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Impact of lifelong exercise training on endothelial ischemia-reperfusion and ischemic preconditioning in humans

Running title: Exercise and ischemia-reperfusion in older age

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ABSTRACT

Reperfusion is essential for ischemic tissue survival, but causes additional damage to the endothelium (*i.e.* ischemia-reperfusion [IR] injury). Ischemic preconditioning (IPC) refers to short repetitive episodes of ischemia that can protect against IR. However, IPC efficacy attenuates with older age. Whether physical inactivity contributes to the attenuated efficacy of IPC to protect against IR injury in older humans is unclear. We tested the hypotheses that lifelong exercise training relates to 1) attenuated endothelial IR and 2) maintained IPC efficacy that protects veteran athletes against endothelial IR. In 18 sedentary male individuals (SED, <1 exercise hour/week for >20 years, 63±7 years) and 20 veteran male athletes (ATH, >5 exercise hours/week for >20 years, 63±6 years), we measured brachial artery endothelial function with flow-mediated dilation (FMD) before and after IR. We induced IR by 20-minutes of ischemia followed by 20-minutes of reperfusion. Randomized over 2 days, participants underwent either 35-minute rest or IPC (3 cycles of 5-minutes cuff inflation to 220 mmHg with 5-minutes of rest) before IR. In SED, FMD decreased after IR (median [interquartile range]): (3.0% [2.0-4.7] to 2.1% [1.5-3.9], P=0.046) and IPC did not prevent this decline (4.1% [2.6-5.2] to 2.8% [2.2-3.6], P=0.012). In ATH, FMD was preserved after IR (3.0% [1.7-5.4] to 3.0% [1.9-4.1], P=0.82) and when IPC preceded IR (3.2% [1.9-4.2] to 2.8% [1.4-4.6], P=0.18). These findings indicate that lifelong exercise training is associated with increased tolerance against endothelial IR. These protective, preconditioning effects of lifelong exercise against endothelial ischemia-reperfusion may contribute to the cardio-protective effects of exercise training.

Key words: Endothelial cell function; flow-mediated dilation; vascular physiology; ischemic preconditioning; exercise physiology;

INTRODUCTION

Cardiovascular disease is the world's leading cause of mortality, with ischemic disease representing the most prevalent cause (47%) of cardiovascular death (35). Reperfusion therapy (*e.g.* percutaneous coronary intervention) is a common and effective strategy to restore blood flow to ischemic tissue after coronary artery occlusion (33). Paradoxically, reperfusion causes significant additional damage (*i.e.* ischemia-reperfusion [IR] injury) to the endothelium, which may limit the prognosis after myocardial infarction (50). Although reperfusion therapy induces damage, it is mandatory in order to prevent further tissue damage. Attenuating the deleterious effects of IR is therefore of utmost importance to further improve outcomes after myocardial infarction.

Ischemic preconditioning (IPC; *i.e.* short repetitive episodes of non-injurious ischemia and reperfusion) has been recognized as a potent strategy to reduce the severity of endothelium IR (22, 31, 49). The majority of research on IPC is conducted in healthy animals or young healthy humans, whereas ischemic heart diseases generally occur in older humans with different comorbidities that may interfere with the efficacy of IPC (6, 13). Various traditional cardiovascular risk factors may affect the efficacy of IPC and/or the magnitude of IR (6, 13, 21). Lifestyle changes and appropriate pharmacotherapy can modify some risk factors such as smoking, obesity, hyperlipidemia, and type 2 diabetes mellitus. However, some risk factors cannot be modified, such as ageing. A recent *in vivo* study compared the effectiveness of IPC between 15 young (20–25 years) *versus* 15 older (68–77 years) men, and demonstrated that older age was associated with an attenuated protective effect of IPC against IR (46). It is unclear whether this attenuated efficacy in older humans results from the ageing process *per se*, or whether physical inactivity contributes to these observations (1).

