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Impact of handgrip exercise and ischemic preconditioning on local and remote protection against endothelial reperfusion injury in young men.

Impact of handgrip exercise and ischemic preconditioning on local and remote protection against endothelial reperfusion injury in young men

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Abstract

Aims/Hypothesis: Ischemic preconditioning (IPC), cyclical bouts of non-lethal ischemia, provides immediate protection against ischemic injury, which is evident both locally and remotely. Given the similarities in protective effects of exercise with ischemic preconditioning, we examined whether handgrip exercise also offers protection against endothelial ischemia-reperfusion (IR)-injury, and whether this protection is equally present in the local (exercised) and remote (contralateral, non-exercised) arm.

Methods: Fifteen healthy males (age 24±3 years; BMI 25±2 kg/m^2) attended the laboratory on 3 occasions. Bilateral brachial artery flow-mediated dilation (FMD) was examined at rest and following a temporary IR-injury in the upper arm. Prior to the IR-injury, in the dominant (local) arm participants performed (randomised, counterbalanced); *i.* 4x5 minutes unilateral handgrip exercise (50% maximal voluntary contraction), *ii.* 4x5 minutes unilateral IPC (220 mmHg), or *iii.* 4x5 minutes rest (control). Data were analysed using repeated measures general linear models.

Results:

Allometrically scaled FMD declined after IR in the control condition (4.6 ± 1.3% to 2.2 ± 1.7%, P<0.001), as well as following handgrip exercise (4.6 ± 1.6% to 3.4 ± 1.9%, P=0.01), however was significantly attenuated with IPC (4.5 ± 1.4% to 3.8 ± 3.5%, P=0.14). There were no differences between the local and remote arm.

Conclusion: Our findings reinforce the established protective effects of IPC in young, healthy males, and also highlight a novel strategy to protect against IR injury with handgrip exercise, which warrants further study (**Word count:** 226)

Keywords: *Ischemic preconditioning, exercise preconditioning, vascular function, ischemia-reperfusion injury.*

Declarations: N/A

Abbreviations:

BMI *Body mass index*

CVD *Cardiovascular disease*

DBP *Diastolic blood pressure*

ExPC Exercise preconditioning

FMD *Flow-mediated dilation*

IPC *Ischemic preconditioning*

IR *Ischemia reperfusion*

MAP *Mean arterial pressure*

NIRS *Near-infrared spectroscopy*

SBP *Systolic blood pressure*

Introduction

Cardiovascular disease remains the world's leading cause of mortality and morbidity [1]. Within the development of cardiovascular events, the presence of ischemia-reperfusion (IR) injuries play a central role. IR injuries present a pathophysiological paradox whereby re-establishing blood flow is essential to limit ischemic related injury yet the reperfusion of blood flow itself causes further cellular damage[2]. IR injuries can occur in unplanned prominent clinical scenarios (e.g. myocardial infarction) and during planned cardiac ischemia (e.g., cardiac angioplasty, coronary artery bypass surgery). Since IR-injury plays a central role in mediating tissue injury and damage, strategies to mitigate these effects have significant clinical potential. Brief, cyclical periods of ischemia separated by periods of reperfusion, termed ischemic preconditioning (IPC), confers cardioprotection in local tissue [3] as well as in remote regions [4]. These cardioprotective effects of IPC are evident from 1-72 hrs, in a biphasic pattern [4, 5], and have demonstrated potential clinical relevance [6].

Similar to IPC, a single bout of acute exercise may also offer cardioprotection against IR injuries, which is present in local and remote tissue, immediately and up to 72 hours following exercise [7]. For example, 30 minutes of running in mice reduced infarct size from a subsequent IR injury immediately following the exercise with the protective effects lasting up to 60 hours post exercise [8]. The protective effects were more persistent when multiple exercise bouts were performed [9]. In a human study which employed intermittent running exercise, preconditioned blood was used as a dialysate to perfuse an isolated rabbit heart, which resulted in a ~50% reduction in infarction size[10]. Similarly, Seeger et al reported that a single bout of lower limb intermittent exercise offered protection against endothelial IR-injury of the upper limb[11], highlighting that the cardioprotective effects of a single bout of exercise are systemic.

