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Lower Extremity Amputation and Leg Blood Flow

Time-course of thigh muscle contraction-induced blood flow magnitude in amputated lower limb with prosthesis during dynamic knee extensions: A case study

Abstract

Background: The increase in exercising leg blood flow (LBF) is directly proportional to the workload performed, in relation to the interplay between cardiovascular regulation and muscle energy metabolism. However, little information is available on exercise LBF in thigh muscle contractions in amputated lower legs (ALL) via the stump fitting prosthesis.

Case presentation: A 79-year-old male patient performed 3-min one-legged repeat/dynamic knee extensor exercise at a target muscle contraction frequency (1-s thigh muscle contraction and 1-s relaxation, 90 repetitions) on each leg (left ALL as trans-tibial amputation using a patellar tendon-bearing prosthesis and right non-ALL) at six different contraction intensities. Simultaneous measurement of blood velocity/flow (Doppler ultrasound) in the femoral artery, blood pressure, leg vascular conductance and muscle strength were performed at pre-exercise, during 3-min exercise and 5-min recovery. There was a 12% reduction in the maximum thigh circumference in the ALL compared to non-ALL. LBF was lower in the ALL than non-ALL at pre-exercise and during exercise. In non-ALL, there was a good positive linear relationship (r=0.922, P<0.01) between mean peak muscle strength and mean exercising LBF during 30-s steady-state before the end of exercise, but the same relationship was not seen (r=0.220, P=ns) in the ALL.

Conclusions: The present clinical intervention study showed no significant thigh LBF increase in the ALL with incremental workload (peak muscle strength). This might be due to a mismatch of the workload-dependent LBF increase in the disused and/or atrophic thigh muscle-resection stump of the ALL compared to non-ALL, which in ALL may be due to remaining muscle contractile effort with a reduced arterial inflow and/or lack of venous return in relation to the arteriovenous pressure gradient and/or hydrostatic pressure through the lower leg.

Keywords: Exercising blood flow, knee amputation, dynamic knee extensor exercise, prosthesis, Doppler ultrasound

Background

It is important to prevent loss of daily activity following lower leg amputation [1-3]. In general, exercise therapists commonly recommend resistance training for subjects with amputation below the knee. Exercise therapy may include repeated hip/knee extensor-flexor motion [4]. Such habitual exercise training may promote walking ability by increasing thigh muscle strength [5,6].

In exercise physiology, the stimulation of muscle blood flow due to physical activity may have a major role in oxygen transport for the muscle metabolism in the limb, which is closely related to exercise tolerance as systemic maximum oxygen uptake and/or muscle strength power. During leg exercise, increased leg oxygen uptake is directly proportional to the work performed [7,8]. The leg oxygen uptake is calculated as the product of "working leg arterial blood flow" and the arteriovenous oxygen difference to the exercising leg.

Thus, the determination of working leg blood flow (LBF) dynamics feeding the contracting muscles to the incremental exercise workload can contribute to understanding of the muscle blood flow supply and vasodilation due to exercise as circulatory factors limiting work capacity in not only the non-amputated leg [9-12], but also the thigh stump of the amputated leg. Furthermore, evaluation of thigh muscle stump contraction-induced peripheral circulatory adjustment may potentially be required for exercise prescription such as muscle contraction intensity and/or frequency.

However, there have been no reported investigations of the magnitude of temporal beat-to-beat LBF dependence during incremental thigh muscle-stump contraction strength/workload in the amputated lower leg from onset to steady-state of exercise. This may be due to the difficulty of stable LBF measurement and its evaluation during constant voluntary repeated/rhythmic muscle contractions of the thigh-stump at a target workload. In addition, it is difficult to compare the magnitude of LBF between the amputated and non-amputated leg during unilateral leg exercise because of dissimilarity in the exercise model without prosthesis due to the loss of function in lower leg muscle mass, limitation of range of motion in the knee joint and/or stable load-setting for the thigh muscle strength.

