Intermittent compression induces transitory hypoxic stimuli, upstream vasodilation and enhanced perfusion of skin capillaries, independent of age and diabetes.

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Running title: *Vasodilation and enhanced capillary perfusion with IPC*

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ABSTRACT

The benefit of enhanced shear stress to the vascular endothelium has been well documented in conduit arteries but is less well understood in skin microcirculation. The study aim was to provide physiological evidence of the vascular changes in skin microcirculation induced by intermittent pneumatic compression (IPC) of 1 second cuff inflation (130 mmHg) every 20 seconds to the palm of the hand for 30 minutes. The oxygenation and hemodynamics of dorsal mid-phalangeal finger skin microcirculation were assessed by laser Doppler fluximetry and reflectance spectroscopy before, during and after IPC in 15 young (18–39 years) and 39 older (40-80 years) controls and 32 older subjects with type 2 diabetes mellitus. Each individual cuff inflation induced: 1) brief surge in flux immediately after cuff deflation followed by 2) transitory reduction in blood oxygen for ~4 second and 3) a second increase in perfusion and oxygenation of the microcirculation peaking ~11 seconds after cuff deflation in all subject groups. With no significant change in blood volume observed by reflectance spectroscopy, despite the increased shear stress at the observed site this second peak in flux and blood oxygen suggests a delayed vasoactive response upstream inducing increased arterial influx in the microcirculation which was higher in older controls and subjects with diabetes compared to young controls ($p<0.001$, $p<0.001$ respectively) and achieving maximum capillary recruitment in all subject groups. Transitory hypoxic stimuli with conducted vasodilation may be a mechanism through which IPC enhances capillary perfusion in skin microcirculation independent of age and type 2 diabetes mellitus.

KEYWORDS: intermittent pneumatic compression, skin microcirculation, shear stress, vasodilation, type 2 diabetes mellitus.

NEW& NOTEWORTHY

This study demonstrates that hand intermittent pneumatic compression evokes transitory hypoxic stimuli in distal finger skin microcirculation inducing vasodilation of arterial inflow vessels, enhanced perfusion and maximum capillary recruitment in young and older subjects and older subjects with type 2 diabetes mellitus. Enhanced shear stress in the microcirculation did not appear to induce local skin vasodilation.

INTRODUCTION

In health the cardiovascular system responds spontaneously to perturbations in blood flow and oxygen demand, whether evoked by exercise, increased metabolic demand or disease, to maintain homeostasis and this has been linked to vasomotion (58). Multiple signalling mechanisms may be triggered relating to the accumulation of vasodilator metabolites (17, 41); the existence of oxygen sensors (18, 20, 26, 35, 48); conducted vasodilation inducing ascending vasodilation in feeding vessels (53, 57) and conversely the veno-arteriolar reflex causing upstream vasoconstriction in the presence of increased local venous pressure (23). Integral to these mechanisms, flow-induced shear stress induces endothelial derived vasodilators (5, 22, 39, 42, 46) where both the duration of the shear stress, whether transient or sustained, and vessel size have been shown to activate different vasodilators and different responses (6, 40, 62, 66, 67).

Clinical tools that have been developed to assess this vascular function include flow mediated dilatation (FMD) in large vessels (14, 22, 36) and post occlusion reactive hyperaemia (PORH) in the skin microcirculation (6, 24, 34). Both these techniques measure vascular health as indicated by a vasodilation in response to increased shear stress to the endothelium following the release of an arterial occlusion. However the underlying mechanism are likely to differ as in FMD the occlusion is distal to the vessel of interest and an increased shear stress is the main stimulus whereas in PORH a proximal cuff is used which will induce vascular responses due to the accumulations of metabolites as well as a functional hyperaemia in response to the increased shear stress on release of the cuff (34). Research has predominantly focussed on the effects of shear stress on the vascular endothelium in large peripheral conduit arteries by FMD (2). Although a standard FMD protocol uses a 5 minute arterial cuff to increase shear stress, some investigators have advocated more physiologically relevant sustained shear stress stimuli to explore flow mediated vasodilation such as through exercise (47), intermittent pneumatic compression (55), heating (28); and distal vasodilatory perfusion (40)

Despite our increased understanding of the role of shear stress in the regulation of blood flow in conduit arteries its significance in the microcirculation is less well understood. Therefore the purpose of this study was to use an externally applied pressure-oscillating system (intermittent pneumatic compression (IPC)) to reduce venous filling and thus downstream resistance, in order to study the acute effect of altered shear stress in the microcirculation with minimal perturbation of metabolic demand, thus reducing the contributions from metabolic vasodilators (52). Single pneumatic compression to the foot has been shown to increase shear rate and induce vasodilation in the popliteal artery (55) and in a similar manner single acute mechanical deformation of forearm blood vessels, with a fast inflating cuff, has also been shown to elicit a rapid vasodilation in the brachial artery (30) suggesting similar mechanisms might operate in both the foot and hand.

The aims of this study in the skin microcirculation of the hand were to use IPC with a combination of non-invasive optical techniques to: 1) quantify physiological changes in the skin microcirculation following a reduction of venous pooling downstream and thus a local increase in shear stress and 2) compare acute and sustained effect of shear stress during and after a single period of IPC for 30 minutes, on the skin microcirculation, 3) examine whether the effects of IPC were altered by age and the presence of type 2 diabetes mellitus (T2DM).

METHODS

Participants were recruited from a database of research volunteers (Exeter 10000) held by the National Institute of Health Research (NIHR) Exeter Clinical Research Facility. The study protocol was approved by the NHS Research Ethics Committee South West - Exeter (study reference 11/SW/0320). Written informed consent was obtained from all participants prior to inclusion on the study according to the Declaration of Helsinki. Fifteen young controls (18 – 40 years), 39 older controls (40 – 80 years) and 32 older subjects with confirmed T2DM treated by diet and/or oral hypoglycaemic agents only (40 – 80 years) participated in the study. Of the 32 subjects with T2DM, 62.5% received antihypertensive medication, the majority received ACE inhibitors or ARBs, either alone (21.9%) or in combination with calcium channel blockers (12.5%) and/or diuretics (12.5%). One subject

required the further addition of a beta blocker. One subject received an alpha blocker with ace inhibitor alone. One subject received a calcium channel blocker and diuretic and a further patient was on beta blocker alone. Exclusion criteria were: smokers; established coronary heart disease and/or peripheral artery disease that was clinically symptomatic with Ankle-Brachial Pressure Index (ABPI) > 0.8; superficial wounds on hand or foot and Body Mass Index > 40 kgm^{-2}. Healthy controls were excluded if they were on anti-hypertensive, or cholesterol lowering agents; whilst subjects with T2DM were excluded if they were on insulin or glucagon-like peptide analogue treatment.

Study protocol. The participants attended the NIHR Exeter CRF Diabetes and Vascular Research Centre for the study assessment having taken no food or drink for a minimum of 2 hours before arrival. A comprehensive medical examination was performed during the screening and a blood sample taken to measure their renal function and HbA1c. The relevant characteristics of study participants are shown in Table 1. The participants sat in a relaxed seated position for the duration of the study with their hand supported approximately 20cm below the heart to ensure continuous venous filling. Subjects were acclimatised in a temperature controlled laboratory at a room temperature of 24.1 ± 1.2°C (mean ± SD) for a 30 minute period with forearm and finger skin temperature monitored throughout the study (Fluke 52, Washington, USA.). The right index and middle finger were cleaned with alcohol and bilateral blood pressures were measured.

A VADOplex hand pad (OPED GmbH, Germany) was fitted to the palm of the right hand (secured gently by Velcro straps) for the delivery of intermittent impulse compression of 130 mmHg (inflation within < 0.5 seconds) and duration of 1 second which repeated at 20 second intervals or 3 cycles per minute, 0.05Hz (Figure 1). A digital pressure cuff was placed between the metacarpophalangeal and proximal interphalangeal joints of the right index finger for the measurement of maximum capillary density (MCD) as described below. To assess skin blood oxygenation by reflectance spectroscopy and skin perfusion by laser Doppler fluximetry an Oxygen2See combined probe (O2C, LEA Medizintechnik, Giessen, Germany) was attached, by double-sided adhesive tape, to the dorsum of the right middle finger in the mid-phalangeal region. Data from the O2C were recorded continuously

during 20 minutes of baseline (with no intermittent compression), during 30 minutes of IPC and for 30 minutes post IPC. Analysis of skin oxygenation and flux was performed on 16.7 minutes (1000 seconds) of artefact free data collected at 2Hz at the start of baseline and 16.7 minutes at the end of the post IPC period; and for periods of 100 seconds at 40Hz at the start of intermittent impulse compression. Capillary video microscopy (Microscan, MicroVision Medical, Amsterdam) was performed after an acclimatisation period, in the mid-phalangeal region of the index finger and recorded three time (see skin capillary density detailed section and Figures 1 and 2).

Skin oxygenation and perfusion. The hemodynamics and oxygenation of dorsal finger skin microcirculation were measured by optical reflectance spectroscopy (ORS) using the combined O2C and an LF-2 surface probe. Continuous white light (500–850 nm) is guided to the skin via an optical fiber, and backscattered light collected by a detector fiber encapsulated in the same probe at a distance of 2 mm from the light source. The sampled volume of tissue is taken to be at a depth of approximately half the probe spacing; therefore, a total skin thickness of 1 mm is sampled (11). The collected light is spectrally analysed in steps of 1 nm (500–620 nm) to derive relative concentrations of oxy- and deoxyhemoglobin. The sum of the derived concentration parameters [HbO_2] and [Hb] provides a measure of changes in blood volume in the skin [rHb] and the mean blood oxygen saturation is derived from the relationship: $S_{mb}O_2 = ([HbO_2] \times 100)/([HbO_2]+[Hb])$. However, it is important to recognise that ORS calculates the mean values of [HbO_2] and [Hb] across all vessels in the microcirculation of the skin, therefore the derived $S_{mb}O_2$ is a measure of mean blood oxygen saturation across arterioles, capillaries and venules. Incorporated within the O2C probe is an optical fiber that guides the output from a 830 nm (30 mW) laser diode to the surface of the skin allowing the measurement of skin blood flux. The probe separation is the same as for optical reflectance spectroscopy, and therefore the volume of tissue sampled by laser Doppler fluximetry overlaps that studied by ORS.

Skin capillary density. MicroScan (MicroVision Medical, Amsterdam) a handheld tool that uses sidestream dark field illumination to produce a real-time black and white video image of red blood cells was used to image red blood cells in the capillaries of the skin.