Regular exercise training is one of the most potent strategies to improve the cardiovascular risk profile (30), lower the risk for cardiovascular diseases (12, 26, 30, 43), and promote longevity. Whether exercise training also influences the magnitude of IR and/or maintains the efficacy of IPC

is less frequently studied. DeVan *et al.* suggested that habitual resistance exercise protects against IR in young, asymptomatic adults (9). Other studies found that exercise mimics the effects of IPC in young healthy individuals (29, 38), and attenuates the magnitude of IR on endothelial function. Animal studies demonstrated that exercise training can restore the attenuated efficacy of IPC in aged rat hearts (1, 48). To date, no previous study explored the impact of exercise training on IR and IPC in older individuals. Examining veteran athletes, *i.e.* those who exercised a large part of their lives, may provide novel insight to understand whether exercise training is associated with protection against IR and/or maintained efficacy of IPC in older humans. Therefore, by comparing veteran athletes *versus* older sedentary individuals, we tested the hypothesis that lifelong exercise training relates to an attenuated endothelial IR. Secondly, we evaluated whether lifelong exercise training relates to a maintained IPC efficacy that protects veteran athletes against endothelial IR.

METHODS

Ethical approval

This study was approved by the local Ethics committee of the region Arnhem-Nijmegen (CMO no. 2011-079) and conducted in accordance with the standards set by the Declaration of Helsinki. All participants gave their written informed consent before study participation. The study was registered at ClinicalTrials.gov (NCT01606410).

Participants

We included 20 veteran male athletes (ATH, >5 exercise hours/week for more than 20 years, 63±6 years) and 18 sedentary male individuals (SED, <1 exercise hour/week for more than 20 years, 63±7 years. We assessed exercise history of the athletes over 5 age-periods: I) 20-29 years, II) 30-39 years, III) 40-49 years, IV) 50-59 years, and V) >60 years. Each period consisted of 2 queries: 1) type of activity (*e.g.*, running, cycling, etc., or nothing) and 2) exercise time (hours) per activity per week. Veteran athletes performed mostly lower limb endurance exercise activities (*e.g.* running and

cycling). All participants were non-smokers, free of any cardiovascular disease, diabetes mellitus, and were not treated for hypertension or hypercholesterolemia.

Experimental design

Participants visited our laboratory twice on the same time of day to study the effect of forearm ischemia and reperfusion on endothelial function in the presence and absence of preceding preconditioning by short periods of forearm ischemia and reperfusion. A washout period of at least 7 days and a maximum of 30 days was set between day 1 and day 2 to eliminate any residual effects of IPC (34). During each visit, participants underwent vascular measurements. Brachial artery endothelial vasodilator function was quantified before and after IR using flow-mediated dilation (FMD), measured with ultrasound. We induced IR by 20 minutes of forearm ischemia by upper arm cuff inflation to 220 mmHg, followed by 20 minutes of reperfusion. In a randomized order, each participant underwent either 35-minute rest or IPC (3 cycles of 5-minute cuff inflation to 220 mmHg with 5 minutes of reperfusion) before the prolonged period of ischemia and reperfusion (Figure 1). The randomization procedure was programmed via a random-number generator in Microsoft Excel 2016.

Screening. Participants were medically screened for eligibility during the first visit. Height (cm), body mass (kg), and waist circumference (cm) were assessed. Blood pressure was measured twice after 15 minutes of rest in supine position followed by assessment of cholesterol and glucose levels via a capillary blood sample (35µL blood, Mission, ACON Laboratories, Inc., San Diego, USA). The Framingham Risk Score was calculated to obtain insight in the cardiovascular risk profile of the participants (7).

Vascular measurements. Participants were asked to abstain from high intensity exercise for 24 hours, any food intake for ≥6 hours, and from dietary products known to alter endothelial function

for ≥18 hours before the testing sessions (*i.e.* caffeine, vitamin C, alcohol) according to guidelines to assess peripheral vascular function (41). Measurements were performed in a temperature-controlled room (22°C) with the participants in the supine position.

Brachial artery flow mediated dilation (FMD). Assessment of the FMD as measure of endothelial function is a validated model to explore the potential protective effects of several strategies on IR in humans *in vivo* (3, 23). FMD was measured by positioning the Echo-Doppler probe on the brachial artery using a T3000 ultrasound system (Terason Teratech Corporation, Boston, United States) equipped with a 10-MHz 12L5 linear transducer. A rapid inflating/deflating pneumatic cuff (E20 rapid cuff inflator, Hokanson, Bellevue, USA) was placed on the right forearm, distally from the imaged artery. Diameter and flow velocity were recorded at the baseline during 1-minute, followed by 5 minutes of ischemia by inflating the pneumatic cuff to 220 mmHg. Diameter and flow velocity recordings resumed 30 seconds before deflating the cuff, and continued for 3 minutes during the reperfusion (41). All FMD measurements were taken by a single experienced sonographer to reduce variation (47).