Ultrasound Doppler devices can provide high temporal resolution measurement of blood velocity. Pulsatile blood velocity profile in the conduit artery at systole and diastole may be detected at rest, synchronized with the heart beat and blood pressure [13-15]. Based on this technique, rapid changes in the blood velocity profiles in the conduit artery can be measured with muscle contraction and relaxation and/or beat-to-beat in different states of exercise, muscle contraction time/frequency and workload, and in relation to vasodilatation/vasoconstriction.

Following our previous reports for healthy legs with muscle blood flow regulation during leg exercise using Doppler ultrasound, a positive linear correlation between unilateral whole LBF and muscle workload has been observed during steady-state repeated rhythmic unilateral leg exercise [9,11,12,16,17]. However, in ALL there is still a lack of information regarding the thigh muscle contraction-induced LBF response. Particularly, during knee extensions, the workload-dependent magnitude in the thigh muscle-resection stump blood flow should initially be investigated because of the lack of circulatory effect due to the absence of the lower leg as well as potential low muscle strength.

Using the above-mentioned Doppler technique, the present clinical trial as a case study attempted to measure exercising limb circulatory response in amputated and non-amputated lower legs (ALL and non-ALL) in order to understand 1) whether the time course in LBF can be determined during repeated/dynamic knee extensor exercise of the thigh muscle-resection stump with prosthesis compared to the non-amputated leg, 2) whether there is a relationship between LBF and muscle contraction strength in not only non-ALL but also ALL, and 3) how other hemodynamic parameters such as blood pressure and heart rate may be altered.

Case presentation

Participant

A male (79 yr 11 mo, 158 cm, 54 kg) with trans-tibial amputation of the left lower leg due to diabetic foot gangrene (at age 65 yr 1 mo) and with amputation of the right second and third toes (at 73 yr 9 mo) participated in the study. The participant had past history of coronary artery bypass graft due to asymptomatic myocardial ischemia (at 78 yr 5 mo), and right total knee arthroplasty (at 78 yr 8 mo) due to right knee pyogenic arthritis. The right ankle-brachial index was 1.13. The length of the resection-stump was 17.5 cm from the knee joint space to the stump-end, which supported walking by using a patella tendon-bearing (PTB) prosthesis and crutch. The weight of the PTB was 1.4 kg. The range of knee angle motion in the ALL was maintained for activity of daily life using the PTB. The circumference of the thigh was 44.0 cm at maximum, 39.0 cm at 10 cm above the patella and 36.0 cm at 5 cm above the patella in non-ALL, and 38.6 cm at maximum, 33.0 cm at 10 cm above the patella and 30.5 cm at 5 cm above the patella in ALL. The length between the greater trochanter and the knee joint space was 40 cm in both legs. The lower leg length was 38 cm in non-ALL. His cardiovascular condition and diabetic mellitus were well controlled. The study was conducted according to the principles of the Declaration of Helsinki (1964) and with approval of the Institutional Ethics Committee of the authors' institution (approval No. 2016-080). The participant gave written consent and was informed of the nature and purpose of the study and for further publication, as well as potential risks and discomfort. The participant was informed that withdrawal from the study was possible at any time without consequences.

Dynamic knee extensor exercise

Previous studies have reported exercising LBF measured by the invasive thermodilution technique, in relation to one-legged, dynamic knee-extensor exercise [7,8]. The present exercise model allows stable measurements of blood velocity in the femoral artery using Doppler ultrasound [9-12]. Thermodilution measurements obtained under similar experimental conditions by Andersen *et al.* [7] were similar to those obtained by Doppler ultrasound [9]. The muscle contraction is confined to the quadriceps muscle group, which represents the activation of the large thigh muscle group (**Figure 1**). The knee extensor model to determine muscle blood flow uses only the knee extensors of the thigh quadriceps muscle group. Previous research on LBF in relation to dynamic knee extensions has contributed to investigations of the magnitude of thigh muscle contraction-induced blood flow increase [10-14,18].