Previous work has shown that both strenuous exercise or IPC, likely through humoral factors released from the ischaemic arm in IPC and active muscles with exercise, trigger physiological processes that mediate the protective effects of a preconditioning stimulus[10]. Whilst some previous work related to the protective effects of acute exercise has focused on whole-body exercise (e.g., running, cycling)[11], other forms of exercise may lead to protection. A local 'mismatch' between oxygen supply and demand may seem relevant in mediating such protective effects. Exercise performed in a cyclical manner (repeated) can induce this 'mismatch' (e.g. handgrip exercise or squats) and this may also have local and remote protective effects. From a practical perspective, local exercise might be easier to perform, be more acceptable to individuals, and in some instances might not require any equipment (e.g. squats). To explore this notion, the primary aim of this study was to examine whether handgrip exercise can offer comparable protection in the both the exercised (local) *and* contralateral (remote) arm, as demonstrated previously with ischemic preconditioning (i.e., IPC), against brachial artery endothelial IR-injury in healthy individuals. We expect that handgrip exercise, like IPC, demonstrates local and remote protection against endothelial IR-injury.

Methods

Participants

Fifteen healthy male participants (Table 1) who were free from cardiovascular and metabolic diseases, did not have any arm injury, non-smokers and were not taking any regular medication were recruited. Participants were informed of the study protocol verbally and in writing before providing fully informed verbal and written institutionally approved informed consent. Due to the exploratory nature of this proof-of-concept study we did not include an *a priori* power calculation. Sample size was based on previous research studies [5,11] that adopted similar or comparable approaches to assessing preconditioning stimuli, including IPC and exercise, in the

prevention on endothelial IR-injury. As such, we targeted to include fifteen participants. The study was approved by the University ethics committee (18/SPS/063). and adhered to the standards set out in the *Declaration of Helsinki*[12] .

Research Design

Participants attended the laboratory on three separate occasions. The three experimental visits, performed at the same time of day, separated by at least 3 days having fasted overnight (12 hrs), refraining from alcohol and exercise for 24 hrs and caffeine for 12 hrs before each visit. At the start of the exercise preconditioning (ExPC) visit forearm maximal voluntary contraction (MVC) was assessed. During each visit, bilateral brachial artery endothelial function [using flow-mediated dilation (FMD)] was examined at rest prior to any intervention and following an endothelial ischemia reperfusion (IR)-injury (15 min of arm ischemia and 15 min of reperfusion). Following the resting FMD, participants then either rested in the supine position for 40 min (Control), performed handgrip exercise (ExPC: 4 x 5 min of unilateral handgrip exercise, separated by 5 min rest) or were administered IPC (4 x 5 min forearm cuff inflation separated by 5 min reperfusion) [Figure 1]. The order of the intervention (control, ExPC and IPC) was administered in a randomised counterbalanced order on the dominant arm.

Measurements

Brachial artery endothelial function.

Bilateral brachial artery endothelial function was assessed using the FMD technique adhering to the latest published guidelines [13]. Following 20 minutes of supine rest, both arms were extended and positioned 80° from the torso. A rapid inflation/deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was placed around the forearm (immediately distal to the olecranon) to produce the stimulus of forearm ischemia. A 15-MHz multifrequency linear array probe,

