ACTA PHYSIOLOGICA

Acta Physiol 2011, **201**, 427–434

The impact of exercise training on the diameter dilator response to forearm ischaemia in healthy men

D. H. J. Thijssen,[1,2] T. M. Tinken,[1] N. Hopkins,[1] E. A. Dawson,[1] N. T. Cable[1] and D. J. Green[1,3]

1 Research Institute for Sport and Exercise Science, Liverpool John Moores University, Liverpool, United Kingdom

2 Department of Physiology, Radboud University Nijmegen Medical Centre, the Netherlands

3 School of Sport Science, Exercise and Health, The University of Western Australia, Crawley, Western Australia

Received 30 June 2010,
revision requested 31 August 2010,
final revision received 19 October 2010,
accepted 24 October 2010

Correspondence:
Dr D. H. J. Thijssen,
Research Institute for Sport and Exercise Science, Liverpool John Moores University, 15-21 Webster Street, Liverpool L3 2ET, United Kingdom.
E-mail: d.thijssen@ljmu.ac.uk

Abstract

Aim: Recent studies found differences between groups in the rate of diameter increase following the flow-mediated dilation (FMD). Whilst exercise training alters the magnitude of the FMD, little is known about the impact of exercise training on the rate of diameter increase. The aim of this study is to examine post-cuff deflation changes in brachial artery diameter following 5 min forearm ischaemia every 2 weeks across 8-weeks of a handgrip exercise training regimen.

Methods: Post-deflation changes in brachial artery diameter following 5-min of ischaemia were examined before, after and every 2-weeks across an 8-week handgrip training programme in healthy young men (n = 11) using echo-Doppler.

Results: The magnitude of dilation increased at week 2–4–6, but returned towards baseline values at week 8 (ANOVA: P = 0.001). The time-to-peak diameter (42 ± 15 s) demonstrated a significant prolongation at week 4 (77 ± 32 s), but returned towards baseline values at weeks 6 and 8 (ANOVA: P < 0.001). The rate of diameter increase did not differ across the intervention.

Conclusion: Exercise training in healthy subjects is initially characterized by a larger dilation. Since the rate of dilation did not change, a longer time-to-peak dilation was necessary to achieve the increase in magnitude of dilation. As exercise training continues, the timing and magnitude of the peak diameter response returns to near baseline levels.

Keywords diameter pattern, exercise training, high resolution ultrasound, shear rate.

Exercise training is associated with beneficial effects on the vasculature in humans (Green *et al.* 2004), which may eventually contribute to the cardioprotective effects of exercise (Green *et al.* 2008, Joyner & Green 2009). These vascular changes lead to improvement in vascular function, but also can alter dynamic responses of the vasculature during periods of increased metabolic demand. This refers to the ability of the vascular system to adequately respond to physiological stimuli, such as ischaemia and exercise.

Flow-mediated dilatation (FMD) describes the dilator response of a conduit artery to elevations in shear stress (Celermajer *et al.* 1992). Exercise training positively increases the magnitude of the FMD in healthy subjects (Tinken *et al.* 2008) and in patients with cardiovascular disease (Green *et al.* 2004). However, the FMD may also be associated with changes in the dynamic diameter response. For example, a slower vasodilation following ischaemia is found in older men (Black *et al.* 2008) and patients with type II diabetes (Irace *et al.* 2008). Given

the beneficial effects of exercise training on the magnitude of the FMD response, exercise training may also alter the dynamic diameter response to ischaemia. However, little is known about the impact of exercise training on the dynamic diameter responses to ischaemia in humans. Therefore, the purpose of the present study was to examine the brachial artery dynamic dilator response to ischaemia across an 8-week period of handgrip training in healthy subjects. Specifically, we hypothesized that an exercise training induced increase in the magnitude of the post-deflation response would be accompanied by a more rapid diameter response.

Methods

Subjects

Eleven healthy, recreationally active men were recruited and allocated to an 8-week exercise training intervention (Table 1). Subjects were all young and healthy; none had been diagnosed with cardiovascular disease, diabetes, insulin resistance or cardiovascular risk factors (i.e. hypercholesterolaemia or hypertension). Subjects who smoked or were on medications of any type were excluded. The study procedures were approved by the Ethics Committee of Liverpool John Moores University and adhered to the Declaration of Helsinki. Informed consent was gained from all participants prior to the experimental procedures.