Experimental protocol

Prior to exercise, the maximum voluntary contraction (MVC) in the isometric knee extensor was measured using a strain-gauge (see the section on MVC measurement). The participant's thigh was positioned horizontally, with the knee joint bent (90 degree flexion) in a sitting position.

Before the exercise test, the participant was familiarized with the exercise to maintain the target knee joint angle for repeated dynamic knee extensions. Following 1 min of pre-exercise, the participant performed 3-min one-legged repeat/dynamic knee extensor exercise at the target muscle contraction frequency <1-s thigh muscle contraction (active knee extension) and 1-s relaxation (passive knee flexion)>, for a total of 90 repetitions on each leg (left ALL using the PTB or right non-ALL) at six different contraction strengths (exercise intensities) using a rubber resistance band in the sitting position (**Figure 1**). There was a 5-min recovery phase after the end of each exercise session.

The muscle contraction interval (1 s on/1 s off) during 3-min exercise was maintained by following an audible metronome every 2 s for the muscle contraction and muscle relaxation-phases (90 duty kicking cycles). The participant attempted to kick with his toe a target point corresponding to 60 degree flexion from 90 degree flexion following the pace of the metronome at the target muscle contraction frequency (**Figure 1**). The required range of motion in the knee angle was only 30 degrees, which may be appropriate for stable repeated knee extensor exercise with PTB. The absolute value for muscle contraction strength due to knee extensor exercise was displayed in real time on a monitor connected to the strain-gauge and amplifier.

The exercise intensity and required muscle contraction strength was adjusted using thin, medium, heavy, extra heavy, special heavy, and super heavy rubber bands (see the section on rubber bands). The recovery time was sufficient for the hemodynamic parameters to return to resting control levels between exercise sessions in the 45-min experimental period. The parameters (blood velocity, blood pressure, heart rate and voluntary muscle contraction strength) were simultaneously recorded at 1-min pre-exercise, during 3-min exercise and 5-min recovery (**Figure 1**). Steady-state during exercise was defined from 150 s to 180 s (76th- 90th duty kicking cycles) for the evaluation of workload-dependent of LBF.

Rubber bands for muscle contraction strength (exercise intensity)

Six different TheraBands® were used to manage the muscle contraction strength (exercise intensity): thin (yellow, 1.3 kg), medium (red, 1.7 kg), heavy (green, 2.1 kg), extra heavy (blue, 2.6 kg), special heavy (black, 3.3 kg), and super heavy (silver, 4.6 kg), which values formally represent the degree of strength required to stretch the rubber band 30 cm to 60 cm in the Thera-Band® product catalogue [19]. Validation of the stiffness of each band was previously reported by the manufacturer [20-22].

We evaluated the accuracy of resistance of the rubber bands by stretching each band with an applied weight. When an iron weight (1 kg) was hung on each rubber band tied in a loop (total length, 60 cm) connected to a strain-gauge, the changes (delta) in length of the rubber bands were 10 cm for thin (yellow), 8 cm for medium (red), 6 cm for heavy (green), 5 cm for extra heavy (blue), 3 cm for special heavy (black) and 1 cm for super heavy (silver). These changes elongation were inversely and linearly related ($r=0.970$, $P=0.0013$, $n=6$) to the resistance (in kg) at 100% of elongation in the previously published product information [19,20].

It may not be possible for a subject to consistently demonstrate a specific target strength due to variations in the kicking duty cycle influencing in the mean/peak muscle contraction strength when using a rubber band. Therefore, the precise value of muscle contraction strength was evaluated by using a strain-gauge and amplifier.

The rubber band was tied in a loop, enclosing the ankle, with a sensor fixed in the chairstrut connected to the strain-gauge with amplifier (Meiko Co. Ltd, Tokyo, Japan), and values were continuously recorded on a computer using a PowerLab data acquisition system (**Figure 1**).