attached to a high-resolution ultrasound machine (T3300; Terason, Burlington, MA), was then used to image the brachial artery in the distal third of the 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 the lumen–arterial wall interface. The ultrasound was also used to attain simultaneous continuous Doppler velocity using the lowest possible insonation angle (60°). A recording of resting diameter and velocity was taken for 1 minute, then the forearm cuff was inflated (>200 mm Hg) for 5 minutes. Both diameter and velocity recordings resumed 30 seconds before cuff deflation and continued for 3 minutes post deflation. Analysis of brachial artery diameter was performed using custom designed edge-detection and wall-tracking software, which is largely independent of investigator bias. Previous articles contain detailed descriptions of our analytical approach [14, 15]. From synchronized diameter and velocity data, blood flow (the product of lumen cross sectional area and Doppler velocity) were calculated at 30 Hz. Shear rate (an estimate of shear stress without viscosity) was calculated as four times mean blood velocity/vessel diameter. Reproducibility of diameter measurements using this semi-automated software is significantly better than manual methods, significantly reduces observer error, and possesses within-day coefficient of variation of 6.7%[15]. All FMD measurements were performed by two sonographers who performed measurements on right and left arms, respectively. Sonographer 1 had a coefficient of variation in FMD% of 19% and a coefficient of variation of 2% for baseline artery diameter. Sonographer 2 had a coefficient of variation in FMD% of 18% and a coefficient of variation of 3% for baseline artery diameter. These values for coefficient of variation were close to recommended guidelines for FMD in consecutive scans[13]. FMD was performed bilaterally at rest and following a temporary endothelial IR-injury.

Interventions

Exercise Preconditioning. On the initial visit, participants performed short (3 second) MVC's; with each effort separated by 90 seconds rest. Each participant produced three efforts in total. A dynamometric handheld force transducer (Stoelting, USA) was used to determine force generation. The maximum-recorded value (kg) from these three efforts was used to determine MVC. For the exercise intervention, handgrip exercise intensity was performed at 50% of MVC unilaterally on the dominant arm. Participants were in a seated position, with the dominant arm placed at a 90° angle on a table. Participants performed 5-minutes of rhythmic (using a metronome) handgrip contractions on a dynamometric handheld force transducer (Stoelting, USA), completing 60 reps/min, followed by 5 min rest. Participants were provided with visual feedback, using a marker on the analogue dynamometer gauge to reach with each contraction.

Ischemic Preconditioning. Unilateral IPC was performed in the supine position with a pressure cuff inflated around the upper dominant arm to a pressure set at 220 mm Hg with the use of a rapid inflator (E20) and air source (AG101) (Hokanson, Washington, USA). IPC protocol consisted of 5 minutes inflation, 5 minutes deflation, repeated 4 times (40 minutes total)

Control. Participants lay rested in a supine position for 40 minutes.

Assessing deoxygenation between preconditioning protocols

A pilot study was conducted in order to observe if levels of deoxygenation (a proxy for ischemia) exercise and IPC interventionswere similar.,. Four males, (26 ± 8 yrs.; BMI 25 ± 1 $kg.m^2$), had a near-infrared spectroscopy (NIRS) device (MOXY, USA) attached to the extensor carpi radialis longus in order to measure muscle oxygenation during a range of different handgrip exercise protocols. Devices were attached over the muscle body and held in place using micropore tape, protocols were conducted in a darkened room to avoid light interference. The range of protocols were tested with intensity between 30-75% MVC and 30-

60 reps/min. This was then visually compared to NIRS data from traditional IPC (220 mmHg) in order to evaluate whether the temporal pattern of deoxygenation and reperfusion of tissue is comparable between exercise and IPC. Devices captured live SMO_2 every two seconds which was analysed using the MOXY PC Application which has now been discontinued. The most feasible, well-matched protocol was 60 reps/min at 50% MVC (Figure 2).

Ischemia reperfusion. Following a 20-minute rest period after the intervention was administered, a temporary bilateral endothelial IR-injury was induced by inflating a cuff around the upper arms to 220 mmHg for 15 min using a rapid inflation pneumatic device. This was followed by a 15 min reperfusion period before the FMD protocol was repeated.

