1992; Laughlin et al. 1998). Exercise training improves smooth muscle cell sensitivity in animals via the regulation of intracellular Ca^{2+} and an enhanced K^{+} channel regulation of tone (Bowles et al. 2000). It is unknown if these adaptations are time-dependent (Bowles et al. 2000). Future research should examine the potential of exercise training to alter NO sensitivity of smooth muscle cells in humans.

We found no differences in vascular function or structure between middle-aged type 2 diabetes patients and controls. As baseline values for vascular function in both groups were lower than typically reported in young healthy subjects in previous studies, this finding suggests that we included 2 groups with *a priori* endothelial dysfunction. Furthermore, the similarity in FMD between groups suggests that the type 2 diabetes patients included in our study have no additional impairment in vascular function relative to their age-matched peers. As a result, our findings in time-course between middle-aged and young subjects may relate to the impact of age, rather than to a pathological mechanism in type 2 diabetes.

A limitation of this study is that it has been powered to answer our primary research question, to detect changes in brachial artery FMD in the type 2 diabetes group. As we have not powered our study on the other outcome parameters, including the derivative outcome parameter FMD/GTN, we cannot make any conclusive statements regarding its power.

In conclusion, exercise training leads to rapid improvement in brachial artery vascular function in type 2 diabetes and controls. Contrary to previous findings in healthy young subjects, in whom vascular function normalises when exercise training continues, the impact of exercise training on vascular function was preserved after 8 weeks exercise training in middle-aged type 2 diabetes patients and controls. These data suggest a distinct time-course in vascular adaptations in middle-aged type 2 diabetes patients and controls compared with previous observations in young healthy subjects which warrants further investigation.

ACKNOWLEDGEMENTS

DHJT is financially supported by the Dutch Heart Foundation (E Dekker stipend, 2009T064) and Actelion BV (2009-46 Actelion Endothelin Research Award).

DJG is supported by the National Heart Foundation of Australia.

CONTRIBUTION STATEMENT

THAS, MTEH and DHJT designed the study. THAS collected and analysed the data. THAS, DJG, JN, MTEH and DHJT interpreted the data. THAS, DJG, MTEH and DHJT wrote the manuscript. DHJT had primary responsibility for final content. DHJT is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript. No conflicts of interest exist.

REFERENCES

Ades PA, Savage PD, Lischke S, Toth MJ, Harvey-Berino J, Bunn JY, Ludlow M, Schneider DJ (2011) The effect of weight loss and exercise training on flow-mediated dilatation in coronary heart disease: a randomized trial. Chest 140 (6):1420-1427. doi:10.1378/chest.10-3289

Atkinson G, Batterham AM, Thijssen DH, Green DJ (2013) A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research. Journal of hypertension 31 (2):287-291. doi:10.1097/HJH.0b013e32835b8164

Babbitt DM, Diaz KM, Feairheller DL, Sturgeon KM, Perkins AM, Veerabhadrappa P, Williamson ST, Kretzschmar J, Ling C, Lee H, Grimm H, Thakkar SR, Crabbe DL, Kashem MA, Brown MD (2013) Endothelial activation microparticles and inflammation status improve with exercise training in african americans. International journal of hypertension 2013:538017. doi:10.1155/2013/538017

Balady GJ, Arena R, Sietsema K, Myers J, Coke L, Fletcher GF, Forman D, Franklin B, Guazzi M, Gulati M, Keteyian SJ, Lavie CJ, Macko R, Mancini D, Milani RV, American Heart Association Exercise CR, Prevention Committee of the Council on Clinical C, Council on E, Prevention, Council on Peripheral Vascular D, Interdisciplinary Council on Quality of C, Outcomes R (2010) Clinician's Guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. Circulation 122 (2):191-225. doi:10.1161/CIR.0b013e3181e52e69

Barone Gibbs B, Dobrosielski DA, Bonekamp S, Stewart KJ, Clark JM (2012) A randomized trial of exercise for blood pressure reduction in type 2 diabetes: effect on flow-mediated dilation and circulating biomarkers of endothelial function. Atherosclerosis 224 (2):446-453. doi:10.1016/j.atherosclerosis.2012.07.035