Measurements

Maximum voluntary contraction (MVC)

Prior to the experiment, the MVC was measured as the maximum muscle contraction strength throughout a single (one-legged) knee extensor isometric muscle contraction of each leg with the subject's thigh positioned horizontally and the knee joint bent (90 degree flexion) in the sitting position, following the previously validated procedure [18]. The MVC (in kg) was determined from the average of five repeated measures using a strain-gauge connected to a strain amplifier and gauge meter (Meiko Co. Ltd, Tokyo, Japan) and was continuously recorded on a computer using a PowerLab data acquisition system (Chart v.4.2.3 software; ADInstruments, Sydney, Australia) (**Figure 1**). The MVC was defined as the peak value of maximum voluntary contraction on the strength curve. The peak muscle strength during one-legged repeat/dynamic knee extensor exercise was also evaluated by the relative (percentage of) MVC (peak muscle strength/MVC×100, %) at six different exercise intensities, i.e., with the six kinds of rubber band. Beforehand, the accuracy of the strain-gauge was tested by repeated measures. The coefficient of variation for repeated measures using a 1 kg iron weight was 0.47% (range: 1.01-0.99 kg).

Blood velocity and diameter in the femoral artery

The measurement of blood velocity in the femoral artery feeding the active thigh muscles, using ultrasound where LBF is determined by the product of blood velocity and cross-sectional area, has been validated and shown to produce accurate absolute values both at rest and during incremental leg exercise such as rhythmical/dynamic thigh muscle contractions [9-15,18]. The high temporal resolution of Doppler ultrasound enables continuous measurement of blood velocity (a time- and space-averaged and amplitude-weighted" mean blood velocity") throughout the kicking cycle during one-legged dynamic knee extensor exercise [9-15,18,23,24].

The beat-to-beat blood velocity profile (fast Fourier transformation) and its mean value (the average of the separate variables calculated automatically on a beat-by-beat basis for each cardiac cycle) in the femoral artery was continuously measured by 7.5 MHz pulsed Doppler ultrasound (GE Logiq 3, Tokyo, Japan) with a videotape recorder (AG-7350-P, Panasonic, Tokyo, Japan). Any noise or interrupted blood velocity profile data was automatically omitted for calculation of the mean value. The minimum value of the coefficient of variation (<5%) for the repeated blood velocity (including individual physiological variations) measurements represented the criteria for quality control of the operator's technique (first author) at pre-exercise as well as during exercise [10-14,18,24-30].

The diameter of the target femoral artery is not affected by the muscle contractions and relaxation at this site, which is located proximal to the thigh muscle. The femoral arterial diameter was measured in the pre-exercise state under perpendicular insonation (longitudinal two-dimensional mode). This location minimizes turbulence from the femoral bifurcation and the influence of blood velocity from the inguinal region.

The probe position was stabilized (<60°) by the operator's hand throughout pre-exercise, exercise and recovery, and was precisely positioned by the first author in the center of the vessel and adjusted to cover the diameter of the vessel in the femoral artery above the bifurcation into the branches of the superficial and deep femoral arteries [10-14,18,24-30].

The mean vessel diameter (the distance between proximal and distal intima in the artery) at the pulsatile diastolic phase for each beat was calculated over approximately five beats. The best theoretical axial resolution corresponds to ~0.1 mm, i.e., one-half of the spatial wavelength [$\lambda = c/(f)$, where c is the velocity of sound in soft tissue (1540 m/s) and f is the imaging frequency (7.5 MHz)].

The value of the vessel diameter at pre-exercise was used to calculate the femoral arterial LBF at pre-exercise, during one-legged repeated dynamic knee extensions, and during recovery, since the diameter does not significantly vary between pre-exercise and knee extensor exercise [1,17,25,31,32].

Blood pressure, heart rate and peak muscle contraction strength

Blood pressure and heart rate were simultaneously measured using an auricular plethysmography device with oscillometric calibration, through a cuff tourniquet placed on the upper right arm (RadiaPress RBP-100, KANDS, Aichi, Japan).