Statistical Analysis

To answer our primary research question and assess whether handgrip exercise offers comparable local and remote protection to IPC, a three-factor general linear model was employed with condition (3 levels: control, ExPC and IPC), time (2 levels: rest and post-IR injury), and location (2 levels: local, exercised arm and remote, contralateral arm) for FMD data. These analyses were performed on FMD data as well on allometrically scaled FMD data. For the latter analysis, we included baseline brachial artery diameter (log transformed) and shear rate area under the curve (SRAUC) as covariates in the model (ANCOVA) to account for changes in these variables across trials, as this may affect the magnitude of change in FMD [16, 17]. Statistically significant interactions were followed up with the Bonferroni approach to correct for multiple comparisons. Analysis was conducted using Statistical Package for Social Sciences (Version 26: SPSSS Inc., Chicago, IL). Statistical significance was delimited at $P<0.05$ and exact P values are cited (P-values of '0.00' provided by the statistics package are

reported as '<0.01'). Data are presented in the text as mean and 95% confidence intervals (95%CI).

Results

To answer our primary research question, that handgrip exercise and IPC offer comparable local and remote vascular protection against brachial artery endothelial IR-injury, the results of the three-way general linear model revealed no condition*time*arm interaction effect (P=0.92, Table 2). This suggests there were no significant differences in the impact of the intervention between the local and remote site. Allometrically scaled FMD with baseline diameter and SRAUC as co-variates, did not alter the outcomes of the three-factor general linear model (P=0.91).

The allometrically scaled three-factor general linear model, revealed a significant main effect of time, indicating a decline in FMD following IR (P<0.01). The main effect of condition did not reach statistical significance (P=0.07). This model did however indicate a significant condition*time interaction (P=0.02, Table 2). Corrected pairwise comparisons show, in the control condition, FMD declined significantly following IR-injury (4.6 ± 1.3% to 2.2 ± 1.7%, P<0.001). In the handgrip exercise intervention, the decline in FMD was also significant (4.6 ± 1.6% to 3.4 ± 1.9%, P=0.01). There was no significant difference between pre and post IR injury in the IPC intervention (4.5 ± 1.4% to 3.8 ± 3.5%, P=0.14), (Figure 3). Resting diameter did not change following IR (P=0.34), with no significant effect of condition (P=0.79) or condition*time interaction (P=0.94). SRAUC (10^3) was reduced by 2.9 (1.0, 4.8) following IR-injury (time: P<0.01), but there was no main effect of condition (P=0.71) or condition*time interaction (P=0.10).

Discussion

The aim of this study was to examine the impact of local handgrip and IPC on the ability to provide immediate protection against endothelial IR-injury in healthy individuals, and whether this protection was present at both local and remote locations. Our study supports previous evidence that IPC can attenuate IR injury, and importantly, adds additional insight into the use of handgrip exercise as a potential tool to prevent this injury as well.While the potential preconditioning effects of exercise remain elusive in the present study, it is clear that further research is needed to interrogate whether this stimulus can be adjusted to provide protection against IR injury. Consequently, local (handgrip) exercise could be useful in a clinical environment to prevent and/or attenuate endothelial IR injuries.

As observed in several previous studies, our work reinforces that forearm IR-injury induces a significant and marked decline in endothelial function measured with the FMD in healthy participants. Our data is comparable to previous studies who have implemented this temporary IR-injury model [18-22]. During the control experiment, IR-injury reduced brachial artery FMD by ~2.5%. This is largely in line as observed in other studies (2.0 to 3.8%) [19, 21, 23]. More importantly, our study found that the decline in FMD was markedly attenuated when IR-injury was preceded by the ischemic preconditioning stimulus and also to some extent with forearm exercise. In a previous study, our laboratory found that interval cycling exercise (which includes intermittent periods of rest) can negate brachial artery endothelial IR-injury [11], which highlighted the ability of acute bouts of exercise to mediate remote protection against endothelial IR-injury. Our study adds the novel insight that even localised, small muscle group, handgrip exercise may be able to attenuate endothelial IR-injury. The simplicity and low participant burden of handgrip exercise, especially when compared to cycling exercise, represents an important clinical advantage.