Experimental design

To examine the impact of 8-weeks of handgrip training, subjects reported to the laboratory for initial assessment of anthropometric and vascular measurements. Subsequently, we examined brachial artery blood flow and diameter responses to a 5-min distal ischaemic stimulus [commonly referred to as the flow-mediated dilation (FMD%)]. We also examined the diameter response to sublingual glyceryl nitrate [400 μg, a nitric oxide (NO) donor], an endothelium-independent vasodilator. These procedures were repeated every 2-weeks until the end of an 8-week handgrip training period. Maximal voluntary contraction (MVC) was also assessed every 2-weeks, with anthropometric measures repeated at the end of the 8-week training period. A part of this data has been published previously to examine the impact of shear rate to mediate vascular adaptations during exercise training (Tinken *et al.* 2010).

Table 1 Baseline characteristics of exercise training subjects (n = 11) before (0 weeks) and after the exercise intervention (8-weeks)

	0 weeks	8 weeks
Age, years	22 ± 2	
Weight, kg	82 ± 12	
Height, cm	181 ± 6	
SBP (mmHg)	128 ± 10	128 ± 7
DBP (mmHg)	59 ± 6	60 ± 4
MAP (mmHg)	83 ± 7	83 ± 5
HR (beats min^{-1})	55 ± 11	55 ± 6
MVC (kg)	42 ± 10	54 ± 9*
Forearm volume (mL)	1400 ± 284	1472 ± 250*
Forearm girth (cm)	27.8 ± 0.6	28.6 ± 0.6*

Values are means ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; MVC, maximal voluntary contraction.
*Significant from pre-training at $P < 0.05$ (paired t-test).

Experimental procedures

Vascular assessments were conducted in a quiet, temperature-controlled environment. Each visit for a given subject was performed at the same time of day. Subjects were asked to fast for >4 h, abstain from alcohol and caffeine for 16 h, and not to perform any exercise for 24 h (including handgrip exercise bouts).

Flow-mediated dilatation (FMD%). Subjects rested in the supine position for a period of at least 15-min to facilitate baseline assessment of heart rate and blood flow. Heart rate and mean arterial pressure were determined from an automated sphygmomanometer (GE Pro 300V2; Dinamap, Tampa, FL, USA). To examine brachial artery FMD, the arm was extended and positioned at an angle of approx. 80° from the torso. A rapid inflation and deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA, USA) was positioned on the forearm, immediately distal to the olecranon process to provide a stimulus for forearm ischaemia (Corretti *et al.* 2002). A 10-MHz multi-frequency linear array probe, attached to high resolution ultrasound machine (T3000; Terason, Burlington, MA, USA), was used to image the brachial artery in the distal 1/3rd of each upper arm. When an optimal image was obtained, the probe was held stable and the ultrasound parameters were set to optimize the longitudinal, B-mode image of lumen-arterial wall interface. Continuous Doppler velocity assessments were also obtained using the ultrasound, and were collected using an insonation angle which was kept at <60°. Following baseline assessment, the forearm cuff was inflated (>200 mmHg) for 5-min. Diameter and flow recordings resumed 30 s prior to cuff deflation and continued for 3 min thereafter. This is in accordance with recent technical specifications (Corretti *et al.* 2002, Black *et al.* 2008), as the peak diameter is typically observed within 120 s after cuff deflation.

Endothelium-independent vasodilation. Following a >15-min rest period, a 1-min baseline recording of diameter, flow and shear stress was taken from the brachial artery. Subsequently, brachial artery endothelium-independent vasodilation was examined after administration of a single spray of sublingual glyceryl trinitrate (GTN, 400 μg), a NO donor. This was followed by 10 min continuous recordings of the diameter images in both arms.

Anthropometry measurements. Maximal forearm girths were assessed using a Lufkin diameter tape (Lufkin, Mexico, USA) and forearm volume in both arms was determined by immersion of the forearm to the cubital crease. Three measurements of girth and volume were taken on each arm and the mean was derived. MVC of both forearms was assessed as the mean of three measurements using a handgrip force dynamometer (Stoelting, Wood Dale, IL, USA).

Exercise training. Exercise training was performed over an 8-week period with subjects visiting the laboratory three times a week and performing one session at home. Each laboratory session was supervised and consisted of 30-mins isometric handgrip exercise (30-contractions per min) at 30% MVC for 4-weeks, 40% for 2-weeks and the final 2-weeks at 50% MVC. Across the 8-week exercise training period, there was 90% adherence to the training sessions.