These values and the muscle contraction strength curve (muscle contraction - relaxation phase) from the stretched rubber band with strain-gauge connection, strain amplifier and gauge meter (Meiko Co. Ltd, Tokyo, Japan) were continuously recorded on a computer using a PowerLab data acquisition system (Chart v.4.2.3 software; ADInstruments, Sydney, Australia) at 1-min pre-exercise, during the 3-min exercise and for 5-min recovery (**Figure 1**). The mean values of blood pressure and heart rate (defined as R-R interval of blood pressure curve) were extracted at the same time as the determinants of beat-by-beat blood velocity value. The peak muscle contraction strength was defined as the peak value at maximum amplitude of the muscle contraction strength curve (muscle contraction-relaxation cycle) during active knee extensor kicking via the acquisition system.

LBF and leg vascular conductance (LVC)

The time- and space-averaged and amplitude-weighted "mean blood velocity" in the femoral artery was determined by automatically averaging the separate variables on a beat-by-beat basis for each cardiac cycle. LBF in the femoral artery was calculated by multiplying the cross-sectional area [area= $\pi \times$(pre-exercise vessel diameter/2)^2] by mean the blood velocity at pre-exercise, during exercise and recovery. The LVC was calculated as LBF divided by blood pressure (LBF/blood pressure) using the unit ml/min/mmHg.

Evaluations and statistics

Mean values of LBF, blood velocity, blood pressure, LVC and heart rate were determined as the average of every 30 s from the time of the start of exercise (t=0 in figures), at pre-exercise, during 3-min exercise and during 5-min recovery. The mean LBF, LVC and peak muscle contraction strength at steady-state for the 30-s period before the end of exercise were evaluated. Statistical comparisons with a linear-curve fitting regression correlation coefficient (r), and P value were conducted between mean LBF and mean LVC, and the mean peak muscle contraction strength (%MVC) was examined (Microsoft Excel 2010). A P value <0.05 was considered significant. All values are mean ± standard deviation (SD).

Results

There was a 12% reduction in maximum thigh circumference in the ALL compared to non-ALL. Repeated measures of MVC were12.8±0.9 kg in non-ALL and 9.3±0.1 kg in ALL. The diameter of the femoral artery was 8.12±0.21 mm in non-ALL and 7.16±0.23 mm in ALL. The LBF values at pre-exercise were 379±62 ml/min in non-ALL and 234±46 ml/min in ALL. The steady-state for 30 s before the end of exercise indicated a stable peak muscle strength as well as the times for the muscle contraction-relaxation cycles in **Table 1**. During exercise, the peak muscle contraction strength at each kick linearly declined from onset to the end of exercise (P<0.0001, solid line and r value: yellow, 0.425; red, 0.608; green, 0.696; blue, 0.717; black, 0.675; silver, 0.946 for non-ALL and red, 0.742; green, 0.719; blue, 0.799; black, 0.967; silver, 0.926 for ALL), except for low intensity (yellow band) exercise in ALL in **Figure 2**. Simultaneous recording of all hemodynamic parameters (heart rate, blood pressure, LVC, femoral artery blood velocity and LBF) are shown for pre-exercise, during exercise and recovery in **Figure 3** (high time resolution data at heavy intensity) and