The exposure to repeated cycles of (local) ischemia may represent an important shared stimulus between (handgrip) exercise and IPC, mediating the protective effects. By design, both handgrip and IPC stimuli in the current study induced ischemia, reflected by changes in deoxygenation in the forearm during both protocols (n=4), to a similar extent, whilst also a comparable temporal pattern of deoxygenation and reperfusion was observed (Figure 2). Similarly, the study presented by Seeger, Lenting [11] showed that high-intensity interval exercise (associated with a cyclic pattern of tissue deoxygenation), but not moderate intensity attenuated the damage following an IR injury [7]. Furthermore, others have found that resistance exercise training, which involves muscular contractions which can render downstream tissue ischemic, was associated with substantially reduced endothelial damage following an IR injury compared to a non-resistance trained group [24]. At least, this work suggests that the pattern (i.e. intermittent) of local tissue hypoxia induced by exercise may be relevant to consider when exploring or identifying the optimal exercise stimulus for preconditioning benefits in humans. Our study was not designed to examine mechanisms that relate to exercise preconditioning. Nonetheless, we have recently examined potential mechanisms underlying both exercise and ischemic preconditioning at a microvascular level and suggest the mechanisms responsible for IPC are different to those responsible for ExPC with prostacyclin formation a key mediator of IPC but not ExPC [25].

Methodological considerations. Our model of ischemia/reperfusion was used to mimic IR-injury in the upper limb, this model has been used in previous studies to produce a temporary ischemic injury [18-22]. This model only represents a surrogate index of cardiac tissue, nevertheless, applying this technique significantly decreases plasma nitrite and plasma nitrate concentrations, indicating that any change following this endothelial IR injury is due to a reduction in NO bioavailability [26] and thus provides relevant insight. Finally, our data is limited

to individuals who are young, healthy men. Work by Shenouda et al[27] found that functional responses did not differ across the menstrual cycle, therefore controlling for menstrual phase may not be required in conducting this work in women. Previous work suggests that IPC is similarly effective in preventing IR injury in women[5],however inclusion of women would allow further comparisons for handgrip exercise in particular. Since previous evidence suggests that IPC is less effective in older individuals [21] or those with cardiovascular disease, future studies are also warranted to explore the hypothesis that exercise possesses preconditioning effects in those vulnerable groups.

Clinical perspectives. Re-establishing blood flow to an ischemic area is crucial in order to attenuate damage, but the reperfusion that follows can itself cause further cellular damage [28]. Endothelial cells are particularly sensitive to IR injury, leading to endothelial injury and swelling [29]. IR injuries represent a serious clinical complication, which are encountered through various acute vascular occlusions (e.g. stroke and myocardial infarction), but also during planned routine procedures (e.g. cardiopulmonary bypass surgery and organ transplantation) [30]. Based on previous work, outlining that exercise may be a useful intervention in providing protection against IR-injury [8-11, 31], our results raise the concept that (handgrip) exercise, by virtue of increasing the intensity or frequency of exercise, may enhance the preconditioning stimulus. This ability to increase the preconditioning stimulus is especially relevant, since we recently found that 12-week exercise training attenuates IR-injury in subjects with heart failure [31].

Conclusion

In summary, our data reinforces the established protective effects of IPC against IR-injury. Whilst we show that a single bout of handgrip exercise did not offer protection against endothelial IR-injury in young, healthy individuals, whether this can be achieved with adjusting

313 the stimulus (frequency, intensity) warrants study. Consequently, (local) handgrip exercise
314 may potentially be useful in a clinical environment to prevent and/or attenuate endothelial IR
315 injuries, an interesting hypothesis that requires further investigation.
316
317 **Author Contributions**
318 DJB, HJ, DJH and DAL contributed to the conception and design of the study. DJB, JDM and
319 FTM contributed to acquiring data, performing data reduction, and interpretation of data. DJB,
320 YBS contributed to statistical analysis and interpretation. DJB, HJ, DJH DAL and JDM
321 prepared and critically revised the manuscript. All gave final approval of the manuscript
322 **The authors declare that there is no conflict of interest**
323
324 **No source of Funding**
325

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Table 1. Descriptive *characteristics of participants*

	N = 15
Age (*years*)	24 ± 3
Weight (*kg*)	79 ± 11
BMI (kg/m^2)	25 ± 2
MAP (mmHg)	78 ± 6
SBP (*mmHg*)	115 ± 8
DBP (*mmHg*)	60 ± 5

Values are means ± SD. Abbreviations; BMI, Body Mass Index; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure.