Birk GK, Dawson EA, Atkinson C, Haynes A, Cable NT, Thijssen DH, Green DJ (2012) Brachial artery adaptation to lower limb exercise training: role of shear stress. J Appl Physiol 112 (10):1653-1658. doi:10.1152/japplphysiol.01489.2011

Black MA, Cable NT, Thijssen DH, Green DJ (2008) Importance of measuring the time course of flow-mediated dilatation in humans. Hypertension 51 (2):203-210. doi:10.1161/HYPERTENSIONAHA.107.101014

Blair SN, Morris JN (2009) Healthy hearts--and the universal benefits of being physically active: physical activity and health. Annals of epidemiology 19 (4):253-256. doi:10.1016/j.annepidem.2009.01.019

Bowles DK, Woodman CR, Laughlin MH (2000) Coronary smooth muscle and endothelial adaptations to exercise training. Exercise and sport sciences reviews 28 (2):57-62

Brown MD (2003) Exercise and coronary vascular remodelling in the healthy heart. Experimental physiology 88 (5):645-658

Colberg SR, Stansberry KB, McNitt PM, Vinik AI (2002) Chronic exercise is associated with enhanced cutaneous blood flow in type 2 diabetes. J Diabetes Complications 16 (2):139-145

Dunstan DW, Mori TA, Puddey IB, Beilin LJ, Burke V, Morton AR, Stanton KG (1997) The independent and combined effects of aerobic exercise and dietary fish intake on serum lipids and glycemic control in NIDDM. A randomized controlled study. Diabetes care 20 (6):913-921

Ginsberg HN, MacCallum PR (2009) The obesity, metabolic syndrome, and type 2 diabetes mellitus pandemic: Part I. Increased cardiovascular disease risk and the importance of

atherogenic dyslipidemia in persons with the metabolic syndrome and type 2 diabetes mellitus. Journal of the cardiometabolic syndrome 4 (2):113-119. doi:10.1111/j.1559-4572.2008.00044.x

Green DJ (2009) Exercise training as vascular medicine: direct impacts on the vasculature in humans. Exercise and sport sciences reviews 37 (4):196-202. doi:10.1097/JES.0b013e3181b7b6e3

Green DJ, Maiorana A, O'Driscoll G, Taylor R (2004) Effect of exercise training on endothelium-derived nitric oxide function in humans. The Journal of physiology 561 (Pt 1):1-25. doi:10.1113/jphysiol.2004.068197

Green DJ, O'Driscoll G, Joyner MJ, Cable NT (2008) Exercise and cardiovascular risk reduction: time to update the rationale for exercise? J Appl Physiol 105 (2):766-768. doi:10.1152/japplphysiol.01028.2007

Green DJ, Spence A, Halliwill JR, Cable NT, Thijssen DH (2011) Exercise and vascular adaptation in asymptomatic humans. Experimental physiology 96 (2):57-70. doi:10.1113/expphysiol.2009.048694

Green DJ, Walsh JH, Maiorana A, Best MJ, Taylor RR, O'Driscoll JG (2003) Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. Am J Physiol Heart Circ Physiol 285 (6):H2679-2687. doi:10.1152/ajpheart.00519.2003

Honkola A, Forsen T, Eriksson J (1997) Resistance training improves the metabolic profile in individuals with type 2 diabetes. Acta diabetologica 34 (4):245-248

Janka HU (1996) Increased cardiovascular morbidity and mortality in diabetes mellitus: identification of the high risk patient. Diabetes research and clinical practice 30 Suppl:85-88

Jones H, Lewis NC, Thompson A, Marrin K, Green DJ, Atkinson G (2012) Diurnal variation in vascular function: role of sleep. Chronobiology international 29 (3):271-277. doi:10.3109/07420528.2012.654554

Joyner MJ, Green DJ (2009) Exercise protects the cardiovascular system: effects beyond traditional risk factors. The Journal of physiology 587 (Pt 23):5551-5558. doi:10.1113/jphysiol.2009.179432

Kannel WB (2002) The Framingham Study: historical insight on the impact of cardiovascular risk factors in men versus women. J Gend Specif Med 5 (2):27-37

Langille BL, O'Donnell F (1986) Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. Science 231 (4736):405-407