Brachial artery diameter and blood flow analysis

Post-test analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software which is independent of investigator bias (Woodman *et al.* 2001, Black *et al.* 2008). Recent papers contain detailed descriptions of our analysis approach (Black *et al.* 2008, Thijssen *et al.* 2009a). From synchronized diameter and velocity data, blood flow [the product of lumen cross-sectional area and Doppler velocity (v)] was calculated at 30 Hz. Shear rate (an estimate of shear stress without viscosity) was calculated as four times mean blood velocity/vessel diameter (Parker *et al.* 2008). Reproducibility of diameter measurements using this semi-automated software is significantly better than manual methods and reduces observer error significantly (Woodman *et al.* 2001, Green *et al.* 2002). Assessment of the peak diameter following this 5-min ischaemic stimulus using this software result in a coefficient of variation (CV) of 6.7–10.5% (Thijssen *et al.* 2009b).

Data analysis

Flow-mediated dilatation and GTN-mediated dilation are presented as the absolute (mm) and relative (%) rise from the preceding baseline diameter and are calculated based on standardized algorithms applied to data which had undergone automated edge-detection and wall-tracking, and were therefore observer-independent (Black *et al.* 2008). The rate of diameter increase was measured across various time windows (from the time of deflation up to; (i) 30 s, (ii) 60 s, and (iii) the time of peak diameter). We also measured the rate of diameter decrease from the time of peak diameter up to 150 s (end of recording). In addition, we continuously examined the post-deflation changes in brachial artery diameter and blood velocity at 30 Hz for 150 s in all subjects. During this post-deflation time-window we identified the time of peak diameter and we calculated the area-under-the-curve for the changes in mean wall shear rate (AUC_{SR}) from time of deflation up to time of peak diameter as the relevant shear rate stimulus for dilation (Pyke & Tschakovsky 2007). We also calculated the area-under-the-curve of the diameter response from cuff deflation until 150 s.

Statistics

Statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, IL, USA) software. All data are reported as mean (SD) unless stated otherwise, while statistical significance was assumed at $P < 0.05$. Repeated measures ANOVA (with time as the independent factor) and *post hoc* analysis (with Least Square Difference-correction for multiple comparisons) were used to assess the changes in brachial artery dilation during the FMD response throughout the 8-week intervention period. A group size of 11 allows us to detect a 1.3% change in FMD (Woodman *et al.* 2001) and 28 s change in time-to-peak diameter (based on 80% power and an alpha of 0.05).

Results

Subject characteristics are presented in Table 1. MVC increased significantly after 8-weeks of handgrip training ($P < 0.001$) and the forearm demonstrated a significant increase in volume and girth ($P < 0.05$). Baseline brachial artery diameter did not change across the 8-week training period (Table 2).

Flow-mediated vasodilation

As reported previously (Tinken *et al.* 2010), localized handgrip training induced a significant change in brachial artery FMD% (ANOVA, $P = 0.001$, Table 2). Brachial artery FMD% values at 2, 4 and 6 weeks were significantly higher compared with baseline, but returned to near baseline values at week 8 (Table 2).

Table 2 Brachial artery characteristics throughout an 8-weeks exercise intervention measured at 2-week intervals. The *P* value refers to a one-way ANOVA to examine changes across the 8-week handgrip training

	Week 0	Week 2	Week 4	Week 6	Week 8	*P* value
Resting diameter (mm)	3.9 ± 0.4	4.0 ± 0.4	4.1 ± 0.3	4.0 ± 0.4	4.1 ± 0.3	0.21
FMD (%)	4.4 ± 1.2	6.5 ± 1.8*	6.9 ± 1.5**	5.8 ± 1.3*	4.6 ± 0.7	0.001
Rate of increase 0–30 s (% s^{-1})	0.11 ± 0.06	0.12 ± 0.14	0.12 ± 0.07	0.16 ± 0.08	0.08 ± 0.10	0.41
Rate of increase 0–60 s (% s^{-1})	0.06 ± 0.03	0.06 ± 0.07	0.06 ± 0.02	0.09 ± 0.05	0.07 ± 0.04	0.54
Rate of increase 0-ttp (% s^{-1})	0.11 ± 0.04	0.14 ± 0.07	0.10 ± 0.04	0.12 ± 0.04	0.09 ± 0.03	0.12
Time to peak diameter (s)	42 ± 15	50 ± 20	77 ± 32*	54 ± 22	54 ± 19	0.005
Diameter AUC (0–150 s)	51 ± 265	388 ± 197*	406 ± 170*	329 ± 166	297 ± 195	<0.001
AUC_{SR} 0-ttp (10^3):	27.8 ± 2.9	24.9 ± 11.0	29.2 ± 9.4	27.9 ± 6.6	26.6 ± 14.6	0.94
GTN (%)	14.6 ± 6.0	14.9 ± 4.1	16.0 ± 3.6	16.5 ± 4.4	16.5 ± 5.5	0.42