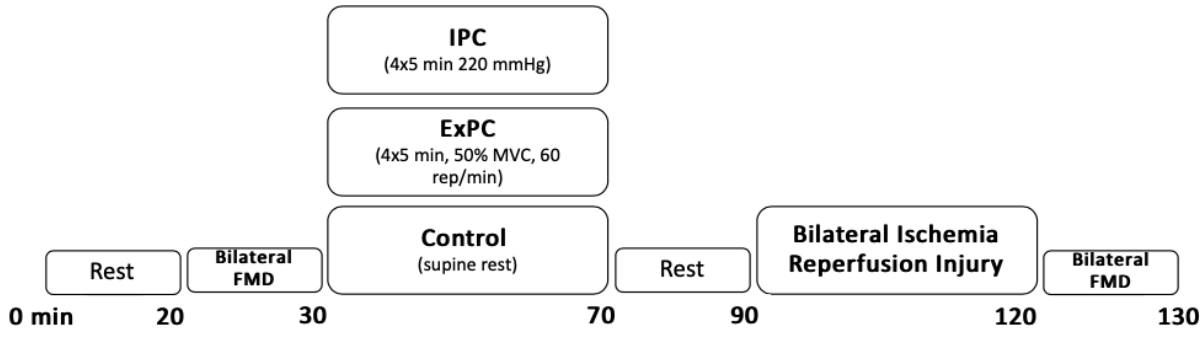

Figure 1. *A schematic of the research design. FMD, flow-mediated dilation; IPC, ischemic preconditioning; ExPC, handgrip exercise preconditioning*

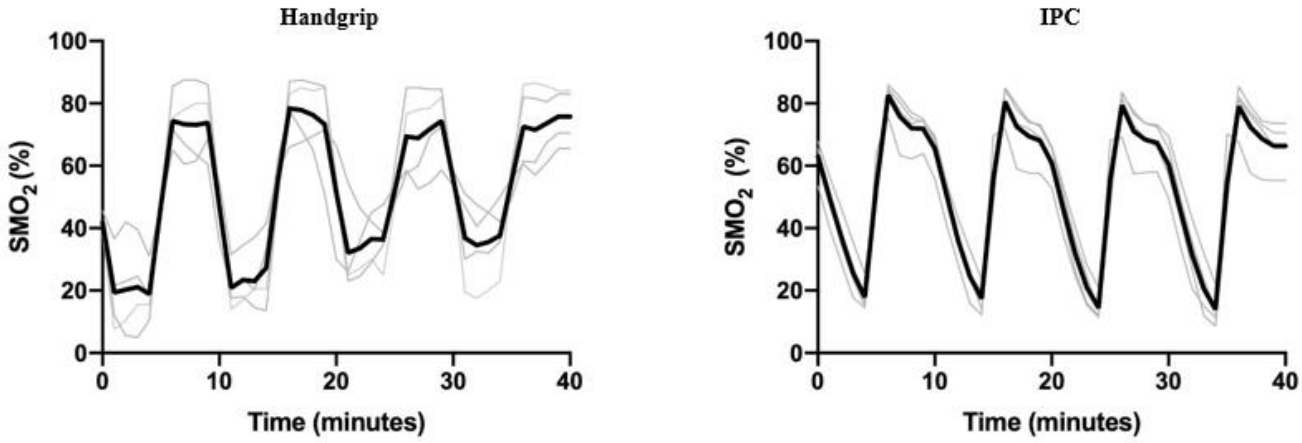

Figure 2. *Muscle oxygen saturation (SMO_2%) data to show the comparable cyclic pattern of tissue deoxygenation for the intervention protocols. Handgrip and IPC shows SMO_2% of the carpi radius longus*