Capillaroscopy can only image vessels containing red blood cell therefore as skin capillaries are perfused intermittently, the observed capillary density changes with time and the tissue haemodynamics. Mean baseline capillary density in the literature for healthy dorsal mid-phalangeal finger skin ranges from 80 to 135 n.mm^{-2} (19, 21, 60) and it has been suggested that this wide range in resting capillary density arises from the heterogeneity of skin microcirculation (50) and the effects of vasomotion. To account for the effect of such variation, our study calculated mean capillary density from an average of six consecutive 20 second videos of adjacent areas of skin between the proximal and distal phalangeal joints of the index finger at three time points to determine maximum capillary density (MCD) (capillary density counted after venous cuff occlusion for 5 minutes to fill all capillaries), baseline capillary density (BCD) and after intermittent impulse compression (IPCCD) as described above. The mean capillary density at each time point for each subject was averaged from the number of perfused capillaries (those containing red blood cells) observed over the 20 second in each of the 6 videos. The change within an individual due to IPC was assessed by: 1) absolute change in capillary density from baseline to post use of IPC (IPCCD-BCD) n.mm^{-2} and 2) %Recruitment as (IPCCD – BCD)/BCD expressed as a percentage (both changes between baseline and after IPC were tested using a paired t-test). The maximum change, within an individual, induced by a venous cuff occlusion was assessed by: 1) absolute change in capillary density from baseline to 5 minutes of a venous cuff (MCD - BCD) n.mm^{-2} and 2) %Recruitment as (MCD – BCD)/BCD expressed as a percentage (paired t-test).

Statistics. The power calculation was based on capillary recruitment data from a large study population of 524 individuals aged between 40 – 75 years with BCD (72 ± 16 n.mm^{-2}) and MCD by venous occlusion (104 ± 17 n.mm^{-2}) which demonstrated that capillary density increased by almost 2 SD from baseline to maximum capillary density in health (64). Our study was powered to detect an increase in capillary density from baseline to post IPC of 0.5 SD as a pragmatic approach as there are no pilot data on which to base this calculation of the effect of IPC. The 0.5 SD or ~8 capillaries mm^{-2} is considered to be a physiologically meaningful increase. It is for example the mean difference seen in MCD between normotensive and hypertensive subjects (54). Data were analyzed using IBM Statistics 24

(SPSS Inc., Chicago, USA) with data sets tested for normality using the Kolmogororv-Smirnov test. Standard 2-tailed Student's t-tests and Mann-Whitney U and Wilcoxon tests were used as appropriate with significance defined as $P < 0.05$. Values presented as mean ± SD with boxplots presented as median and interquartile range.

RESULTS

Baseline subject characteristics

Older subjects, with and without diabetes, had higher blood pressures than the younger subjects (SBP: 131.1 ± 14.6 and 125.3 ± 12.5 vs 114.2 ± 13.1 mmHg ($p>0.001$, $p<0.005$, respectively). Subjects with T2DM had higher BMI, %body fat and HbA1c than the older controls as expected and were older (Table 1). Baseline skin flux in the young and older subjects was positively correlated with skin temperature ($p<0.008$ (young controls), $p<0.01$ (older controls)) but this correlation was lost in older subjects with diabetes (ns) (data not presented). Older subjects with T2DM demonstrated a higher and wider range of baseline fluxes (66.6 ± 39.6 au) compared to young and older subjects without diabetes (30.0 ± 23.4 41.8 ± 30.1, respectively) despite no significant difference in finger temperature between the 3 groups. This trend was similarly reflected in the mean red blood cell velocity (Table 2). Older controls and older subjects with diabetes both had a significantly higher baseline (before IPC) capillary density compared to the healthy young controls, but differences between the two older groups were not significant:101.0 ± 14.1 and 103.3 ± 16.74 $n.mm^{-2}$ (older healthy subjects and subjects with diabetes respectively) vs 90.4 ± 10.7 $no.mm^{-2}$ young controls, ($p<0.03$, $p<0.03$, Table 1). Baseline capillary density did not correlate with skin temperature (data not presented).

Multiple linear regression analysis was used to evaluate the association of SBP, age, sex, BMI, T2DM, ace inhibitors/angiotensin receptor blocker and calcium channel blockers with the study outcomes. Across the whole cohort, age was the only significant predictor of the observed increase in blood flux (F_{AUC}) following each impulse cuff compression ($p<0.001$) and linear regression analysis demonstrated age to be positively associated with F_{AUC} ($r = 0.44$, $p<0.001$). The only significant predictor of BCD and MCD was sex (Mean difference, 95% CI) 9.8 (3.3 – 16.3) $n.mm^{-2}$ $p<0.04$ and 14.3 (7.3 – 21.4) $n.mm^{-2}$ $p<0.001$

respectively with females having a significantly higher BCD and MCD than males. There was no statistical effect of SBP on BCD and MCD and antihypertensive medication taken only in subjects with T2DM was not a significant confounding variable.

Acute effects of IPC on skin perfusion and oxygenation.

The acute effects induced by IPC on the hemodynamics and oxygenation of blood in the microcirculation of finger skin were recorded by a combined optical spectroscopy and laser Doppler fluximetry probe (Figure 3 and 4A). For each subject detailed analysis of the acute effects of IPC was undertaken from the average of 5 compression cycles recorded during the first 100 seconds of IPC when data was collected at 40Hz. With each short 1 second 130 mmHg intermittent pneumatic compression there was an instantaneous movement artefact followed by a temporary rapid surge in flux for ~2 seconds defined as $Peak_{mech}$. After a delay from cuff deflation of ~4 seconds (T_{delay}) there followed a steady increase in flux and mean red blood cell velocity in the finger microcirculation (Table 3, Figure 4B). The rise time in flux was 7.7 ± 1.9 s (young subjects), 7.2 ± 2.2 s (older subjects) and 6.5 ± 2.3 s (subject with diabetes) peaking at T_{peak} ~ 11 seconds after cuff deflation. There was no significant different in these flux parameters between the groups (ns, Table 3).

During the acute compression of the cuff and emptying of the venous compartment the mean blood oxygenation across all vessels in the skin microcirculation fell from baseline ($\Delta_{IPC}S_{mb}O_2$) in all subject groups (Figure 4B). This fall was then immediately followed by a rise in blood oxygenation ($\Delta_{VASO}S_{mb}O_2$) simultaneous with the second surge in flux ($Peak_{vaso}$). Neither $\Delta_{IPC}S_{mb}O_2$ nor $\Delta_{VASO}S_{mb}O_2$ were significantly different between the groups (ns, Table 3). With the increase in blood oxygenation $\Delta_{VASO}S_{mb}O_2$ there was an increase in concentration of oxyhemoglobin [HbO_2] in the skin microcirculation, an equal and opposite decrease in deoxyhemoglobin [Hb] and a small change in total blood volume [rHb] contemporaneous with the change in [HbO_2]. This is indicative of an influx of arterial blood into the microcirculation starting approximately 4 seconds following the completion of each cuff inflation/deflation cycle. This arterial influx is quantified by two terms 1) $flux_{AUC}$: area under curve to flux peak following each intermittent compression and 2) $[HbO_2]_{AUC}$: area under curve of the simultaneous increase in the oxygen content of the

blood [HbO_2] in the skin microcirculation (Figure 5). For young subjects there was a correlation between $flux_{AUC}$ and $[HbO_2]_{AUC}$ ($r=0.82$, $p<0.001$) but not in older subjects with or without diabetes. $Flux_{AUC}$ was highly variable in older subjects with and without diabetes (Figure 6) and on average significantly higher than in younger subjects ($p<0.001$, $p<0.001$) respectively (Table 3 and Figure 6). This increase in flux in response to IPC ($flux_{AUC}$) was associated with age in all control subjects aged 18 – 80 years ($r = 0.44$, $p<0.001$) but not in older subjects with diabetes (ns, data not shown). There was no significant difference in the increase in concentration of oxyhemoglobin in the microcirculation ($[HbO_2]_{AUC}$) following each intermittent compression across the three groups (Table 3).

Sustained effect of intermittent pneumatic compression on skin perfusion and capillary density.

Thirty minutes of IPC did not significantly alter the mean overall skin flux, red blood cell velocity, mean blood volume and mean blood oxygen compared to baseline in all groups as determined by laser Doppler fluximetry and reflectance spectroscopy (Table 4). However this sustained period of 30 minutes of IPC to the palm of the hand did induce the recruitment of more capillaries in the dorsal finger skin of all subjects. Post IPC capillary density (IPCCD) was significantly higher than baseline capillary density for all three groups: IPCCD vs BCD 102.1 ± 12.9 vs 90.4 ± 10.7 $n.mm^{-2}$ (young controls, $p< 0.001$), 110.1 ± 14.4 vs 101.0 ± 14.1 $n.mm^{-2}$ (older controls, $p< 0.001$)), 111.3 ± 19.2 vs 103.3 ± 16.4 $n.mm^{-2}$ (older subjects with diabetes, $p< 0.001$) (Figure 7). In all subjects there was no time effect on the number of capillaries counted across the 6 videos recorded over a 6 minute period (Wilks' Lamda, ns).

Following 30 minutes of IPC across the three subject groups there was no difference in absolute capillary density (IPCCD), increase in capillary density (IPCCD – BCD) or %Recruitment (Table 5). This was despite older controls and older subjects with diabetes both having higher baseline (before IPC) capillary density compared to the healthy young controls. IPCCD %Recruitment was not different in all three groups, independent of age, 9.2 ± 6.0% (older controls) vs 13.2 ± 8.6% (young controls, ns) and diabetes 9.2 ± 6.0% (older controls) vs 8.0 ± 5.6% (older subjects with diabetes, ns, Table 5).