Intervention: rest or IPC before IR. In randomized order, 5 minutes after baseline FMD measurement, participants received either a) 35-minute rest or b) IPC, which were both followed by 20 minutes of ischemia and 20 minutes of reperfusion. When the participant received IPC, the pneumatic cuff was positioned proximally around the upper arm. Thus, the brachial artery was within the ischemic zone and was exposed to IR. IPC was performed by applying of 3 cycles of 5 minutes of upper arm ischemia by inflating the pneumatic cuff to 220 mmHg, followed by 5 minutes of rest (in total covering 35-min).

Ischemia reperfusion (IR). Five minutes after either a) 35-minute rest or b) IPC stimulus, participants received upper arm ischemia, which was induced by inflating the pneumatic cuff on the

upper arm to 220 mmHg for 20 minutes, followed by 20 minutes of reperfusion. Finally, brachial artery endothelial function was re-examined using the FMD.

Experimental analyses

Brachial artery diameter, flow velocity, and shear analyses. Analysis of the FMD was performed with a custom-designed edge-detection and wall tracking software written in LabVIEW (LabVIEW 6.02, National Instruments, Austin, United States) as described elsewhere (5). Briefly, from B-mode a region of interest (ROI) was drawn to calibrate the artery diameter. Within this ROI a pixel-density algorithm automatically identified the vessel wall. For the calibration of the flow velocity another ROI was drawn around the Doppler waveform. Baseline diameter was calculated as the mean of data acquired during 1-minute baseline recording, preceding cuff inflation. Peak diameter and peak flow velocity were detected during 3 minutes of reperfusion. FMD was calculated as the relative difference in peak diameter and baseline diameter. Post deflation shear rate data, derived from velocity and diameter measures, were used to calculate the area under the shear rate curve (SR_{AUC}) (5). Analyses were performed by a single, blinded researcher and analyses were subsequently checked by a second blinded researcher. A recent analysis of 672 repeated measures (between multiple laboratories) showed an average reproducibility of the FMD% of 9.3% (coefficient of variation) when measuring asymptomatic volunteers (47).

Power analysis & statistics

In order to assess whether IPC protects against IR, we assumed that a change <1% in FMD after IR (when preceded by IPC) can be regarded as a negligible or absent change and suggests that IPC can effectively prevent a change in FMD. A previous study of our laboratory (46) revealed a significant decrease in %FMD after reperfusion from 3.7%±2.1 to 2.2%±1.1 (Δ1.5% FMD) in older participants. Based on a power of 80% and alpha 5% significance level, we calculated that 20 subjects per group should be included to detect a relevant effect.

Data are presented as mean with standard deviation (SD) unless stated otherwise. Parameters were checked for normality using a Shapiro-Wilk test and Q-Q plots. Group characteristics were analyzed using independent Student's t or Mann-Whitney U test, when appropriate. Within the groups, differences between baseline measurements and after IR were analyzed using linear mixed models with factors time (before *versus* after IR) and group (athletes *versus* sedentary) or the nonparametric Friedman test, when appropriate. In the present study, FMD data were non-normal distributed and we used the non-parametric Friedman test. This statistical approach limited the possibility to co-vary for potentially confounding factors. Data were analyzed using SPSS 22.0 software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Statistical significance was assumed at $p<0.05$ (two-sided).

RESULTS

Characteristics

ATH demonstrated a lower body mass and waist circumference compared to SED, whilst age, height, body mass index, and mean arterial pressure did not differ between groups (Table 1). ATH showed lower levels of total cholesterol and LDL cholesterol compared to SED, whilst no differences were found for HDL cholesterol, triglyceride, and glucose levels between groups (Table 1). Framingham Risk Score was lower in ATH compared to SED (Table 1).