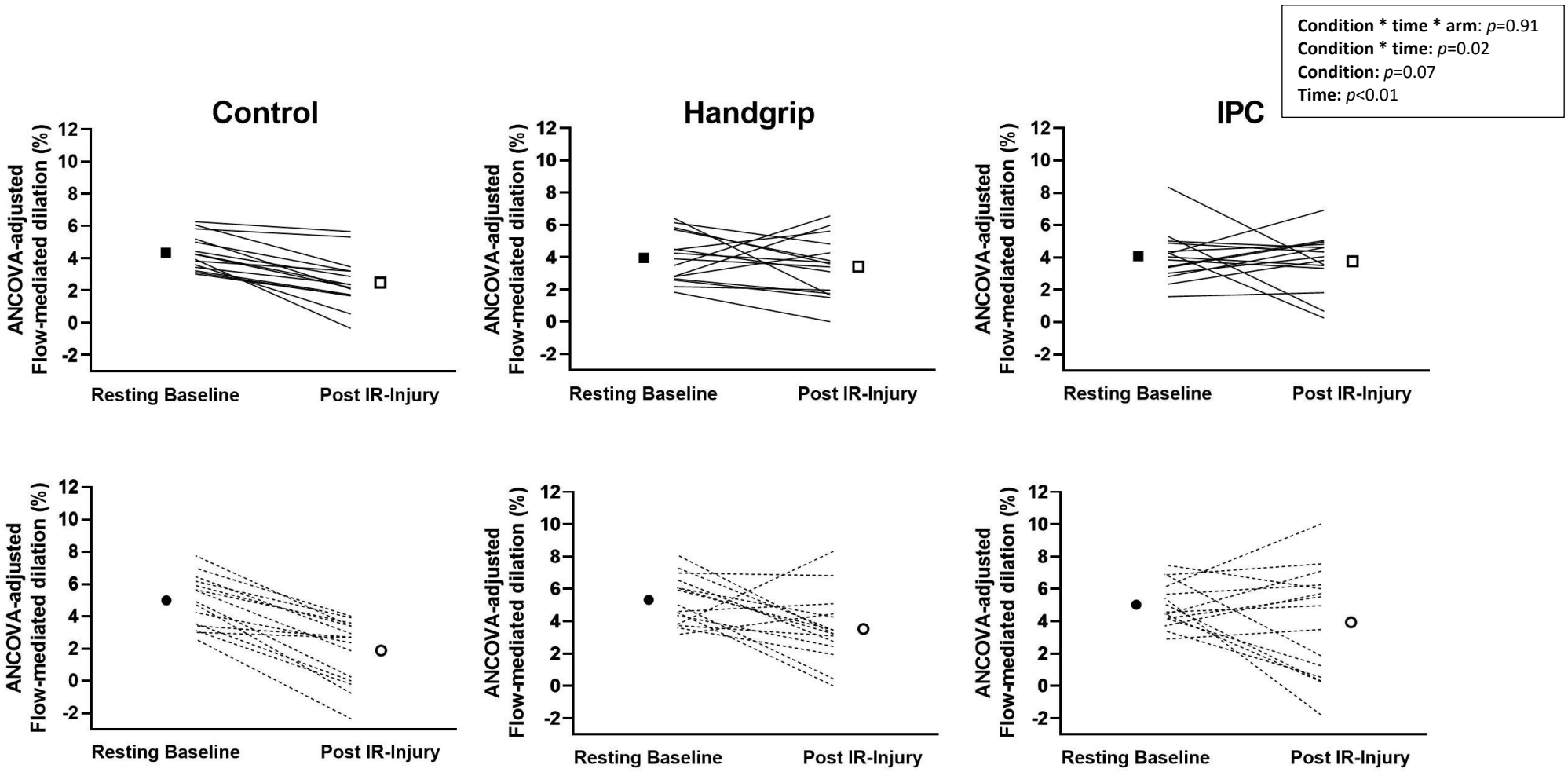

Figure 3. ANCOVA-adjusted mean flow-mediated dilation resting baseline prior to IR injury (solid square) and post IR-injury (open square) at the local arm (solid lines) and remote (circles and dashed lines) in N= 15 young, healthy men for the control, handgrip exercise, and ischemic preconditioning (IPC) conditions. Square and circle symbols denote the mean values.

Table 2. *Brachial artery flow mediated dilation pre and post ischemia-reperfusion (IR) injury after either control, handgrip exercise or IPC in the local and remote arm in 15 young, healthy men. * Denotes significance*

Local	**Control**		**Handgrip**		**IPC**		***3-way linear mixed model, P values***			
	Pre-IR	**Post-IR**	**Pre-IR**	**Post-IR**	**Pre-IR**	**Post-IR**	**Condition**	**Time**	**Condition* Time**	**Condition*Time *Arm**
Resting Diameter (cm)	0.41 ± 0.06	0.42 ± 0.07	0.41 ± 0.06	0.43 ± 0.07	0.41 ± 0.06	0.42 ± 0.07	0.79	0.35	0.94	0.92
Peak Diameter (cm)	0.43 ± 0.06	0.43 ± 0.06	0.43 ± 0.06	0.44 ± 0.07	0.43 ± 0.06	0.43 ± 0.07	0.73	0.93	0.99	0.92