299

300 Maximum capillary density induced by a 5 minute application of a 50 mmHg venous cuff
301 to the base of the finger was significantly higher than BCD for all three groups (Figure 7):
302 MCD vs BCD 104.8 ± 13.7 vs 90.4 ± 10.7 $n.mm^{-2}$ (young controls, $p< 0.001$), 112.0 ± 13.3
303 vs 101.0 ± 14.1 $n.mm^{-2}$ (older controls, $p< 0.001$)), 112.3 ± 19.9 vs 103.3 ± 16.4 $n.mm^{-2}$
304 (older subjects with diabetes, $p< 0.001$, Table 5). Maximum capillary density induced by
305 venous occlusion was not significantly different across the three subject groups whether
306 derived as absolute MCD, increase in capillary density (MCD – BCD) or as %Recruitment
307 (Table 2). This was again despite older controls and older subjects with diabetes both
308 having higher BCD compared to the healthy young controls (Table 5).
309

310 Thirty minutes of IPC achieved an increase in capillary density from baseline (IPCCD)
311 equal in magnitude to the maximum capillary density (MCD) after a 5 minute venous cuff
312 in all subjects except in older controls where IPC did not attain maximum capillary density
313 ($p<0.03$), Table 5). The mean difference between MCD and IPCCD (95% CI) was 0.98
314 (0.93 – 1.03) $n.mm^{-2}$ $p< 0.001$ across the study cohort. Thirty minutes of IPC induced 100.5
315 ± 3.6% of the maximum capillary density in young subjects, 99.0 ± 2.6% in older subjects
316 and 99.6 ± 3.8% in subjects with diabetes and there was no difference in % capillary
317 recruitment either by a venous occlusion cuff or IPC between the groups.
318

319 **DISCUSSION**
320

321 This study provides new insight into the mechanisms through which a transitory reduction
322 in blood oxygen and increased shear stress induced by intermittent pneumatic compression
323 enhances the perfusion of skin microcirculation. The initial brief surge in flux ($Peak_{mech}$)
324 induced mechanically by each cuff inflation with IPC is followed by an acute local
325 transitory fall in blood oxygenation. This induces a delayed second increase in both blood
326 flux ($Peak_{vaso}$) and oxygenation to the finger microcirculation downstream indicative of
327 vasodilation. At the skin measurement site there were minimal changes in blood volume
328 suggesting there is minimal vasodilation in the local skin microcirculation but that the
329 vasodilation is being induced upstream in feeding vessels. At the cessation of IPC this

mechanism does not induce a sustained alteration in skin blood flux and oxygenation but does achieve sustained skin maximum capillary recruitment.

Acute effect of intermittent impulse compression

Here we present novel data that reveals that each one second intermittent pneumatic compression to the palm of the hand induces two separate peaks in flux 1) a brief surge in flux immediately after cuff deflation ($Peak_{mech}$) and 2) a delayed increase in flux (~4 seconds after cuff deflation) consistent with a vasodilation rising to a peak at ~11 seconds post IPC ($Peak_{vaso}$). Between these two peaks there is a period of reduced blood oxygenation in the finger skin microcirculation. We have previously described how vasomotion in skin microcirculation can improve local perfusion through spontaneous intermittent hypoxic vasodilation triggered by a low blood oxygenation (58). That study in dorsal forearm skin demonstrated a spontaneous cyclical fall in the mean blood oxygenation in the microcirculation ($S_{mb}O_2$) of ~7% was followed by a surge of oxygenated blood for ~10-15 seconds. It was hypothesised that this vasomotion may be attributed to hypoxic vasodilation induced by red blood cells oxygen sensors. In this current study we observe brief falls in oxygenations of ~4% induced by IPC which precede surges in flux and mean blood oxygenation in the microcirculation ($S_{mb}O_2$). Sheldon et al have previous shown that following each individual cuff deflation, IPC to the calf increases blood flow and shear rate in the popliteal artery (55). Shear stress is proportional to blood velocity and viscosity and inversely related to vessel diameter. Assuming constant viscosity then the changes we observe in blood flux in the skin microcirculation without a change in blood volume would suggest an increase in shear stress but the lack of increase in total blood volume suggests this occurs without local skin microcirculatory vasodilation.

Studies in large vessels show that IPC induces periods of increased arterial inflow (33, 55) that have been attributed to both mechanical effects and enhanced shear stress (7, 55). We postulate that in our study in the microcirculation distal from the IPC cuff that the surge in flux immediately following cuff deflation ($Peak_{mech}$) is a consequence of the external compression to the venous plexus in the hand displacing blood proximally. This has previously been demonstrated in femoral and popliteal veins with IPC to the foot and calf

(16) and increasing the arteriovenous pressure gradient (15) in the finger. We suggest that the second delayed increase in flux ($Peak_{vaso}$) which follows a brief period of reduced blood oxygenation results from a delayed shear stress-induced dilation in feeding vessels rather than local vasodilation as we do not see a local increase in blood volume. This mechanism would be similar to that described as conducted vasodilation by Segal in muscle (57). However this lack of change in blood volume in the skin microcirculation in our study might also be due to a lack of co-ordinated vasodilation across the microcirculation in the measurement area or a reduction in downstream resistance with capillary recruitment. Surges in blood flux in response to IPC have been reported beneath an intermittent pneumatic cuff in forearm skin peaking at ~ 5 seconds after cuff deflation (52). We believe this shorter time to peak may be due to the probe site being under the cuff in that study rather than downstream as in our study and therefore the response may be dominated by direct compression-induced vasodilation of vessels rather than vasodilation of upstream feeding vessels.

Synchronous with this increased vasodilatory flux response ($Peak_{vaso}$) in all subject groups there is an almost equal increase in concentration of oxyhemoglobin [HbO_2] and decrease in deoxhemoglobin [Hb] resulting in minimal change in blood volume and indicative of increased arterial inflow. This increase in [HbO_2] and washout of [Hb] after each impulse compression may be limited by the capacity of the local microcirculation and/or the metabolic demands of the tissue. In young controls we observe a correlation between the increasing hyperaemic flux (F_{AUC}) with the increase in oxygenated hemoglobin [HbO_2] which would indicate arterial inflow where oxygen demand is already matched. A similar association has been observed in response to IPC in the calf muscle of young subjects but with sustained blood oxygenation for >100 seconds (37). This prolonged increase in blood oxygenation in muscle compared to skin may be a consequence of the eightfold higher capillary density in muscle compared to skin (313 and 317 vs. 38 number/mm^2) (3, 13, 45) sustaining oxygen demand.

Vasodilation responses to IPC in skin compared to muscle studies

In muscle microcirculation similar biphasic responses known as contraction-induced rapid onset vasodilation (ROV) are also observed with the onset of exercise (8, 38). A rapid increase in blood flow within the first second is attributed to an acute mechanical effect of muscle contraction (muscle pump) followed by an additional active dilatation of resistance vessels (56, 63) peaking around 4 seconds and induced by vasoactive metabolites released from contracting fibres (9, 30, 65) and the vascular endothelium (9). Kirby et al demonstrated that a brief isometric muscle contraction resulted in peak vasodilation post contraction in ~4 - 7 cardiac cycles in the muscle bed increasing forearm vascular compliance whilst a single 200mmHg impulse cuff inflation to the forearm muscle evoked a reduced peak vasodilation within 2 cardiac cycles (30). Similar time scales for contraction-induced vasodilation are observed in the leg with peak vasodilation occurring in ~ 5 cardiac cycles (10). The inconsistent temporal patterns in flux induced by IPC and exercise may be explained in part by pioneering research on conducted vasodilation (65). Segal identified two separate endothelial signalling pathways inducing upstream vasodilation in feed arteries at the onset of exercise: 1) endothelium-derived hyperpolarisation in response to a brief tetanic contraction with an instantaneous spread in electrical signal upstream and contract-induced rapid onset vasodilation in < 1 second (4) and 2) a delayed nitric oxide mediated vasodilation in feeding vessels in response to luminal shear stress which increases secondary to arteriolar dilation downstream (57). Segal points out that this second mechanism inducing flow-mediated dilation has a temporal component depending upon the site of measurement. In a standard FMD protocol peak dilation in the conduit vessel occurs around 40 seconds (43) whilst in the present study in the microcirculation, flow-mediated dilation appeared after 8-10 seconds consistent with that observed in feeding arterioles (32). Studies of oxygenation saturation in muscle microcirculation using near infrared spectroscopy also point to a role for hypoxic vasodilation in ROV with a transitory increase in deoxyhemoglobin at the onset of exercise (31, 44, 61).

Sustained effect of intermittent impulse compression

This study demonstrates for the first time that when continuous IPC is applied to young and older healthy volunteers or to individuals with type 2 diabetes mellitus its effect is to:

1) increase skin capillary density 2) produce maximal capillary recruitment in all subjects. This pragmatic study demonstrated that IPC improved capillary perfusion in people with T2DM who were receiving antihypertensive medication as well as those who were not. Although the intermittent increase in skin flux and perfusion of oxygenation blood at a microcirculatory level during IPC is thought to instigate the capillary recruitment, these haemodynamic parameters return to baseline at the end of IPC whilst all capillaries continue to remain perfused. Other studies have reported this return of flux to baseline levels after the cessation of IPC (1, 25, 33).

Effect of age and type 2 diabetes mellitus on response to IPC

Dysfunctional microcirculation is well documented with both increasing age and diabetes and so in this study comparisons were made between young and old subjects and with a group of older subjects with T2DM. We have shown that at baseline older subjects, both with and without diabetes have a higher and wider range of skin flux, higher mean red blood cell velocity and higher capillary density, independent of temperature. Jan et al (27) also report an increased skin flux with age in the dorsal foot and argued that this resulted from a loss of vasoactive control and diminishing microvascular reactivity in T2DM. Indeed the correlation we observed between skin flux and temperature in both the control groups is lost in subjects with diabetes. Age was the only significant predictor of the increase in blood flux (F_{AUC}) with IPC. In all older subjects with and without diabetes, each 1 second pneumatic compression produced a higher increase in flux ($flux_{AUC}$) compared to young subjects suggesting that tight blood flow regulation is perturbed. Surprisingly this greater increase in $Flux_{AUC}$ with age did not equate to a proportional increase in concentration of oxygenated blood into the volume of tissue under investigation. In young controls the increased arterial inflow appears superfluous to oxygen demand and [HbO_2] increases proportionally. In older subjects with higher baseline capillary densities and mitochondrial dysfunction with diabetes (49, 51) potential unmatched oxygen demand may be utilising the increased [HbO_2].

Clinical implications

452 This study suggests for the first time that increased perfusion and capillary recruitment may
453 be a mechanism through which IPC can provide clinical benefits to the skin. Capillary
454 rarefaction has been associated with a range of cardiovascular and metabolic disorders such
455 as hypertension (54), coronary artery disease (60), diabetes (12) and obesity (29). The
456 microvascular dysfunction common to these conditions, where a clinically significant
457 different capillary density occurs at ~ 10 capillaries.mm^{-2} (54, 59, 60), can lead to
458 conditions such as impaired wound healing as seen with diabetic foot ulcers and venous
459 ulceration. Our results suggest that IPC can increase capillary density by ~ 10
460 capillaries.mm^{-2} independent of age and diabetic status.
461
462 Limitations of this study include the complications of blood flow regulation in the skin for
463 thermoregulation. However, there was no significant difference between the subject groups
464 in skin temperature and no significant difference in skin temperature before and after IPC,
465 making the confounding effects of skin thermoregulation unlikely. Data collection was
466 performed at 0.5Hz throughout the study and could only be increased to 40Hz for 100
467 second periods. Collecting data at a higher frequency for longer periods would have
468 allowed the coherence of flux and blood oxygenation to be analysed using tools such as
469 wavelet analysis. This future research would provide further insight into the coupling
470 mechanisms that regulate the delivery of oxygen in the microcirculation.
471
472 In conclusion this study has identified transitory hypoxic stimuli with conducted
473 vasodilation as a possible mechanism through which intermittent pneumatic compression
474 enhances perfusion of oxygenated blood to skin microcirculation, eliciting maximum
475 capillary recruitment. Although IPC induced frequent surges of oxygenated blood flux and
476 associated shear stress in the skin microcirculation there were no significant increases in
477 blood volume suggesting local vasodilation was not observed. This suggests that despite
478 increased shear stress in the microcirculation, the mechanism by which IPC enhanced
479 perfusion in the skin was by delayed vasodilation upstream from the site of study in
480 response to a downstream reduction in blood oxygenation.
481
482 ACKNOWLEDGEMENTS

Authors would like to thank all study participants for their contribution to this study. Participant recruitment was facilitated by the NIHR Exeter Clinical Research Facility, a partnership between the University of Exeter Medical School College of Medicine and Health, and Royal Devon and Exeter NHS Foundation Trust. This study was supported by the National Institute for Health Research (NIHR) Exeter Clinical Research Facility and partially funded by relatives of Mr Gardner, with no involvement in the study, and Dr Fox. Equipment was loaned by OPED GmbH with consumables. The authors have no conflict of interest. The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

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Figure captions

Fig. 1. Schematic diagram of experimental setup.