Laughlin MH (1995) Endothelium-mediated control of coronary vascular tone after chronic exercise training. Med Sci Sports Exerc 27 (8):1135-1144

Laughlin MH, McAllister RM (1992) Exercise training-induced coronary vascular adaptation. J Appl Physiol 73 (6):2209-2225

Laughlin MH, Oltman CL, Bowles DK (1998) Exercise training-induced adaptations in the coronary circulation. Med Sci Sports Exerc 30 (3):352-360

Lehmann R, Vokac A, Niedermann K, Agosti K, Spinas GA (1995) Loss of abdominal fat and improvement of the cardiovascular risk profile by regular moderate exercise training in patients with NIDDM. Diabetologia 38 (11):1313-1319

Maiorana A, O'Driscoll G, Cheetham C, Dembo L, Stanton K, Goodman C, Taylor R, Green D (2001) The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. Journal of the American College of Cardiology 38 (3):860-866

Maiorana A, O'Driscoll G, Goodman C, Taylor R, Green D (2002) Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes. Diabetes research and clinical practice 56 (2):115-123

Maiorana AJ, Naylor LH, Exterkate A, Swart A, Thijssen DH, Lam K, O'Driscoll G, Green DJ (2011) The impact of exercise training on conduit artery wall thickness and remodeling in chronic heart failure patients. Hypertension 57 (1):56-62. doi:10.1161/HYPERTENSIONAHA.110.163022

Marks JB, Raskin P (2000) Cardiovascular risk in diabetes: a brief review. J Diabetes Complications 14 (2):108-115

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 (2013) Nitroglycerine-induced vasodilation for assessment of vascular function: a comparison with flow-mediated vasodilation. Arteriosclerosis, thrombosis, and vascular biology 33 (6):1401-1408. doi:10.1161/ATVBAHA.112.300934

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28 (7):412-419

McAllister RM, Price EM (2010) Effects of exercise training on vasodilatory protein expression and activity in rats. Eur J Appl Physiol 110 (5):1019-1027. doi:10.1007/s00421-010-1584-6

Mora S, Cook N, Buring JE, Ridker PM, Lee IM (2007) Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. Circulation 116 (19):2110-2118. doi:10.1161/CIRCULATIONAHA.107.729939

Naylor LH, Weisbrod CJ, O'Driscoll G, Green DJ (2005) Measuring peripheral resistance and conduit arterial structure in humans using Doppler ultrasound. J Appl Physiol 98 (6):2311-2315. doi:10.1152/japplphysiol.01047.2004

Niebauer J, Cooke JP (1996) Cardiovascular effects of exercise: role of endothelial shear stress. Journal of the American College of Cardiology 28 (7):1652-1660. doi:10.1016/S0735-1097(96)00393-2

Okada S, Hiuge A, Makino H, Nagumo A, Takaki H, Konishi H, Goto Y, Yoshimasa Y, Miyamoto Y (2010) Effect of exercise intervention on endothelial function and incidence of cardiovascular disease in patients with type 2 diabetes. Journal of atherosclerosis and thrombosis 17 (8):828-833

Perneger TV (1998) What's wrong with Bonferroni adjustments. Bmj 316 (7139):1236-1238

Prior BM, Lloyd PG, Yang HT, Terjung RL (2003) Exercise-induced vascular remodeling. Exercise and sport sciences reviews 31 (1):26-33

Raitakari OT, Seale JP, Celermajer DS (2001) Impaired vascular responses to nitroglycerin in subjects with coronary atherosclerosis. The American journal of cardiology 87 (2):217-219, A218

Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze TH (1994) Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. Circulation research 74 (2):349-353

Sun D, Huang A, Koller A, Kaley G (1994) Short-Term Daily Exercise Activity Enhances Endothelial No Synthesis in Skeletal-Muscle Arterioles of Rats. Journal of Applied Physiology 76 (5):2241-2247

Tinken TM, Thijssen DH, Black MA, Cable NT, Green DJ (2008) Time course of change in vasodilator function and capacity in response to exercise training in humans. The Journal of physiology 586 (Pt 20):5003-5012. doi:10.1113/jphysiol.2008.158014