FMD%	4.4 ± 1.1	2.5 ± 1.6	4.0 ± 1.5	3.4 ± 1.8	4.1 ± 1.6	3.8 ± 1.7	0.06	<0.01*	0.03*	0.92
Allometrically scaled FMD%	4.5 ± 1.6	2.3 ± 1.6	3.8 ± 1.6	3.7 ± 1.6	4.1 ± 1.2	3.8 ± 1.6	0.07	<0.01*	0.02*	0.91
Time to peak (sec)	51 ± 21	40 ± 19	41 ± 12	39 ± 19	43 ± 11	42 ± 20	0.61	0.50	0.80	0.77
Shear AUC (10^3)	16.6 ± 8.5	11.7 ± 3.5	13.9 ± 6.6	11.7 ± 5.1	15.1 ± 3.9	13.6 ± 6.7	0.71	<0.01*	0.10	0.73
Remote	**Control**		**Handgrip**		**IPC**					
	Pre-IR	**Post-IR**	**Pre-IR**	**Post-IR**	**Pre-IR**	**Post-IR**				
Resting Diameter (cm)	0.41 ± 0.06	0.42 ± 0.07	0.40 ± 0.6	0.40 ± 0.07	0.40 ± 0.06	0.41 ± 0.06				
Peak Diameter (cm)	0.42 ± 0.06	0.43 ± 0.06	0.42 ± 0.06	0.41 ± 0.07	0.42 ± 0.07	0.42 ± 0.07				
FMD%	5.0 ± 1.6	1.9 ± 2.0	5.3 ± 1.5	3.5 ± 2.1	5.0 ± 1.4	3.9 ± 3.4				
Allometrically scaled FMD%	5.0 ± 1.6	1.8 ± 2.4	5.3 ± 1.6	3.5 ± 2.4	5.0 ± 1.6	3.9 ± 2.4				
Time to peak (sec)	44 ± 15	53 ± 38	45 ± 14	53 ± 46	38 ± 7	48 ± 35				
Shear AUC (10^3)	15.8 ± 6.1	12.9 ± 6.6	19.4 ± 8.2	15.7± 7.5	16.1 ± 5.9	13.8 ± 5.2				

Values are means ± SD. Abbreviations; FMD, flow mediated dilation; AUC, area under curve.