Fig. 2. Schematic diagram of study protocol illustrating the recording of maximum capillary density during the acclimatisation period (MCD: capillary density counted after venous cuff occlusion for 5 minutes to fill all capillaries), baseline capillary density (BCD) and post 30 minutes of intermittent pneumatic compression to the palm of the hand capillary density (IPCCD) in dorsal finger skin. Laser Doppler fluximetry and reflectance spectroscopy were continuously recorded throughout the 90 minute study.

Fig. 3. (A) Cyclical changes in flux, measured by laser Doppler fluximetry, induced in dorsal finger skin microcirculation during intermittent impulse compression (IPC) to the palm of the hand with a cuff inflation of 3 cycles per minute for 30 minutes (data collected at 2Hz). Cuff was inflated to 130mmHg in <0.5 seconds and held for 1 second before deflation and then repeated every 20 seconds. Data collected at 40Hz for the first 100 seconds of IPC demonstrate (B) a temporary immediate surge in flux (black) and mean red blood cell velocity (grey) upon each cuff inflation due to mechanical compression which was followed by a steady increase of flux and mean red blood cell velocity indicative of vasodilation and (C) baseline flux and mean red blood cell velocity in the dorsal finger skin microcirculation without IPC.

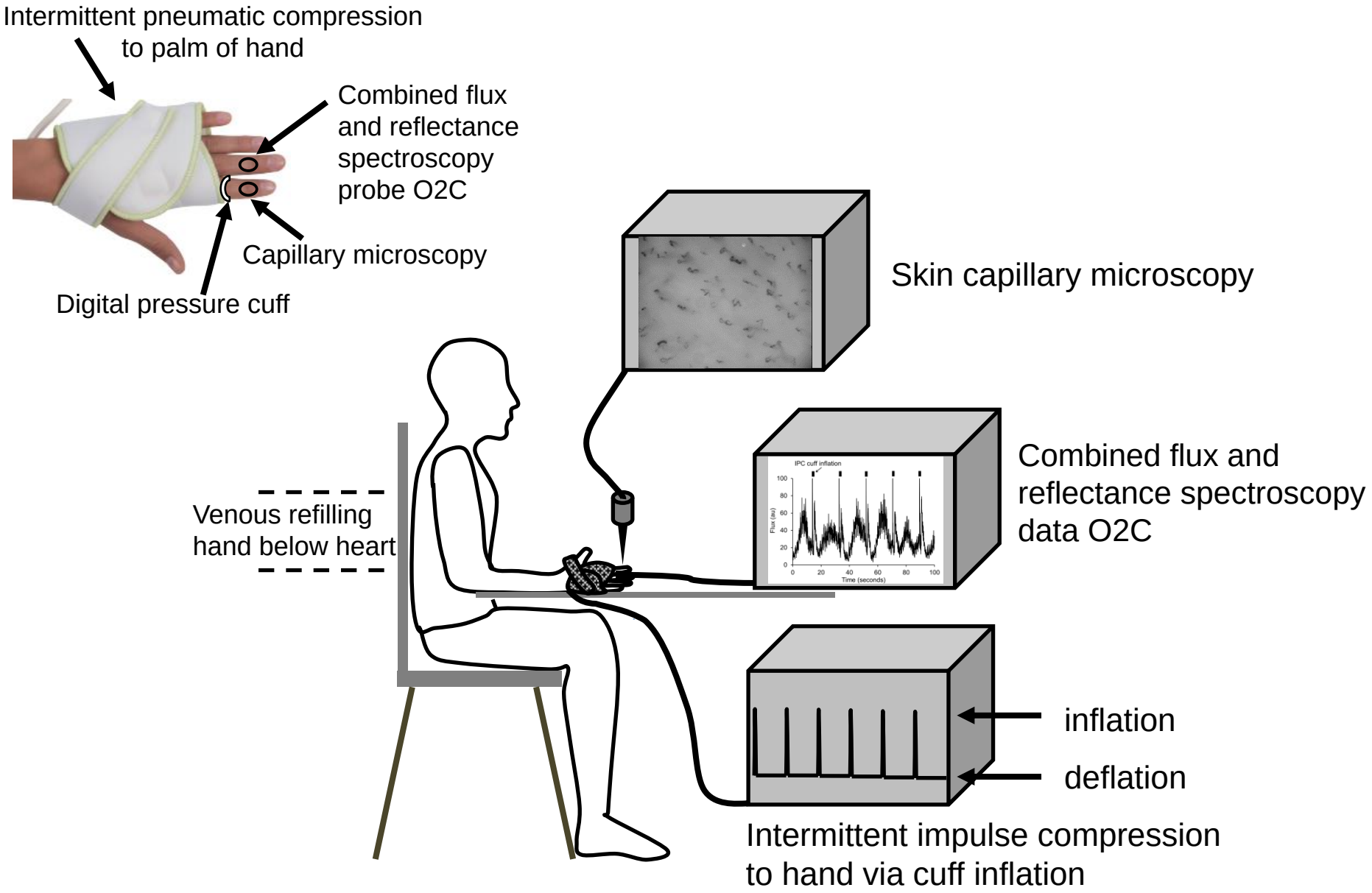
Fig. 4. (A) Effect of intermittent pneumatic compression to the palm of the hand on the oxygenation and hemodynamics of the dorsal finger skin microcirculation: flux (au, dark grey), mean blood oxygenation $S_{mb}O_2$ (%, dashed black), concentration of oxyhemoglobin [HbO_2] (au, black), concentration of deoxyhemoglobin [Hb] (au, dashed grey) and total haemoglobin rHb (au, light grey). (B) Schematic diagram of changes in finger skin flux (au, dark grey) and mean blood oxygenation $S_{mb}O_2$ (%, dashed black) in response to a single one second intermittent pneumatic compression (IPC) to the palm of the hand. The cuff inflation to 130mmHg in <0.5 seconds and held for 1 second induces an instantaneous surge in flux ($Peak_{mech}$) and a steady fall in blood oxygenation ($\Delta_{IPC}S_{mb}O_2$) reaching a minimum mean blood oxygenation $S_{mb}O_2$ after cuff deflation at T_{delay} (s). This hypoxic stimuli is followed by an increase in flux indicative of a vasodilation rising to a maximum ($Peak_{vaso}$) at T_{peak} (s) after cuff deflation.

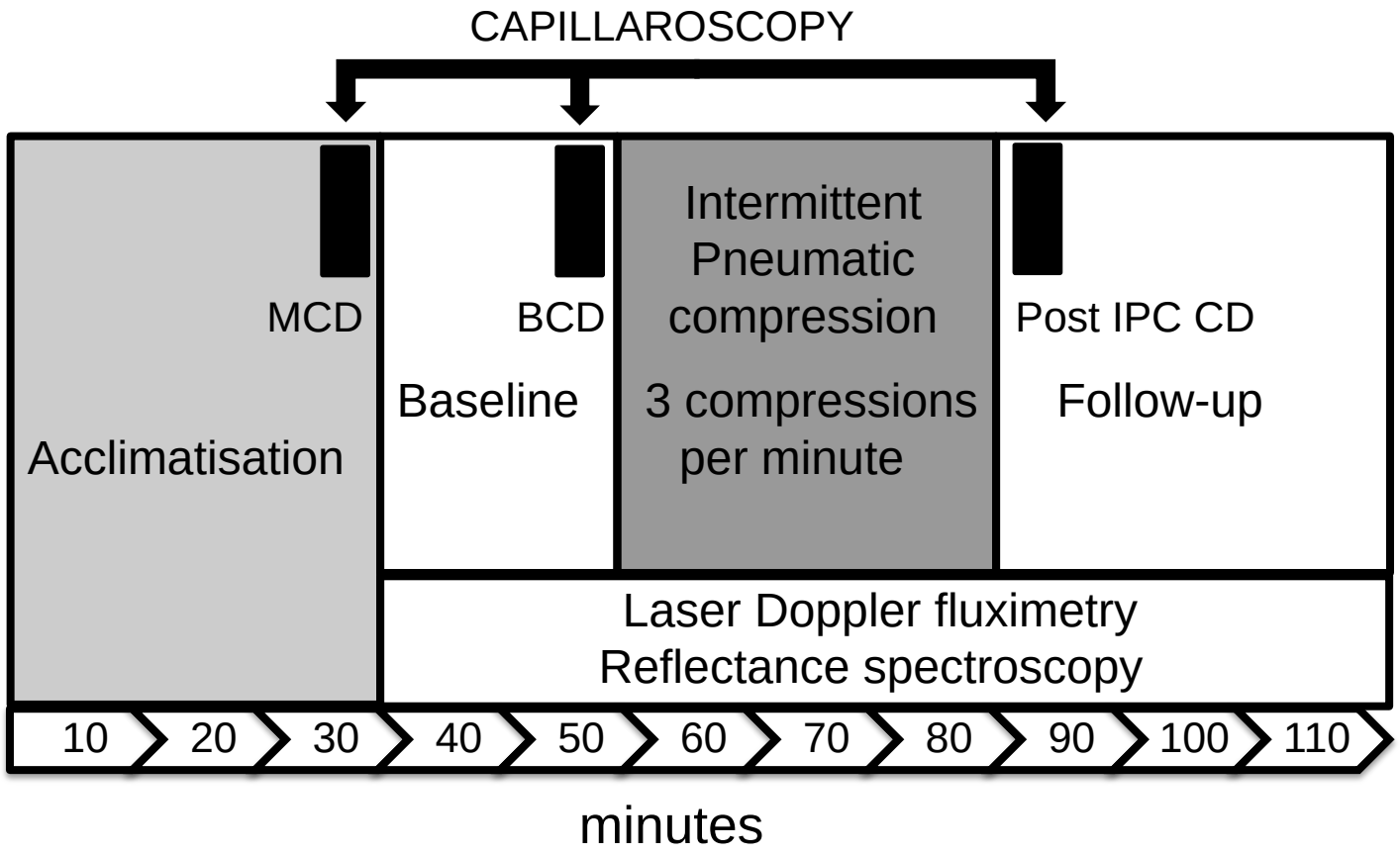
Fig. 5. Method of quantifying the cumulative increase in flux and oxygenation in the dorsal finger skin microcirculation following each one second intermittent pneumatic compression to the palm of the hand. The cumulative increase in flux following each IPC cuff inflation and deflation is derived as an area under curve to peak ($flux_{AUC}$, au.sec, grey shading) from flux (au, dark grey). The cumulative increase in concentration of oxyhemoglobin [HbO_2] following each IPC cuff inflation and deflation is derived as an area under curve to peak ($[HbO_2]_{AUC}$, au.sec, black shading) from concentration of oxyhemoglobin [HbO_2] (au, black). Mean blood oxygenation $S_{mb}O_2$ (%, dashed black), concentration of deoxyhemoglobin [Hb] (au, dashed grey) and total hemoglobin (au, light grey). Area under curve calculated from integral of increase to peak minus area defined