Values are means ± SD. SD, standard deviation; FMD, flow-mediated dilatation; ttp, time-to-peak diameter; AUC_{SR}, shear rate area under the curve; GTN, glyceryl trinitrate to examine endothelium-independent dilation.
Post hoc significant from baseline after Bonferroni correction for multiple comparison at $*P < 0.05$ or $**P < 0.01$.

Diameter and shear rate response pattern

Handgrip training induced a significant change in the time-to-peak dilation of the FMD% (Fig. 1, ANOVA, $P = 0.005$). A significantly longer time-to-peak was found at 4 weeks, which returned towards baseline at week 6 and 8 (Table 2). The rate of diameter increase from deflation to pre-determined time frames (30 and 60 s) or to the individual time of peak dilation did not differ across the 8-week training (Table 2). The area-under-the-curve (AUC) of the diameter response significantly increased at 2 and 4 weeks (Table 2). Although the diameter AUC remained high at 6 and 8 weeks (Fig. 2), this did not reach statistical significance. Finally, no changes were found across the localized handgrip training in the eliciting shear stress stimulus, calculated from cuff deflation to the point of peak diameter (Fig. 2). A significant relation was found between the time-to-peak and magnitude of dilation during the FMD test (Fig. 3).

Endothelium-independent dilation

Localized handgrip training induced no change in brachial artery endothelium-independent dilation (Table 2) (Tinken *et al.* 2009).

Discussion

The purpose of the present study was to examine the brachial artery dynamic dilator response to ischaemia across an 8-week period of handgrip training in healthy subjects. As previously observed (Tinken *et al.* 2008), a larger magnitude of dilation occurred during the first 4 weeks of handgrip training. In the present study we report that no change was evident in the rate of diameter increase during the dilator response to ischaemia. A longer time-to-peak dilation was therefore necessary to achieve the larger dilation with training. As exercise training continued the magnitude of the peak diameter response returned to near baseline levels, whilst the rate of diameter increase remained unaltered and the time-to-peak dilation returned to baseline. The time-to-peak dilation therefore seems to accompany the training duration-dependent changes in the magnitude of dilation in young men across 8-week handgrip training (Tinken *et al.* 2008, 2010).

Previous studies have demonstrated the effects of localized handgrip training on the magnitude of dilation of the brachial artery (Hornig *et al.* 1996, Allen *et al.* 2003, McGowan *et al.* 2007a,b, Dobrosielski *et al.* 2009) and forearm resistance vessels after 5-min ischaemia (Sinoway *et al.* 1986, 1987, Alomari *et al.*

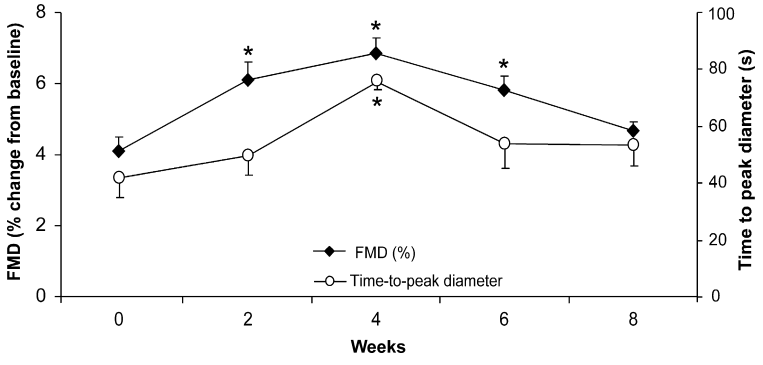

Figure 1 Relative change in brachial artery flow-mediated dilation from baseline (FMD%, ♦) and time-to-peak diameter (○) across the 8-week handgrip exercise training in healthy young men ($n = 11$). Data are presented before, after and at 2-week intervals throughout the 8-week intervention. Error bars represent SEM. *Post hoc* significantly different from 0 weeks at $*P < 0.05$.