IR and IPC: sedentary individuals

Baseline diameter of the brachial artery did not differ before and after prolonged IR, whilst baseline diameter of the brachial artery significantly increased after prolonged IR when preceded by IPC (Table 2). FMD% significantly decreased after prolonged IR in SED (3.0% [2.0-4.7] to 2.1% [1.5-3.9], P=0.046) (Table 2, Figure 2). A similar decrease in FMD% was found after prolonged IR when preceded by IPC (4.1% [2.6-5.2] to 2.8% [2.2-3.6], P=0.012) (Table 2, Figure 2). $SR_{AUC,}$ and

time-to-peak did not differ before and after IR under both conditions (*i.e.* a. 35-minute rest or b. IPC) (Table 2).

IR and IPC: veteran athletes

A significant increase in baseline brachial artery diameter was observed after prolonged IR when preceded by 35-minute rest or the IPC intervention (Table 2). The FMD% did not significantly differ after IR in ATH (3.0% [1.7-5.4] *versus* 3.0% [1.9-4.1], P=0.80) (Table 2, Figure 2). Likewise, the FMD% did not differ after prolonged IR when preceded by IPC (3.2% [1.9-4.2] *versus* 2.8% [1.4-4.6], P=0.18) (Table 2, Figure 2). $SR_{AUC,}$ and time-to-peak did not differ after IR under both conditions (*i.e.* rest or IPC) (Table 2).

DISCUSSION

The purpose of this study was to test the hypotheses that lifelong exercise training is associated with attenuated endothelial IR in humans *in vivo*, and that lifelong exercise training is associated with maintained efficacy of IPC in veteran athletes. Our study presents the following findings. First, we found that brachial artery endothelial function decreases after forearm IR in older sedentary individuals and IPC was unable to prevent this decline in endothelial function. Second, in marked contrast to the sedentary individuals, we found that endothelial function did not decrease after IR in veteran athletes. Furthermore, when preceded by IPC, we found no further attenuation of endothelial function after IR in veteran athletes. These data suggest that lifelong exercise training protects against the detrimental effects of ischemia reperfusion on endothelial function.

We did not observe a significant difference in baseline %FMD between veteran athletes and sedentary controls. This finding is in line with previous work (15, 25), which demonstrated that trained individuals do not necessarily have a superior %FMD compared to their non-athletic peers. Exact mechanisms are not completely understood. Nitric oxide is an important regulator of basal

vasodilator tone of the blood vessels (45). Studies found that exercise promotes the bioavailability of nitric oxide (24, 43). However, athletes do not necessarily demonstrate an elevated basal limb blood flow (40) or basal coronary flow (18). Some evidence suggests that exercise training also increases sympathetic vasoconstrictor tone (2, 32). It is hypothesized that this increased sympathetic vasoconstrictor tone counterbalances the training-induced increase in nitric oxide bioavailability, leading to a preserved basal limb blood flow (16, 18, 40). Similarly, this interaction may also contribute to preservation of the conduit artery endothelium-dependent responses to increased shear during the flow-mediated dilation. Future studies are needed to further explore this hypothesis.

Endothelial Ischemia Reperfusion: impact of a sedentary lifestyle

Endothelial function was significantly lower after IR in sedentary older individuals, which is in line with previous research (46). Despite the fact that previous studies in animals (49) and preclinical work in humans (22, 23) found that IPC attenuates the magnitude of IR, translation of IPC to clinical practice is often challenging (19, 28, 37). This might relate to the inclusion of young, healthy animals/humans, whereas clinical studies mostly include aged individuals with (co)morbidities (28), which may interfere with the efficacy of IPC. Indeed, a recent study found an impaired ability of IPC to prevent the decline in endothelial function after IR in heart failure patients (n=15, 67±10 years) (37). In the current study, we found that IPC could not prevent the decline in endothelial function after IR in asymptomatic aged sedentary individuals. These data reinforce findings of previous work from our laboratory, in which we found that IPC could not prevent the decrease in endothelial function after IR in older individuals (72±4 years) (46). Taken together, our results are in line with previous work, indicating that IR impairs endothelial function and cannot be prevented by IPC in sedentary older individuals.