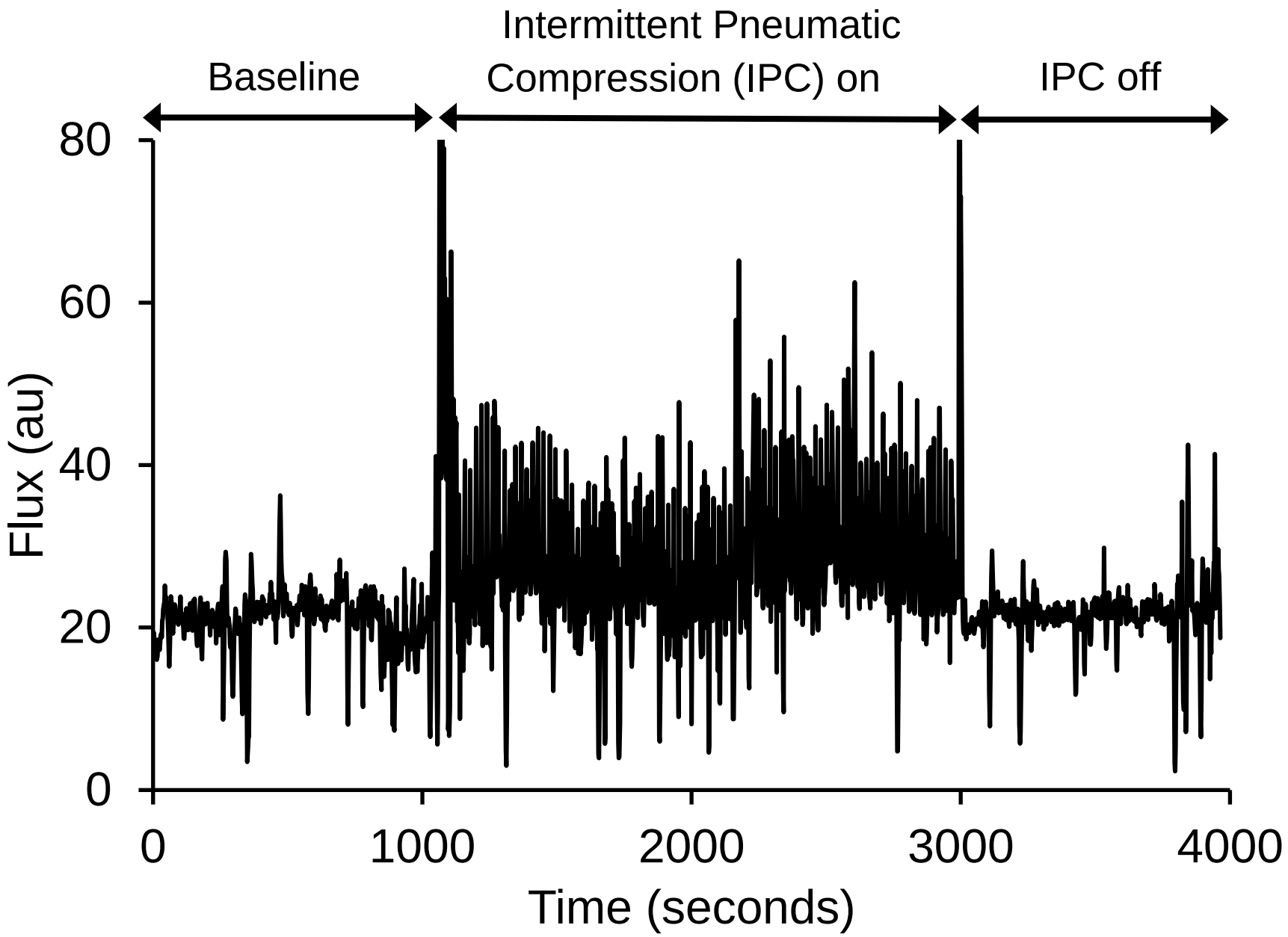
by initial value multiplied by time to peak T_{peak} and averaged over 5 consecutive compression cycles.

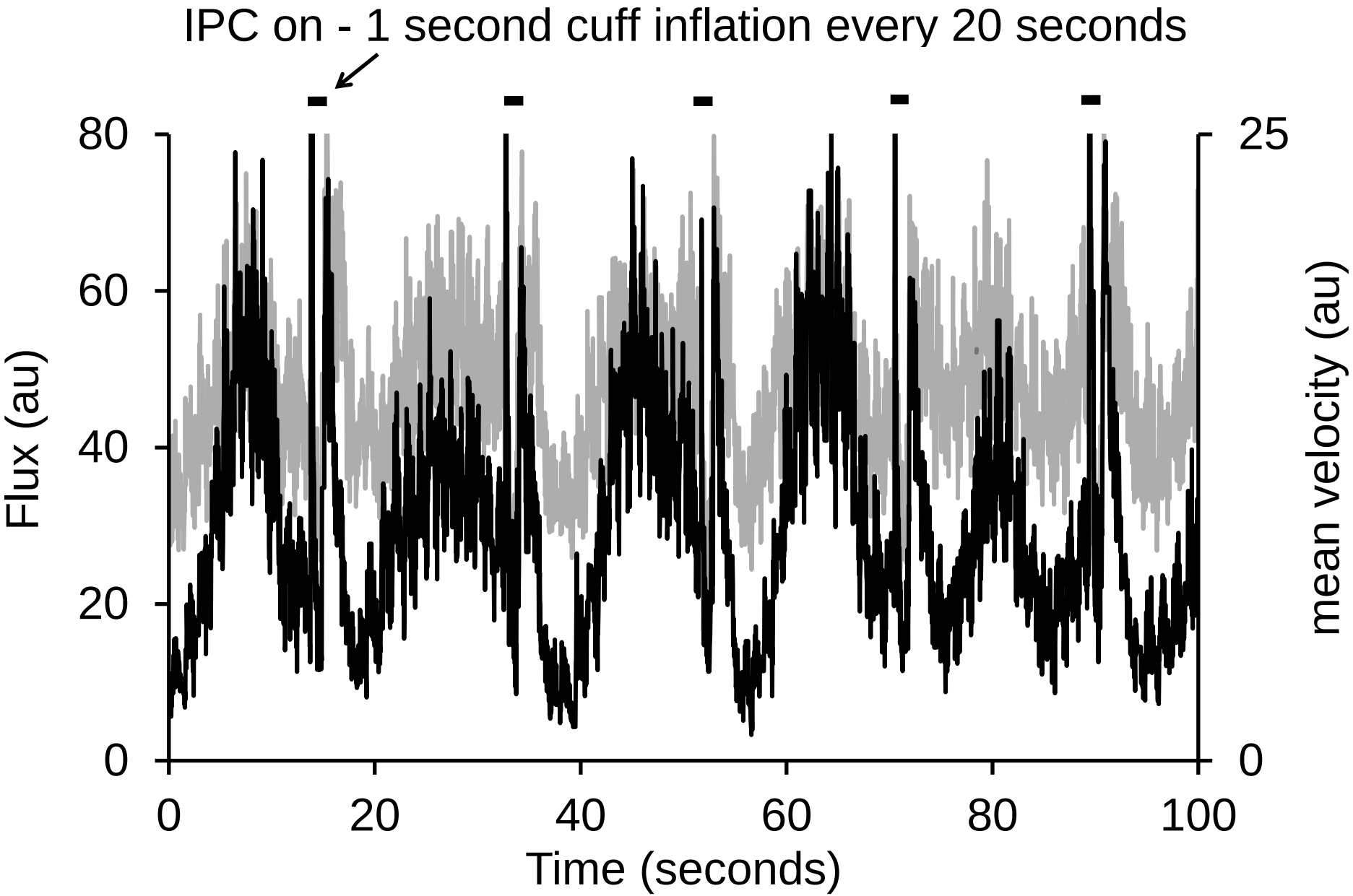
Fig. 6. The cumulative increase in flux following each intermittent pneumatic compression cuff inflation and deflation ($flux_{AUC}$) as recorded by laser Doppler fluximetry (unpaired Student's-t test) for young controls (n=15), older controls (n=39) and older subjects with diabetes (n=32).