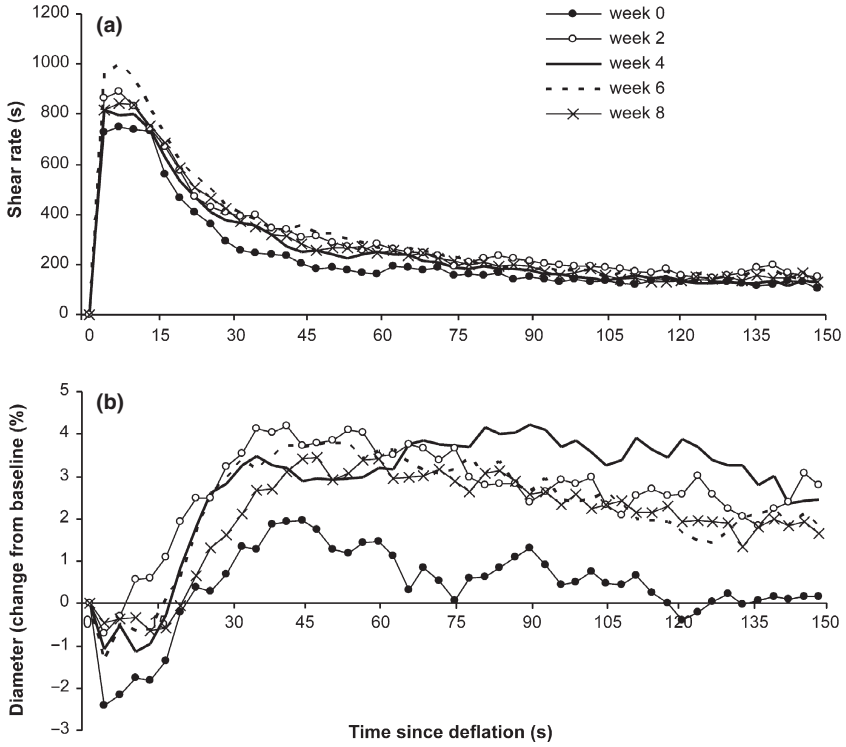

Figure 2 Relative change in brachial artery mean wall shear rate from baseline (in s, a) and diameter (in % from baseline, (b) during the flow-mediated dilation (5-min ischaemia) response from 0 to 150 s after cuff deflation. Data are presented at 3 s intervals and averaged for all 11 subjects and presented for each individual week (0, 2, 4, 6 and 8).

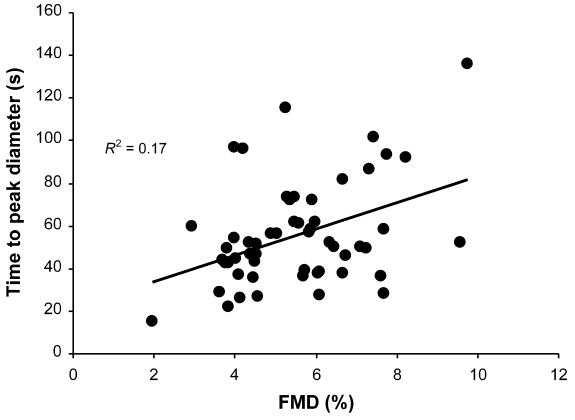

Figure 3 Correlation between time-to-peak diameter (in s) and flow-mediated dilation (FMD), presented for each individual (n = 11) across the 8-week intervention (5 time points).

2001). However, it is only recent that advances in technology, including continuous edge-detection and wall-tracking analysis, have enabled characterization of the temporal or dynamic patterns of artery dilation. Temporal aspects of artery dilation potentially convey valuable information relating to the physiological determinants of conduit artery vasodilation. It is conceivable that exercise training may alter the production and/or transport of endothelial-derived vasodilators (e.g. NO) to the smooth muscle cell, leading to changes in the dilator response. Whilst the underlying mechanisms related to intracellular biochemistry or transport processes are unknown, determining the temporal characteristics of diameter change provides a first step to examine the efficacy of vasodilation upon exposure to a physiological stimulus.