Endothelial Ischemia Reperfusion: impact of lifelong exercise training

In contrast to the decline in FMD in sedentary older humans, we found no significant change in FMD following IR among veteran athletes. This novel finding suggests that lifelong exercise training is associated with preconditioning-like effects. DeVan *et al*. explored whether endothelial IR is present in middle-aged endurance trained athletes, but found a significant decline in endothelial function after IR (8). Differences in methodological design (*i.e.* longer reperfusion in the present study), group characteristics (*i.e.* age) and exercise history may contribute to the different observations between studies. Furthermore, the absence of a decrease in endothelial function after prolonged IR also implies that we could not determine whether lifelong exercise training affected the efficacy of IPC. At least, our results indicated that lifelong exercise training is associated with maintained endothelial function after IR in older humans.

Several previous studies support the hypothesis that exercise possesses preconditioning effects against endothelial IR (38, 39). The similarity of IPC and exercise could relate to the repeated short periods of ischemia/hypoxia (27). These short episodes of ischemia activate signaling pathways involved in the increased tolerance against IR, such as adenosine, bradykinin and opioids (29, 34). Michelsen *et al.* found that this exercise-preconditioning may be driven by a systemic, blood-borne factor that works through opioid receptors (29). The existence of a systemic preconditioning effect of exercise training is supported by our results, as our veteran athletes performed most of the time lower limb exercise training (*e.g.* running or cycling), whilst we observed no significant decrease in brachial artery endothelial function after IR.

Another explanation for vascular protection against IR due to lifelong exercise training in the older participants included in our study may relate to upregulation of nitric oxide (27, 34). In healthy endothelium, an increased blood flow causes release of nitric oxide, leading to dilation of the vessel (14). However, older age is associated with endothelial dysfunction and reduced nitric oxide

bioavailability (10). This latter observation may relate to lower production of nitric oxide, but also to the presence of increased oxidative stress (10) (possibly caused by relatively higher levels of reactive oxygen species (11)). Potentially**,** regular endurance exercise training lowers oxidative stress (36) and prevents the age-related decline in nitric oxide bioavailability (36, 42). Collectively, our study indicates that lifelong endurance exercise training increases IR tolerance.

Benefits of exercise training: beyond traditional cardiovascular risk factors?

The positive effects of regular endurance training on cardiovascular risk factor profile are widely accepted (30). Additional benefits of exercise training may be mediated via improved vasculature (20). Interestingly, endothelial function did not differ at baseline between veteran athletes and their sedentary peers, which seem contradictory as previous studies provided solid evidence that exercise training may enhance endothelial function (42). However, potential benefits of exercise in the lifelong athletes become apparent after the endothelium is exposed to challenging conditions that immediately affect the endothelium (*i.e.* after 20 minutes of ischemia and 20 minutes of reperfusion). Our findings suggest that exercise training may offer cardioprotective effects, in addition to favorable changes in cardiovascular risk profile, through increased tolerance against IR.

Methodological considerations

Strengths of our study include the homogenous asymptomatic sedentary and physically active individuals, blinded observer independent analyses, and we adhered to expert-consensus FMD guidelines (41) to obtain a high reproducibility of the vascular measurements (17, 47). However, our study was inherent to some limitations. There was a high variation in baseline FMD within the groups, which may relate to inter-individual variation between the participants (47). However, primary comparisons were performed within participants, *i.e.* a strong methodological approach and linked to good day-to-day reproducibility (47). Furthermore, FMD data were non-normally distributed. Therefore, we used nonparametric statistical tests to analyze the data. As a consequence

of using non-parametric tests we were unable to co-vary for factors, such as SRAUC and baseline diameter, an approach that is recommended to control for potential within- or between-subject differences in these variables. (4) However, SRAUC did not differ between the pre- and post-measurement of both conditions in both groups, which makes it unlikely that individual differences in SRAUC explain or may alter our main observations. Regarding baseline diameter, we found diameter to significantly increase after IR in both conditions in the ATH group. Although a larger baseline diameter is typically associated with a smaller FMD response, (44) FMD% after IR was preserved in both conditions in ATH. On the contrary, we observed that baseline diameter of the sedentary controls decreased after IR during the IPC-condition. Although a smaller diameter is typically associated with a higher FMD, (44) the FMD significantly decreased in sedentary controls. Despite these observations, our inability to statistically correct for potential variations in baseline diameter is a limitation of our study and should be taken into consideration.