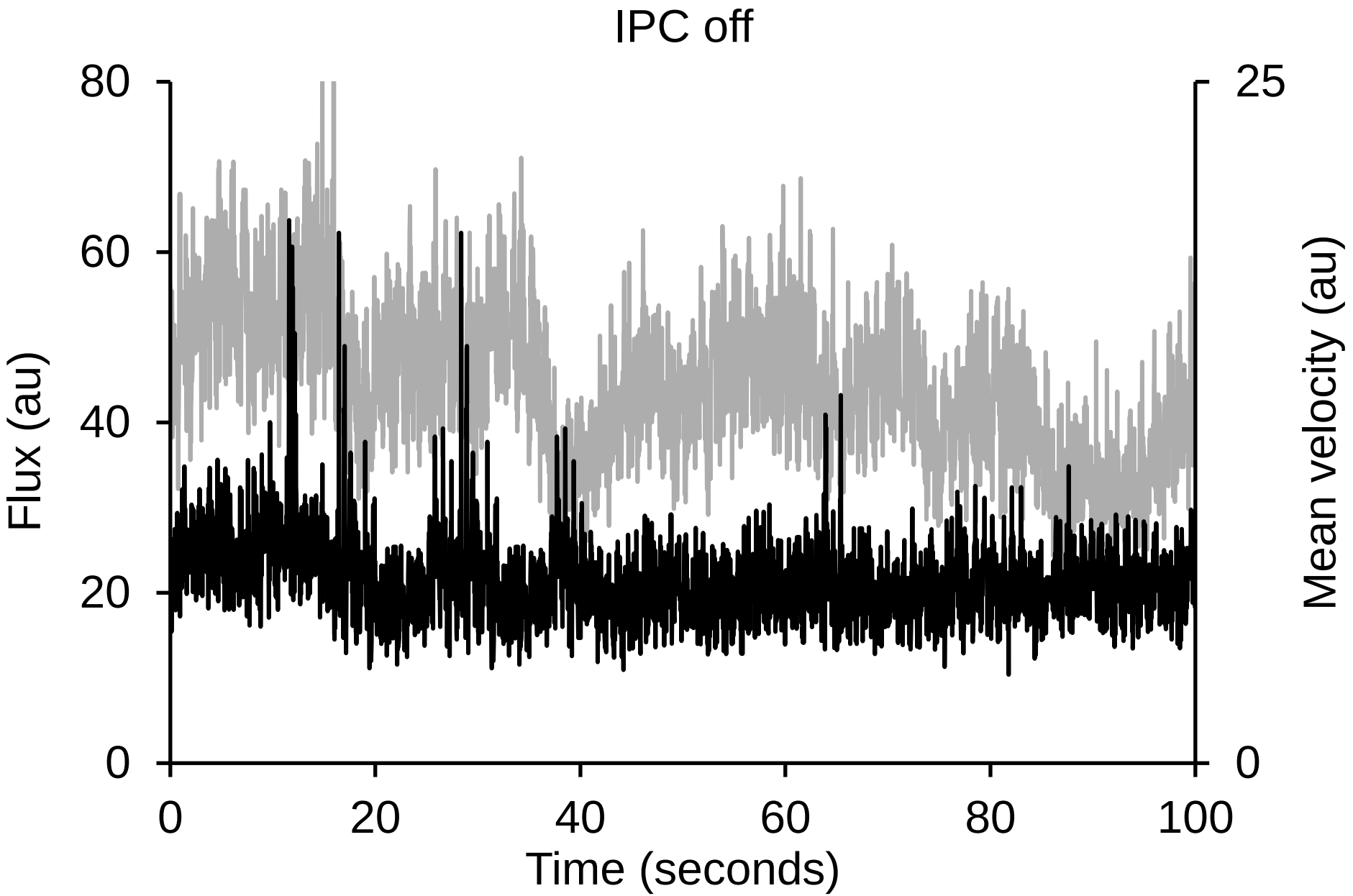
Fig. 7. Baseline capillary density (BCD), maximum capillary density (MCD: capillary density counted after venous cuff occlusion for 5 minutes to fill all capillaries) and post intermittent pneumatic compression capillary density (IPCCD) in dorsal finger skin of young controls (n=15), older controls (n=39) and older subjects with diabetes (n=32).