Recent studies have described differences in the timing of dilation between young and older subjects (Black *et al.* 2008) and in type 2 diabetes patients (Irace *et al.* 2008). Our study, however, is the first to demonstrate that the time-to-peak diameter can change *within* subjects across a training programme. The brachial artery time-to-peak diameter was longer after 4 weeks of handgrip training in young men, which was followed by a return towards pre-training values when exercise training continued. At first glance, this finding seems to be in contrast with a recent study that found no differences in the time-to-peak diameter between sedentary and fit older subjects (Black *et al.* 2008). However, our within training assessment of the time course of the change in time-to-peak diameter revealed reverting to baseline levels as exercise training is continued, which is consistent with the previous cross-sectional observation in chronically trained individuals.

We found a match between the longer time-to-peak and larger peak dilation across the exercise training in young men. This is supported by the significant relation between both factors (Fig. 3). The rate of diameter dilation during the FMD response provides novel information. We found no differences across the various time points during exercise training in the slope of diameter increase from cuff deflation to peak diameter during the FMD response. To achieve the larger artery dilation in young subjects, as observed in our study during the first weeks of exercise training, it simply takes longer to reach this diameter. As no change in the endothelium-independent (i.e. GTN) dilation was found, our FMD findings implicate changes in the endothelial component of the NO dilator system. It therefore appears that adaptations in kinetics of vasodilation occur in the endothelial cell in response to exercise training. However, it is important to note that our finding relate to young healthy men and it is conceivable that different findings may be apparent in subjects with pre-existing cardiovascular disease or after a different intervention.

Whilst the peak dilation is the most robust measurement of the FMD (Donald *et al.* 2008), a more detailed assessment of the post-deflation diameter response, such as the rate of diameter change and the duration of the response may have important and complimentary mechanistic information. Although peak diameter returned towards baseline levels after 8 weeks, the pattern and duration of dilation was not yet returned to baseline values (Fig. 2). In parallel, a previous study found that, despite a similar peak FMD after sildenafil, a prolonged duration of dilation was present (Halcox *et al.* 2002). Another study found that incremental levels of serum CRP in children are associated with a decreased peak FMD, but a similar pattern of dilation (Jarvisalo *et al.* 2002). Taken together, these findings suggest that the pattern and duration of dilation provide complimentary information in addition to the peak FMD. Future studies are supported to further examine this potentially relevant physiological mechanism.

A slower vasodilation in resistance arteries in response to dynamic exercise (Bearden 2007) and pharmacological stimulation (Behnke & Delp 2010) has been described in older animals, whilst prolonged timing of conduit and resistance artery dilation has also been reported in older humans in response to ischaemia (Black *et al.* 2008) or exercise (Carlson *et al.* 2008). In both cases, the age-related longer time to peak was accompanied by an *attenuated* peak dilation. This infers that, the endothelial release of vasodilators and the consequent rate of diameter dilation are impaired with ageing and, therefore, less adequate to respond to acute physiological stimuli. The results of the present study raise the novel hypothesis that, in subjects with impaired vasodilator function, exercise training may increase peak vasodilator responses without necessarily changing the rate at which dilation occurs. This will require further study.

On a technical point, as recently highlighted by Black *et al.*, changes in the time to peak dilation can have important consequences for the interpretation of FMD data if continuous edge-detection and wall-tracking are not undertaken. The use of an arbitrary time point at which to assess FMD, such as the commonly used 60 s post-deflation, would have resulted in an apparent decrease in the magnitude of FMD in the present study, and also to a different change of the FMD across the 8 week exercise training. This, in turn, may have led to a different interpretation regarding the impact of training on the vascular function. Our data therefore reinforce the importance of continuous assessment of diameter change for the determination of the true peak diameter changes after cuff deflation.

Limitations

A potential limitation is that we did not recruit and randomize subjects to a control group. However, previous studies that performed repeated measures using unilateral local handgrip training (Allen *et al.* 2003, McGowan *et al.* 2007a, Dobrosielski *et al.* 2009) demonstrated no changes in brachial artery FMD% and/or eliciting shear rate stimulus in the non-trained arm. Moreover, a recent study found no change in the time to peak diameter in the untrained arm across 4 weeks of unilateral handgrip exercise in older men (Dobrosielski *et al.* 2009). Given these sound literature precedents, we therefore think it is unlikely that our results are compromised. In addition, it seems unlikely that FMD characteristics would alter spontaneously in healthy young subjects across a relatively short time period of 8 weeks. Another limitation relates to the relatively small sample size in our study. However, we have adopted a tightly controlled design with repeated assessment of the diameter responses to 5-min ischaemia and GTN. Finally, our study had a 99% and 94% power to detect the increase in FMD% and time-to-peak diameter at week 4.