Tinken TM, Thijssen DH, Hopkins N, Dawson EA, Cable NT, Green DJ (2010) Shear stress mediates endothelial adaptations to exercise training in humans. Hypertension 55 (2):312-318. doi:10.1161/HYPERTENSIONAHA.109.146282

Tronc F, Wassef M, Esposito B, Henrion D, Glagov S, Tedgui A (1996) Role of NO in flow-induced remodeling of the rabbit common carotid artery. Arteriosclerosis, thrombosis,

and vascular biology 16 (10):1256-1262

Watts K, Beye P, Siafarikas A, Davis EA, Jones TW, O'Driscoll G, Green DJ (2004) Exercise training normalizes vascular dysfunction and improves central adiposity in obese adolescents. Journal of the American College of Cardiology 43 (10):1823-1827. doi:10.1016/j.jacc.2004.01.032

Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA, Green D (2001) Improved analysis of brachial artery ultrasound using a novel edge-detection software system. J Appl Physiol 91 (2):929-937

Zierath JR, Wallberg-Henriksson H (1992) Exercise training in obese diabetic patients. Special considerations. Sports medicine (Auckland, NZ 14 (3):171-189

Table 1. Body characteristics in patients with type 2 diabetes mellitus (T2DM), and controls (Control). Data is presented as mean ± SD. P-values represent a two-way repeated measures ANOVA.

	T2DM (N=13)		Control (N=10)		2-way ANOVA		
Body characteristics	*Pre*	*Post*	*Pre*	*Post*	Training	Group	Training*Group
Age (yrs)	59±6		58±7			0.872	
Height (cm)	179±4		180±5			0.669	
Weight (kg)	103.3±15.4#	103.5±15.8	86.8±8.6	86.0±8.5	0.554	**0.008**	0.341
Body mass index (kg/m^2)	32.4±4.2#	32.5±4.4	26.9±3.5	26.6±3.4	0.533	**0.003**	0.310
Systolic blood pressure (mmHg)	142±17	139±12	132±15	125±10	0.055	**0.042**	0.521
Diastolic blood pressure (mmHg)	88±11	86±9	83±6	80±4	0.077	0.100	0.950
Cholesterol (mmol/L)	4.2±1.0#	4.0±0.9	5.5±1.1	5.4±1.4	0.147	**0.006**	0.929
High-density lipoprotein (mmol/L)	1.0±0.2	1.0±0.2	1.2±0.2	1.2±0.2	0.528	0.088	0.108
Low-density lipoprotein (mmol/L)	2.2±1.0#	2.2±1.0	3.6±1.0	3.5±1.2	0.484	**0.007**	0.449
Triglycerids (mmol/L)	2.0±1.3	2.0±2.0	1.7±1.0	1.7±0.9	0.945	0.627	0.913
Physical fitness							
Peak Oxygen Uptake (mLO_2/min/kg)	23.7±5.8#	25.8±5.4	33.0±7.5	33.8±8.8	**0.031**	**0.010**	0.276
Peak Oxygen Uptake (mLO_2/min)	2381±342	2614±298	2812±338	2876±489	**0.029**	**0.029**	0.169
Peak workload (Watt)	175.0±33.4#	206.7±31.8	232.8±47.5	265.6±46.7	**<0.001**	**0.003**	0.900
Maximal Heart Rate (bpm)	158.2±11.7	159.0±10.9	163.4±13.3	165.3±13.1	0.440	0.266	0.743
Peak lactate (mmol/L)	8.4±2.0	9.8±2.7	11.8±3.0	11.1±3.4	0.689	0.179	0.177
Glucose homeostasis							
Glucose (mmol/L)	8.2±2.8#	6.9±2.3	4.9±0.3	4.8±0.6	0.300	**<0.001**	0.394
Insulin (mmol/L)	18.4±11.8#	24.7±21.7	6.0±3.1	7.0±4.1	0.202	**0.006**	0.351
HOMA-IR (10/%S)	6.6±4.1#	8.7±9.9	1.3±0.7	1.6±1.0	0.331	**0.008**	0.427

#Significantly different between groups at baseline at P<0.05

Table 2. Vascular outcome parameters at 0, 2, 4, 6 and 8 weeks of exercise training in patients with type 2 diabetes mellitus (T2DM), and controls. Data is presented as mean ± SD. P-values represent a 2-way ANOVA for the effect of training (0, 2, 4, 6 and 8 weeks) and group (T2DM vs control).