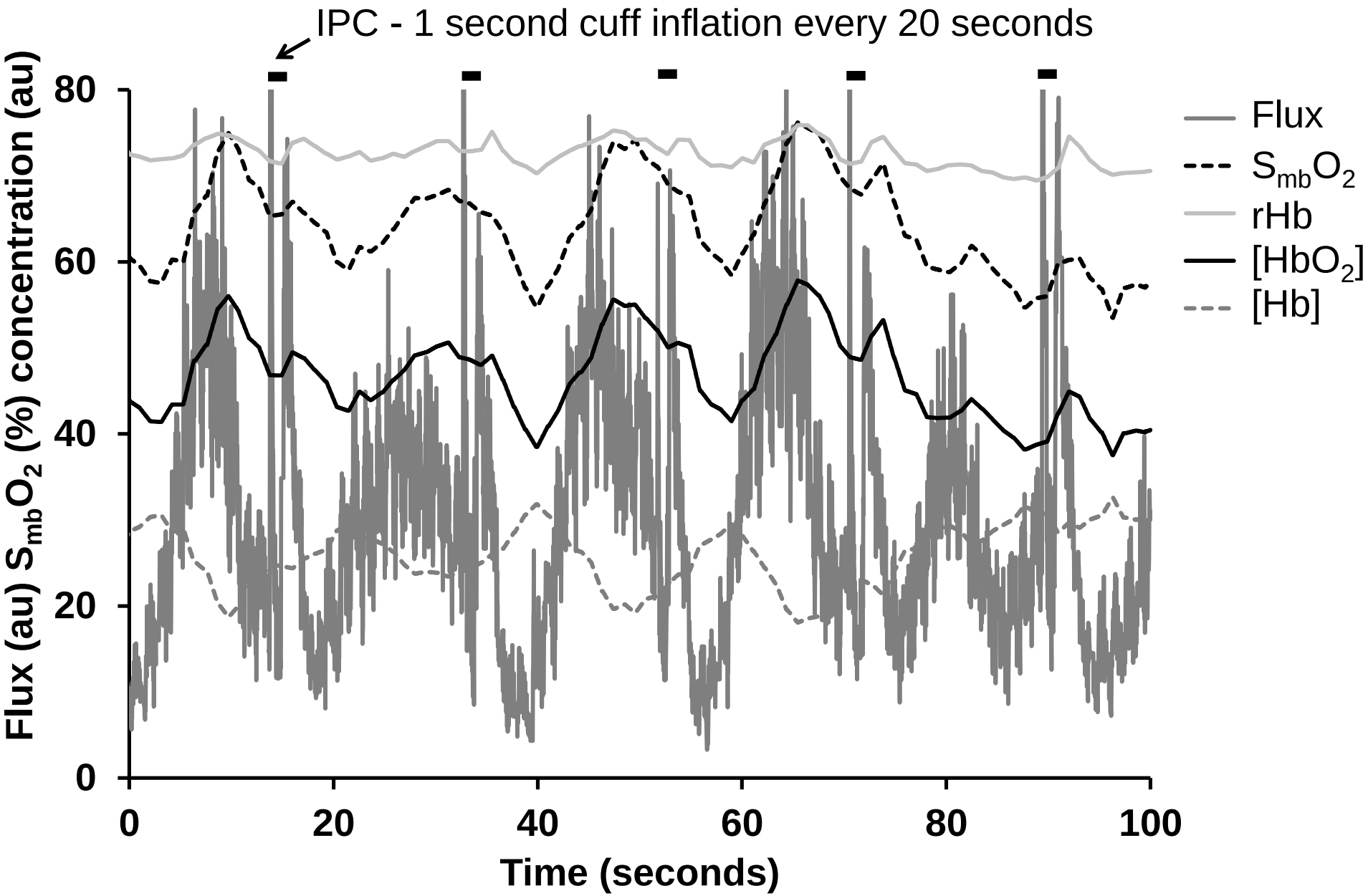


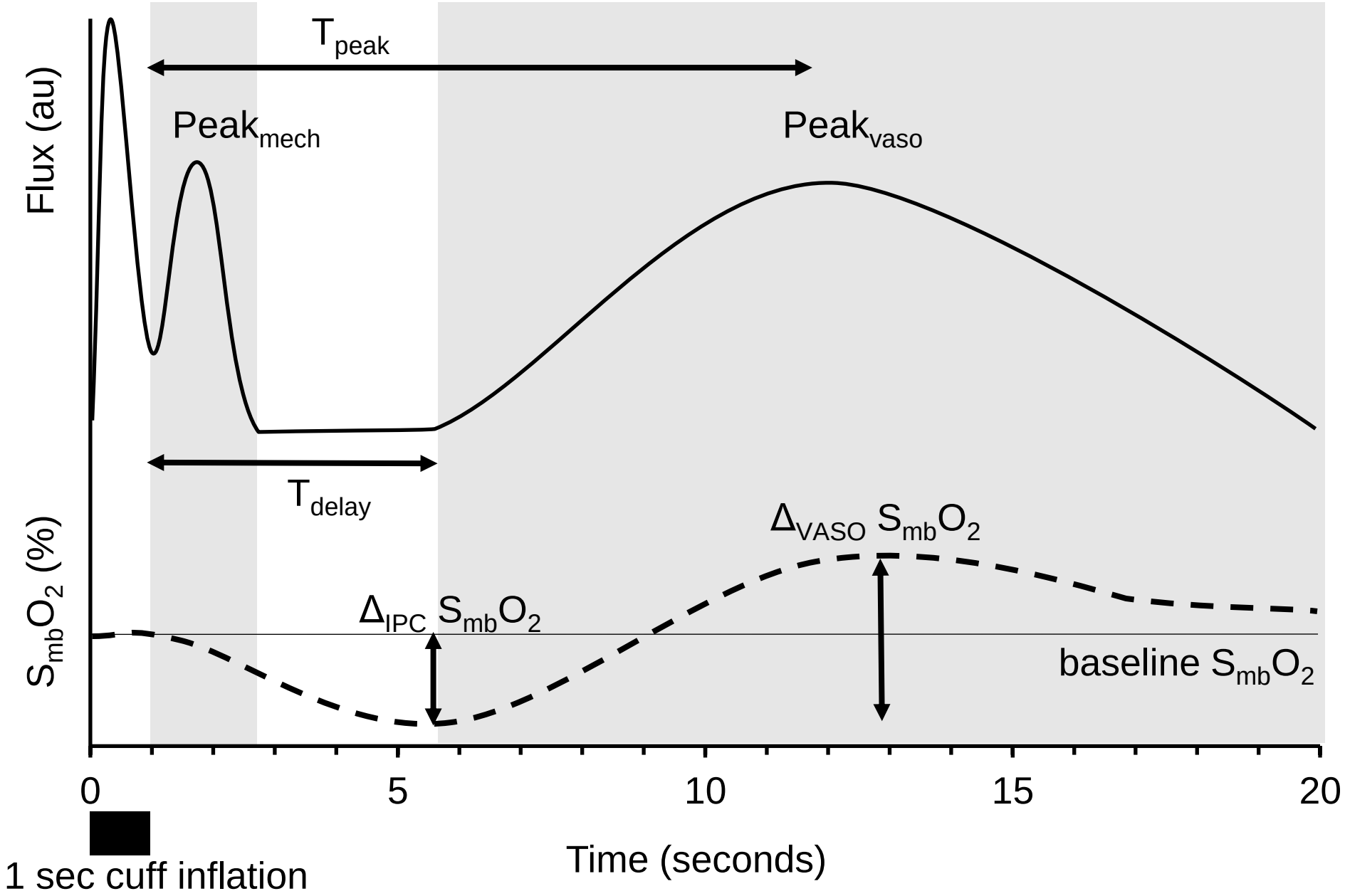


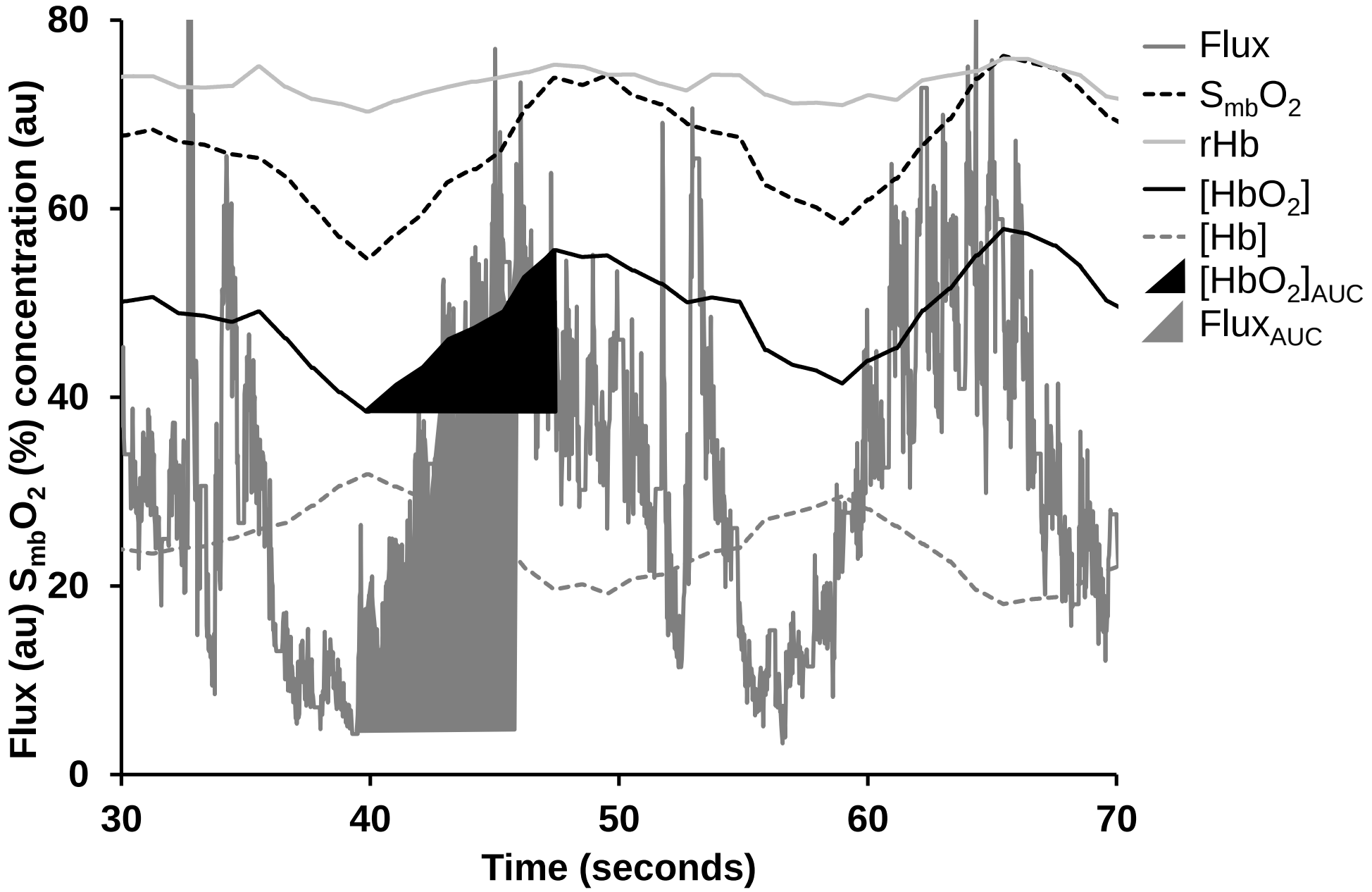


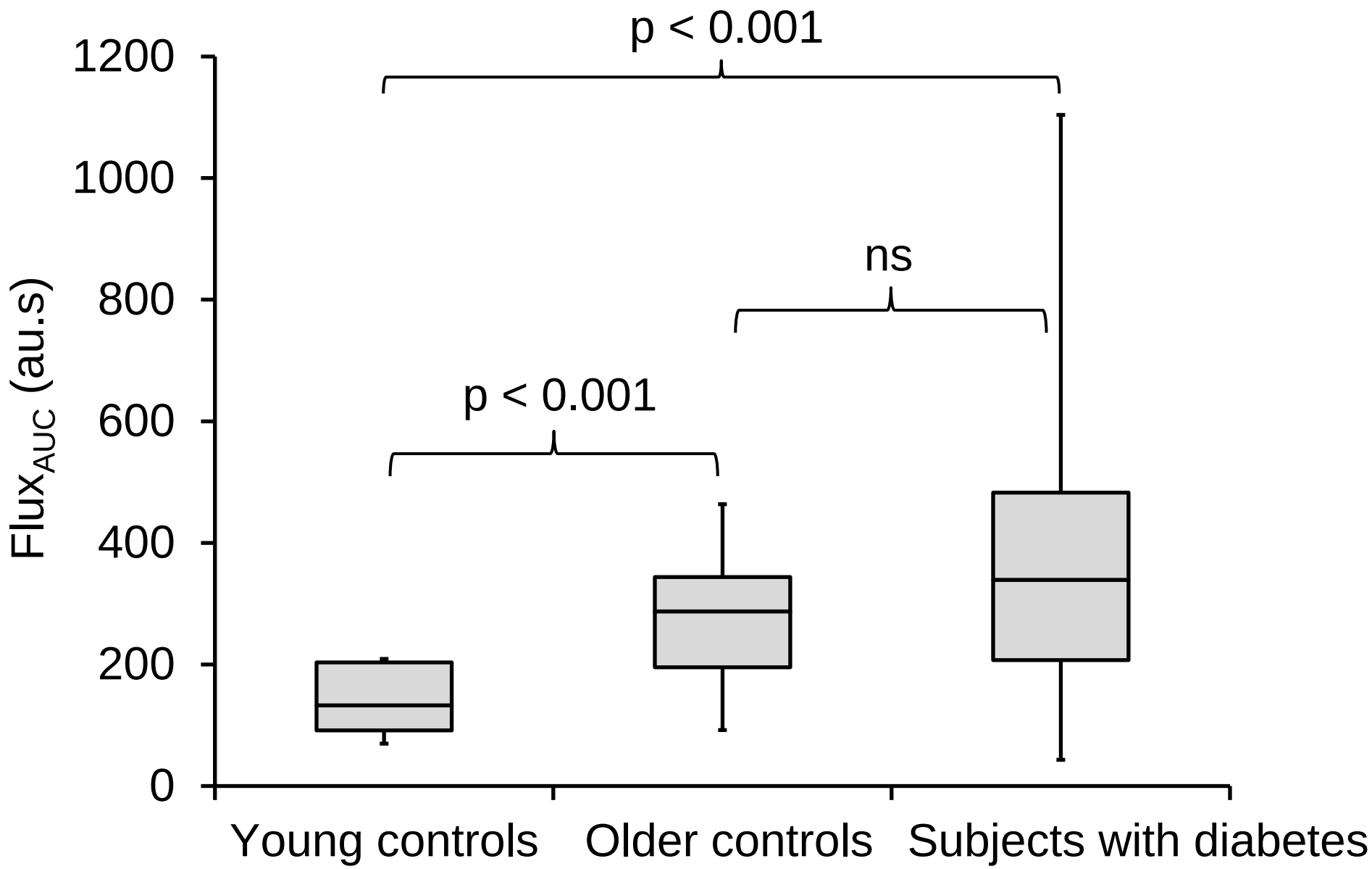












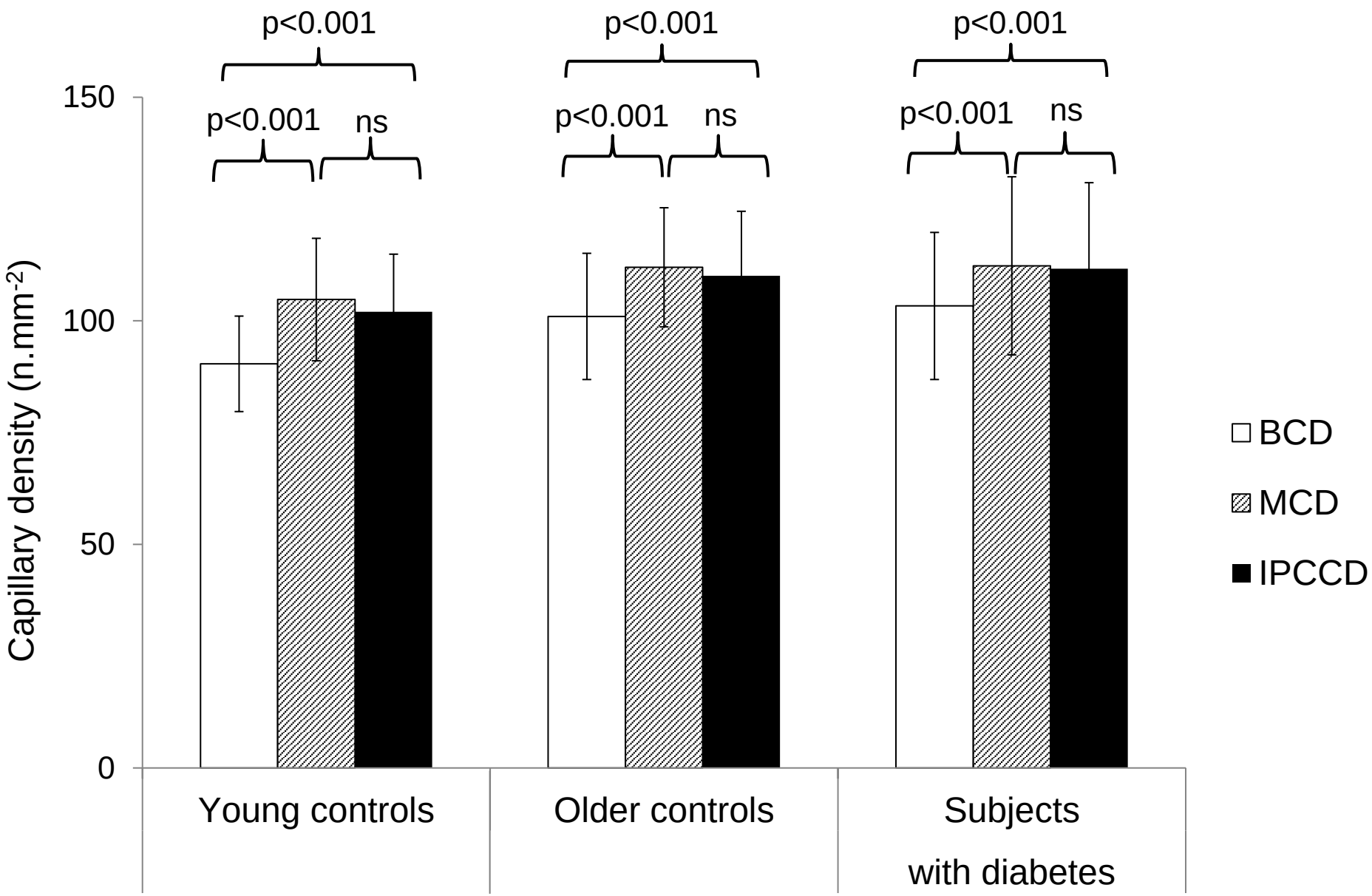


Table 1. *Anthropometric characteristics, skin baseline capillary density and medication of young and older controls and older subjects with type 2 diabetes*