In conclusion, exercise training in healthy subjects is associated with a time-dependent change in the magnitude as well as the dynamic vasodilator responsiveness of the brachial artery FMD response. The initial change is characterized by a larger dilation, followed by a return to near baseline levels when exercise is continued. The rate of diameter increase after cuff deflation does not alter across the 8-week exercise training within young subjects. Consequently, a longer time-to-peak dilation is required to achieve the larger peak dilation during the FMD response observed during the initial

phase of exercise training. These changes are likely to be localized in the endothelial cell, since no changes in the dynamic responses to GTN were observed. Therefore, exercise training in young men impact upon the magnitude and the pattern of dilation in a duration-dependent manner.

Conflict of interest

None of the authors have conflict of interest.

We would like to thank Chris Reed for his assistance with software development. DHJT is financially supported by the Dutch Heart foundation (E. Dekker-stipend).

References

Allen, J.D., Geaghan, J.P., Greenway, F. & Welsch, M.A. 2003. Time course of improved flow-mediated dilation after short-term exercise training. *Med Sci Sports Exerc* **35**, 847–853.

Alomari, M.A., Welsch, M.A., Prisby, R.D., Lee, C.M. & Wood, R.H. 2001. Modification of forearm vascular function following short-term handgrip exercise training. *Int J Sports Med* **22**, 361–365.

Bearden, S.E. 2007. Advancing age produces sex differences in vasomotor kinetics during and after skeletal muscle contraction. *Am J Physiol Regul Integr Comp Physiol* **293**, R1274–1279.

Behnke, B.J. & Delp, M.D. 2010. Aging blunts the dynamics of vasodilation in isolated skeletal muscle resistance vessels. *J Appl Physiol* **108**, 14–20.

Black, M.A., Cable, N.T., Thijssen, D.H. & Green, D.J. 2008. Importance of measuring the time course of flow-mediated dilatation in humans. *Hypertension* **51**, 203–210.

Carlson, R.E., Kirby, B.S., Voyles, W.F. & Dinenno, F.A. 2008. Evidence for impaired skeletal muscle contraction-induced rapid vasodilation in aging humans. *Am J Physiol* **294**, H1963–1970.

Celermajer, D.S., Sorensen, K.E., Gooch, V.M., Spiegelhalter, D.J., Miller, O.I., Sullivan, I.D., Lloyd, J.K. & Deanfield, J.E. 1992. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* **340**, 1111–1115.

Corretti, M.C., Anderson, T.J., Benjamin, E.J., Celermajer, D., Charbonneau, F., Creager, M.A., Deanfield, J., Drexler, H., Gerhard-Herman, M., Herrington, D., Vallance, P., Vita, J. & Vogel, R. 2002. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* **39**, 257–265.

Dobrosielski, D.A., Greenway, F.L., Welsh, D.A., Jazwinski, S.M. & Welsch, M.A. 2009. Modification of vascular function after handgrip exercise training in 73- to 90-yr-old men. *Med Sci Sports Exerc* **41**, 1429–1435.

Donald, A.E., Halcox, J.P., Charakida, M., Storry, C., Wallace, S.M., Cole, T.J., Friberg, P. & Deanfield, J.E. 2008. Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation. *J Am Coll Cardiol* **51**, 1959–1964.

Green, D., Cheetham, C., Reed, C., Dembo, L. & O'Driscoll, G. 2002. Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry. *J Appl Physiol* **93**, 361–368.

Green, D.J., Maiorana, A., O'Driscoll, G. & Taylor, R. 2004. Effect of exercise training on endothelium-derived nitric oxide function in humans. *J Physiol* **561**, 1–25.

Green, D.J., O'Driscoll, G., Joyner, M.J. & Cable, N.T. 2008. Exercise and cardiovascular risk reduction: time to update the rationale for exercise? *J Appl Physiol* **105**, 766–768.