		Weeks of exercise training					**2-way ANOVA**		
Brachial artery		*0*	*2*	*4*	*6*	*8*	Training	Group	Training*Group
Diameter (mm)	*T2DM*	4.7±0.5	4.6±0.5	4.6±0.6	4.5±0.5	4.5±0.6	0.216	0.352	0.958
	Control	4.8±0.3	4.7±0.5	4.9±0.5	4.8±0.5	4.6±0.5			
Flow mediated dilation (FMD, %)	*T2DM*	3.4±2.1	4.4±3.5	4.1±2.1	4.3±1.9	3.9±1.9	0.094	0.225	0.532
	Control	3.9±1.9	5.6±3.3	4.3±2.3	5.4±2.4	6.0±4.0			
Shear rate$_{AUC}$ (s, 10^3)	*T2DM*	15.3±6.0	14.3±7.6	16.0±7.4	19.0±6.4	16.6±6.8	0.228	0.641	0.957
	Control	15.2±10.0	14.4±7.9	13.2±8.2	18.1±9.6	15.7±7.0			
GTN (%)	*T2DM*	13.4±5.9	13.4±7.2	13.1±6.0	10.2±5.2	11.5±6.7	0.489	0.102	0.642
	Control	16.1±5.7	14.4±4.9	16.6±5.2	15.1±5.9	15.8±7.3			
CADC (%)	*T2DM*	12.0±4.6	10.3±3.8	11.8±4.9	10.7±4.1	9.9±4.5	0.322	0.100	0.524
	Control	15.0±6.3	12.7±5.1	13.9±6.0	14.5±6.4	15.0±7.9			
Peak diameter (mm)	*T2DM*	5.2±0.7	5.1±0.7	5.1±0.4	5.4±0.6	5.0±0.7	0.592	0.660	0.125
	Control	5.2±0.4	5.3±0.7	5.1±0.3	5.2±0.3	5.4±0.4			
Peak blood flow$_{AUC}$ (mL/min)	*T2DM*	920±417	868±325	855±323	971±304	844±339	0.674	0.173	0.888
	Control	800±241	682±346	759±272	804±141	813±147			
FMD/GTN	*T2DM*	0.27±0.15	0.41±0.34	0.32±0.14	0.52±0.28	0.53±0.49	**0.036**	0.640	0.903
	Control	0.28±0.22	0.43±0.24	0.29±0.16	0.43±0.32	0.46±0.34			