Characteristic	Control 18 – 39 years n = 15	Control 40 – 80 years n = 39	Diabetic 40 - 80 years n = 32	
Age, years	28.9 ± 7.2	57.7 ± 9.7	65.8 ± 6.7	$p<0.001^C$ $p<0.001^D$
Sex, M/F	5/10	18/21	16/16	
BMI, kgm^{-2}	24.3 ± 3.3	26.6 ± 4.5	29.3 ± 4.2	$p=0.07^C$ $p<0.01^D$
Body fat, %	26.8 ± 5.8	30.5 ± 8.6	34.8 ± 7.7	$p=0.07^C$ $p<0.03^D$
SBP, mmHg	114.2 ± 13.1	125.3 ± 12.5	131.1 ± 14.6	$p<0.005^C$ $p=0.07^D$
DBP, mmHg	67.9 ± 10.7	75.4 ± 9.3	73.8 ± 7.7	$p<0.004^C$ $p=0.41^D$
HbA1c, $mmolmol^{-1}$	34.1 ± 5.9	38.2 ± 5.4	54.4 ± 8.4	$p<0.05^C$ $p<0.001^D$
eGFR, $mlmin^{-1}1.73m^{-2}$	103.9 ± 23.1	80.6 ± 14.3	81.5 ± 15.6	$p=0.07^C$ $p=0.93^D$
ABPI (left leg)	1.24 ± 0.15	1.31 ± 0.11	1.34 ± 0.12	$p=0.06^C$ $p=0.37^D$
(right leg)	1.27 ± 0.22	1.33 ± 0.13	1.29 ± 0.12	$p=0.23^C$ $p=0.16^D$
BCD, $n.mm^{-2}$	90.4 ± 10.7	101.0 ± 14.1	103.3 ± 16.4	$p<0.03^C$ $p=0.52^D$
ACE/ARB, %	0	0	53.1	
CaA, %	0	0	21.9	
Diuretics, %	0	0	21.9	
Alpha Blockers, %	0	0	3.1	
Beta Blockers, %	0	0	6.3	

Subject group characteristics: mean ± SD. ^{C} difference between control groups; ^{D} difference between older controls and older subjects with diabetes (aged 40 – 80 years) unpaired t-test. BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, HbA1C glycated hemoglobin, eGFR estimated glomerular filtration rate calculated from serum creatinine level, ABPI ankle brachial pressure index, BCD baseline capillary density and subject medication: ACE/ARB ACE inhibitors/angiotensin receptor blockers, CaA calcium channel blockers.

Table 2. *Baseline data from the dorsal middle index finger skin of microvascular flux, volume and oxygenation of blood and skin temperature*

Baseline	Control 18 – 40 years n = 15	Control 40 – 80 years n = 39	Diabetic 40 - 80 years n = 32	
Finger skin flux, au	30.0 ± 23.4	41.8 ± 30.1	66.6 ± 39.6	$p=0.17^{C}$ $p<0.004^{D}$
Finger skin mean RBC velocity, au	16.6 ± 6.5	17.8 ± 6.5	23.7 ± 9.9	$p=0.61^{C}$ $p<0.004^{D}$
Finger skin $S_{mb}O_2$, %	62.7 ± 9.3	62.3 ± 11.7	64.4 ± 12.6	$p=0.91^{C}$ $p=0.48^{D}$
Finger skin blood volume rHB, au	61.1 ± 7.8	59.4 ± 7.7	59.0 ± 8.4	$p=0.46^{C}$ $p=0.84^{D}$
Dorsal finger temp, ^{0}C	30.3 ±3.9	30.8 ± 3.5	32.2 ± 2.4	$p=0.63^{C}$ $p=0.09^{D}$
Dorsal forearm temp, ^{0}C	31.2 ± 1.0	31.7 ± 1.1	31.9 ± 1.3	$p=0.16^{C}$ $p=0.23^{D}$

Baseline data (mean ± SD) ^{C} difference between young and older control groups; ^{D} difference between older controls and older subjects with diabetes (aged 40 – 80 years) unpaired t=test. Skin flux and mean red blood cell (RBC) velocity measured by laser Doppler fluximetry and mean blood saturation $S_{mb}O_2$ and blood volume rHb by reflectance spectroscopy.

Table 3. *Hemodynamic response in the microcirculation of dorsal middle finger skin to intermittent pneumatic compressions to the palm of the hand*

	Control 18 – 40 years n = 15	Control 40 – 80 years n = 39	Diabetic 40 - 80 years n = 32	
Flux T_{delay}, s	3.5 ± 1.0	4.5 ± 2.2	4.3 ± 1.4	$p=0.50^{C}$ $p=0.26^{D}$
Flux T_{peak}, s	11.2 ± 2.1	11.5 ± 3.1	11.1 ± 3.0	$p=0.50^{C}$ $p=0.51^{D}$
$Flux_{AUC}$, au.s	140.5 ± 54.5	278.9 ± 98.0	371.5 ± 264.5	$p<0.001^{C}$ $p=0.13^{D}$
$[HbO_2]_{AUC}$, au.s	58.26 ± 18.7	53.0 ± 19.8	50.3 ± 15.2	$p=0.45^{C}$ $p=0.60^{D}$
$\Delta_{VASO}S_{mb}O_{2,}$ %	9.9 ± 5.0	9.6 ± 3.5	8.1 ± 3.6	$p=0.82^{C}$ $p=0.20^{D}$
$\Delta_{IPC}S_{mb}O_2$, %	4.2 ± 8.9	4.5 ±5.2	4.2 ± 5.2	$p=0.28^{C}$ $p=0.25^{D}$

Data (mean ± SD) ^{C} difference between young (aged 18 – 39 years) and older control groups; ^{D} difference between older controls and older subjects with diabetes (aged 40 – 80 years). Hemodynamic response in the microcirculation of dorsal middle finger skin to individual intermittent pneumatic compressions to the palm of the hand to 130 mmHg (inflation within < 0.5 seconds) for 1 second periods at 20 second intervals averaged over 5 compression cycles. After each cuff deflation there was a delay of T_{delay} (s) during which mean oxygenation saturation fell by $\Delta_{IPC}S_{mb}O_2$ before a surge in flux ($flux_{AUC,}$) to a peak at time T_{peak}. This increase in flux was synchronous with an increase in mean oxygenation saturation $\Delta_{IPC}S_{mb}O_2$ due to a rise in concentration of oxyhemoglobin ($[HbO_2]_{AUC}$ and an equal and opposite fall in concentration of deoxyhemoglobin [Hb] measured by reflectance spectroscopy in the skin microcirculation.

Table 4. *Effect of 30 minutes of Intermittent Pneumatic Compression to the palm of the hand on dorsal finger skin flux and oxygenation*

	Controls 18 – 40 years n = 15		Controls 40 – 80 years n = 39		Subjects with diabetes 40 - 80 years n = 32		
	Pre IPC	Post IPC	Pre IPC	Post IPC	Pre IPC	Post IPC	Post IPC
Finger skin flux, au	30.0±23.4	23.6±16.8	41.8±30.1	39.1±31.6	66.6±39.6	61.7±27.8	$p=0.08^{C}$ $p=0.003^{D}$
Finger skin mean RBC velocity, au	16.6 ± 6.5	15.4±6.6	17.8 ± 6.5	17.5±6.4	23.7 ± 9.9	22.6±8.2	$p=0.30^{C}$ $p=0.005^{D}$
Finger skin $S_{mb}O_2$, %	63.0±9.5	59.0±13.5	62.8±11.4	62.6±11.8	64.1±12.8	64.3±13.3	$p=0.34^{C}$ $p=0.56^{D}$
Finger skin rHb, au	60.4±7.5	61.9±7.6	58.7±6.5	59.1±9.1	58.7±8.4	58.1±9.8	$p=0.30^{C}$ $p=0.64^{D}$
Finger temp, ^{0}C	30.3±3.9	30.1±3.6	30.8±3.5	30.7±3.5	32.2±2.4	32.1±2.4	$p=0.95^{C}$ $p=0.07^{D}$

Pre and Post 30 minutes of intermittent pneumatic compression (Mean ± SD), no significant difference in all parameters between pre and post IPC for all subject group. ^{C}significant difference between younger and older controlspost IPC; ^{D}significant difference between older controls and subjects with diabetes (aged 40 – 80 years) post IPC, unpaired t-test. Skin flux and RBC velocity (laser Doppler fluximetry), mean blood saturation $S_{mb}O_2$ and blood volume rHb (reflectance spectroscopy), dorsal finger skin temperature.

Table 5. *Comparison of the effect of intermittent pneumatic compression and a 50 mmHg venous cuff on the increase in capillary density in dorsal finger skin*

	Increase in capillary density from baseline with:				
	50 mmHg venous cuff		30 mins IPC		Venous occlusion vs IPC
	MCD – BCD ($n.mm^{-2}$)	Capillary recruitment (%)	IPCCD – BCD ($n.mm^{-2}$)	Capillary recruitment (%)	
Control 18–39 years n=10	12.4 ± 6.1	13.5±6.0	12.8 ± 7.6	13.2±8.6	ns, ns
Control 40–80 years n=39	10.3 ± 6.3	10.6±7.0	9.2 ± 5.4	9.2±6.0	0.03, ns
Subjects diabetes 40–80 years n=32	9.0 ± 6.3	8.5±5.6*	8.4 ± 5.8	8.0±5.6*	ns, ns

Baseline capillary density (BCD) maximum capillary density (MCD) obtained with a 5 minute 50 mmHg venous cuff to the base of the finger and post 30 minutes of IPC capillary density (IPCCD) in index finger skin. (Paired Student's t-test). %Capillary recruitment = (MCD - BCD)/BCD or (IPCCD – BCD)/BCD as a percentage. There was no significant difference between the three groups in all parameters except that %recruitment by both venous occlusion and IPC were lower in older subjects with diabetes compared to young controls (*$p<0.03$).