Another potential limitation is that we included a healthy (asymptomatic) study population, whilst ischemic events mostly occur in individuals with cardiovascular risk factors. However, we focused on asymptomatic individuals to study the potential effects of exercise and IPC against IR. Whether comparable effects can be found in participants that undergo exercise training *after* an ischemic event or in those with risk factors is a logical next step. Furthermore, due to the absence of a decline in endothelial function after IR in veteran athletes, we were unable to determine whether IPC could prevent endothelial IR in veteran athletes. However, care should be taken when generalizing our findings to other stimuli or arteries, since a stronger ischemic stimulus or different vascular bed may demonstrate different results. Finally, since we did not test endothelium-independent vasodilation, we cannot exclude that our findings are possibly mediated by changes in vascular smooth muscle cell function.

Conclusion

The results of our study indicated that, in marked contrast to the characteristic reduction in endothelial function after IR in older sedentary humans, lifelong exercise training was associated with maintained endothelial function after IR. These findings suggest that veteran athletes have an increased tolerance against IR compared to sedentary individuals. Therefore, lifelong exercise training has protective preconditioning effects against endothelial ischemia-reperfusion that may partially contribute to the cardio-protective effects of exercise training during physiological ageing.

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Disclosures

The authors report no conflicts of interest.

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Figure 1. In randomized order, each participant underwent either A) 35-minute rest or B) IPC (3 cycles of 5-minute cuff inflation to 220 mmHg with 5 minutes of reperfusion) before the prolonged period of ischemia and reperfusion. A washout period of at least 7 days and with a maximum of 30 days was set between day 1 and day 2 to eliminate any residual effects of IPC.

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Figure 2. Individual (circles) and group values (median [IRQ]) of brachial artery FMD before and after ischemia reperfusion (IR) when preceded, in randomized order, by 35-minutes of rest or ischemic preconditioning (IPC) in A. sedentary individuals (n=18) and B. asymptomatic veteran athletes (n=20). FMD of sedentary individuals decreased after 35-min rest as well as IPC, whilst veteran athletes had no change in FMD after either 35-min rest or IPC. P-value refers to *Friedman* test.

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Table 1. Characteristics and cardiovascular risk profile of veteran athletes (n=20) and sedentary individuals (n=18). P-value refers to *Mann-Whitney U* test. Data is presented as median [interquartile range].

	Veteran athletes *n* = 20	Sedentary individuals *n* = 18	
CHARACTERISTICS			
Age (years)	64 [58-67]	63 [57-67]	0.78
Height (cm)	179 [178-183]	182 [176-184]	0.39
Body mass (kg)	77 [72-82]	85 [79-87]	0.009
Body mass index (kg/m^2)	24.5 [22.1-25.7]	25.4 [24.2-25.7]	0.16
Waist circumference (cm)	86 [83-91]	98 [93-103]	<0.01
Mean Arterial Pressure (mmHg)	92 [88-96]	92 [87-98]	0.90
Systolic Blood Pressure (mmHg)	121 [117-131]	123 [117-136]	0.70
Diastolic Blood Pressure (mmHg)	77 [72-80]	76 [71-81]	0.92
CARDIOVASCULAR RISK PROFILE			
Framingham Risk Score (%)	14.1 [9.7-15.5]	18.3 [13.0-23.2]	0.017
Total Cholesterol (mmol/L)	5.4 [4.8-5.8]	6.1 [5.6-6.5]	0.030
HDL (mmol/L)	1.5 [1.4-1.9]	1.3 [1.2-1.5]	0.06
LDL (mmol/L)	3.1 [2.5-3.8]	4.0 [3.3-4.5]	0.033
Triglycerides (mmol/L)	1.1 [0.9-1.5]	1.3 [1.1-1.9]	0.14
Glucose (mmol/L)	4.5 [3.8-4.8]	4.7 [3.8-5.2]	0.30

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Table 2. Brachial artery characteristics before and after ischemia reperfusion (IR) when preceded, in randomized order, by either a) 35-minutes of rest or b) ischemic preconditioning (IPC) in asymptomatic veteran athletes (n=20) and sedentary individuals (n=18). P-value refers to *Friedman* test for difference in change from baseline (rest *versus* IPC). Data is presented as median [IQR].