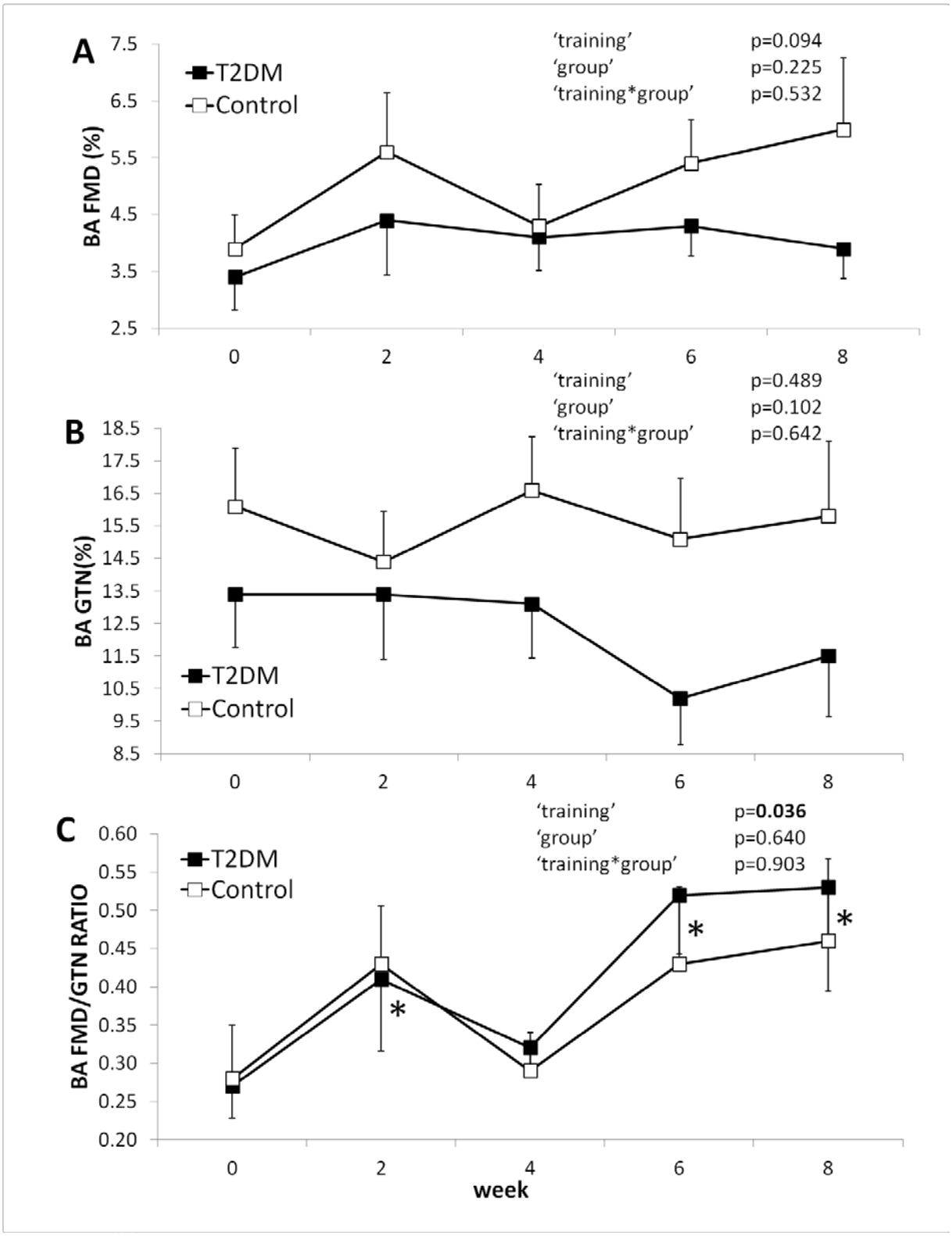
[#]Significantly different between groups at baseline at P<0.05

Table 3. Medication use in patients with type 2 diabetes mellitus (T2DM), and controls (Control). Data is presented as mean ± SD. P-values represent Pearson's χ^2.

Medication use	T2DM (N=13)	Control (N=10)	P-value
Insulin	3	0	0.103
Metformin	11	0	<0.001
Sulfonylurea	6	0	0.012
DPP4 inhibitor	0	0	-
Thiazolidinedione	0	0	-
ACE inhibitor	5	1	0.123
Angiotensin II inhibitor	2	0	0.194
Diuretic	3	2	0.859
Statin	9	2	0.019
Beta-blocker	4	0	0.054
Calcium antagonist	2	0	0.194
Acetylsalicylic acid	2	0	0.194
Coumarin derivative	1	0	0.370

Figure 1. Brachial artery flow mediated dilation (A, FMD (%)), glyceryl trinitrate response (B, GTN (%)), and FMD/GTN ratio (C) at weeks 0, 2, 4, 6, and 8 of an 8-week exercise training program in T2DM (black squares, n=13) and age-matched healthy controls (open squares, n=10) Error bars represent SE. Data for the 2-way ANOVA (main effects for 'training', 'group' and 'training*group') are provided. *Post-hoc significantly different from week 0 at $P<0.05$.

Figure 1


A
BA FMD (%)
7.5
6.5
5.5
4.5
3.5
2.5
T2DM
Control
'training' p=0.094
'group' p=0.225
'training*group' p=0.532
0
2
4
6
8
B
BA GTN(%)
18.5
17.5
16.5
15.5
14.5
13.5
12.5
11.5
10.5
9.5
8.5
'training' p=0.489
'group' p=0.102
'training*group' p=0.642
T2DM
Control
0
2
4
6
8
C
BA FMD/GTN RATIO
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
'training' p=0.036
'group' p=0.640
'training*group' p=0.903
T2DM
Control
*
*
*
0
2
4
6
8
week