Halcox, J.P., Nour, K.R., Zalos, G., Mincemoyer, R.A., Waclawiw, M., Rivera, C.E., Willie, G., Ellahham, S. & Quyyumi, A.A. 2002. The effect of sildenafil on human vascular function, platelet activation, and myocardial ischemia. *J Am Coll Cardiol* **40**, 1232–1240.

Hornig, B., Maier, V. & Drexler, H. 1996. Physical training improves endothelial function in patients with chronic heart failure. *Circulation* **93**, 210–214.

Irace, C., Tschakovsky, M.E., Carallo, C., Cortese, C. & Gnasso, A. 2008. Endothelial dysfunction or dysfunctions? Identification of three different FMD responses in males with type diabetes. *Atherosclerosis* **200**, 439–445.

Jarvisalo, M.J., Harmoinen, A., Hakanen, M., Paakkunainen, U., Viikari, J., Hartiala, J., Lehtimaki, T., Simell, O. & Raitakari, O.T. 2002. Elevated serum C-reactive protein levels and early arterial changes in healthy children. *Arterioscler Thromb Vasc Biol* **22**, 1323–1328.

Joyner, M.J. & Green, D.J. 2009. Exercise protects the cardiovascular system: effects beyond traditional risk factors. *J Physiol* **587**, 5551–5558.

McGowan, C.L., Levy, A.S., McCartney, N. & MacDonald, M.J. 2007a. Isometric handgrip training does not improve flow-mediated dilation in subjects with normal blood pressure. *Clin Sci (Lond)* **112**, 403–409.

McGowan, C.L., Visocchi, A., Faulkner, M., Verduyn, R., Rakobowchuk, M., Levy, A.S., McCartney, N. & MacDonald, M.J. 2007b. Isometric handgrip training improves local flow-mediated dilation in medicated hypertensives. *Eur J Appl Physiol* **99**, 227–234.

Parker, B.A., Trehearn, T.L. & Meendering, J.R. 2009. Pick your poiseuille: normalizing the shear stimulus in studies of flow-mediated dilation. *J Appl Physiol* **107**, 1357–1359.

Pyke, K.E. & Tschakovsky, M.E. 2007. Peak vs. total reactive hyperemia: which determines the magnitude of flow-mediated dilation? *J Appl Physiol* **102**, 1510–1519.

Sinoway, L.I., Musch, T.I., Minotti, J.R. & Zelis, R. 1986. Enhanced maximal metabolic vasodilatation in the dominant forearms of tennis players. *J Appl Physiol* **61**, 673–678.

Sinoway, L.I., Shenberger, J., Wilson, J., McLaughlin, D., Musch, T. & Zelis, R. 1987. A 30-day forearm work protocol increases maximal forearm blood flow. *J Appl Physiol* **62**, 1063–1067.

Thijssen, D.H., Bullens, L.M., van Bemmel, M.M., Dawson, E.A., Hopkins, N., Tinken, T.M., Black, M.A., Hopman, M.T., Cable, N.T. & Green, D.J. 2009a. Does arterial shear explain the magnitude of flow-mediated dilation?: a comparison between young and older humans. *Am J Physiol* **296**, H57–64.

Thijssen, D.H., Dawson, E.A., Tinken, T.M., Cable, N.T. & Green, D.J. 2009b. Retrograde flow and shear rate acutely impair endothelial function in humans. *Hypertension* **53**, 986–992.

Tinken, T.M., Thijssen, D.H., Black, M.A., Cable, N.T. & Green, D.J. 2008. Time course of change in vasodilator function and capacity in response to exercise training in humans. *J Physiol* **586**, 5003–5012.

Tinken, T.M., Thijssen, D.H., Dawson, E.A., Hopkins, N., Black, M.A., Minson, C.T., Newcomer, S.C., Laughlin, M.H., Cable, N.T. & Green, D.J. 2009. Impact of shear rate modulation on vascular function in humans. *Hypertension* **54**, 278–285.

Tinken, T.M., Thijssen, D.H., Hopkins, N., Dawson, E.A., Cable, N.T. & Green, D.J. 2010. Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension* **55**, 312–318.

Woodman, R.J., Playford, D.A., Watts, G.F., Cheetham, C., Reed, C., Taylor, R.R., Puddey, I.B., Beilin, L.J., Burke, V., Mori, T.A. & Green, D. 2001. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol* **91**, 929–937.