	35-minutes of rest			IPC			rest *versus* IPC
	Baseline	Post IR	p-value	Baseline	Post IR	p-value	p-value
ATHLETES							
Baseline diameter (mm)	4.5 [4.2-5]	4.8 [4.3-5.2]	<0.01	4.5 [4.3-4.8]	4.9 [4.7-5.1]	<0.01	0.25
FMD (%)	3.0 [1.7-5.4]	3.0 [1.9-4.1]	0.82	3.2 [1.9-4.2]	2.8 [1.4-4.6]	0.18	0.49
Time-to-Peak (sec)	68 [42-116]	56 [29-89]	0.11	51 [25-71]	52 [34-95]	0.66	0.11
SR_{AUC} ($x10^3$)	12.6 [11.3-18.8]	12.8 [7.3-16.2]	0.49	14.9 [10.7-19.8]	11.6 [7.9-15.3]	0.18	0.49
SEDENTARY AGED INDIVIDUALS							
Baseline diameter (mm)	4.9 [4.4-5.2]	4.7 [4.4-5.8]	0.44	4.6 [4.2-5.1]	5.2 [4.5-5.7]	0.012	0.020
FMD (%)	3.0 [2.0-4.7]	2.1 [1.5-3.9]	0.046	4.1 [2.6-5.2]	2.8 [2.2-3.6]	0.012	0.80
Time-to-Peak (sec)	68 [45-81]	52 [44-62]	0.62	60 [39-86]	66 [42-76]	0.62	0.43
SR_{AUC} ($x10^3$)	15.8 [12.7-18.4]	11.5 [8.9-18.8]	0.62	15.6 [11.1-19.2]	13.7 [8.3-18.9]	0.62	0.62

FMD: flow-mediated dilation; SR_{AUC}: Shear Rate Area Under the Curve

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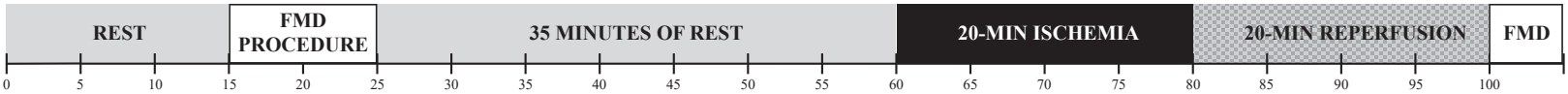
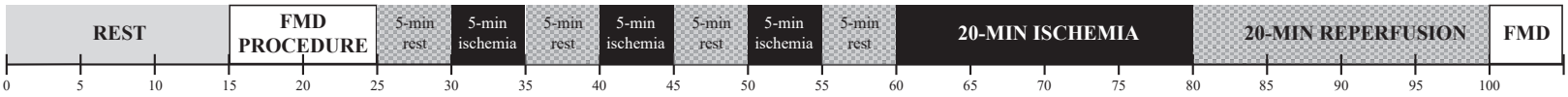
A. Rest

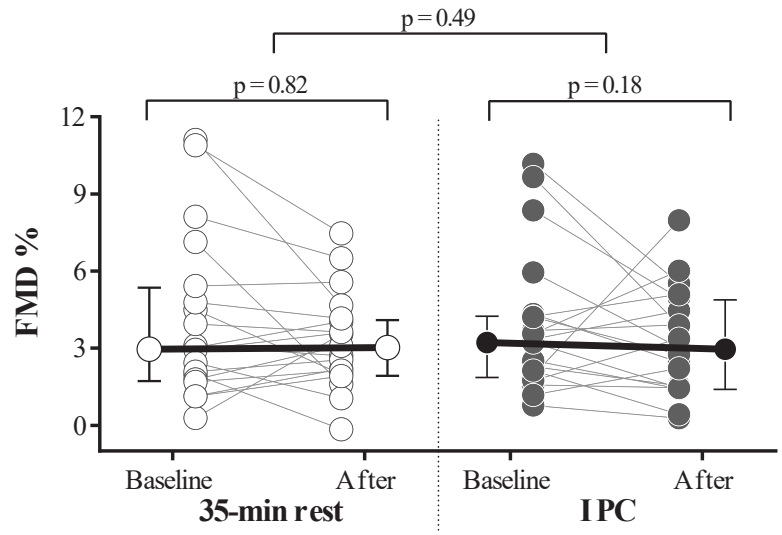


B. Ischemic preconditioning



35 minutes ischemic preconditioning

A. Veteran athletes



B. Sedentary individuals