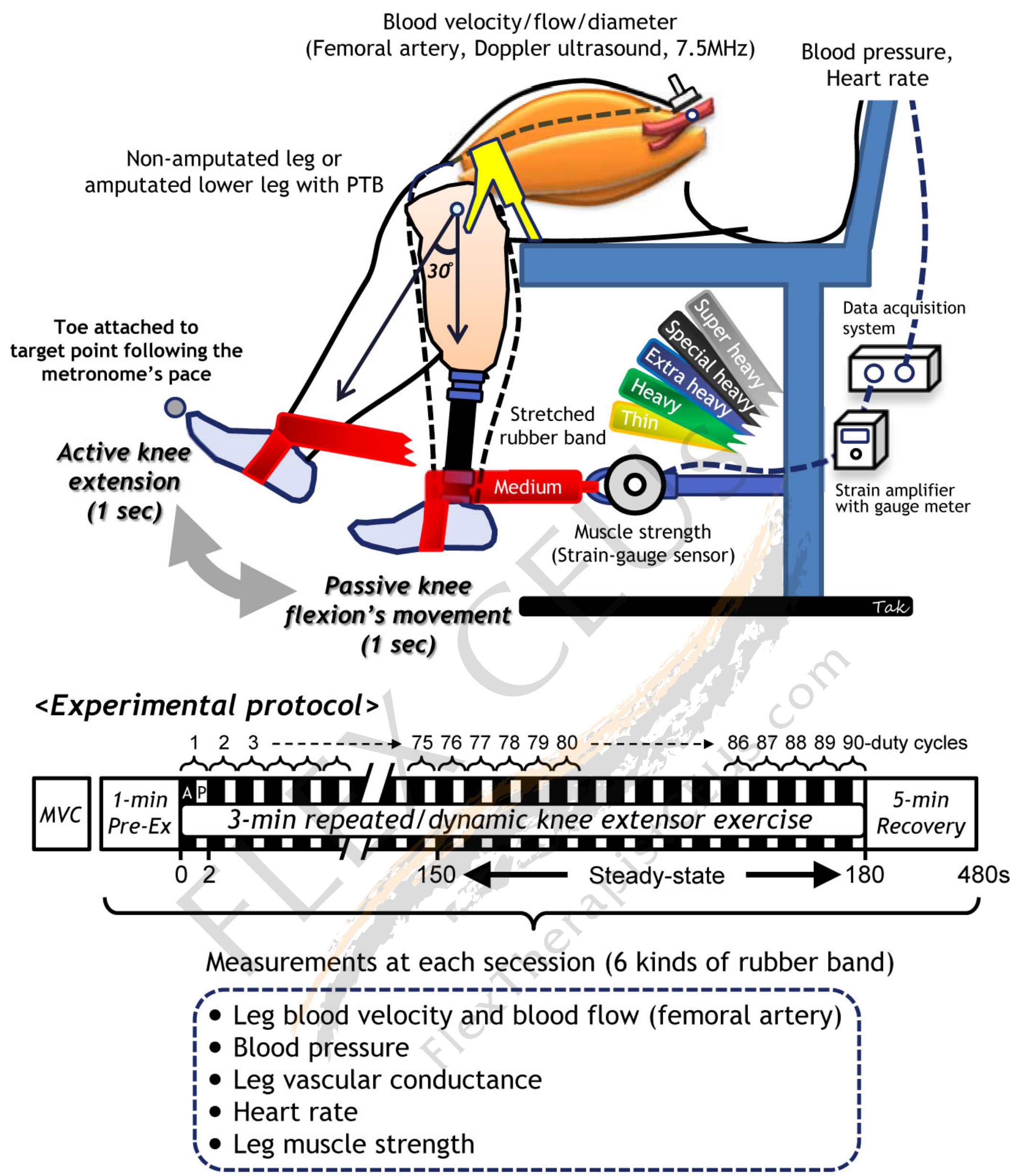

Figure 1. One-legged dynamic knee extensor exercise and experimental protocol.
Prior to the experiment, maximum voluntary contraction (MVC) was measured. Following 1-min pre-exercise (Pre-Ex), a subject performed unilateral repeated/dynamic knee extensor exercise [1-s dynamic-active thigh muscle contraction (knee extension) and 1-s passive muscle relaxation (passive flexion movement): 90-duty cycles] for 3 min using six different rubber resistance bands on each leg. The left amputated lower leg performed the exercise using a patella tendon brace (PTB) prosthesis. Kicking with the toe was directed at a target point corresponding to the 60-degree flexion knee joint angle from 90-degree flexion (knee angle motion: 30 degrees) in time with the pace of an audible metronome at the target muscle contraction frequency. There was a 5-min recovery phase after the end of each exercise session. Blood velocity in the femoral artery (Doppler ultrasound) was continuously measured at pre-exercise, during exercise, and during recovery. Blood flow was calculated as the product of cross-sectional area and blood velocity. Simultaneous recording of muscle strength (strain-gauge sensor), blood pressure, leg vascular conductance (blood flow/blood pressure) and heart rate was also performed via the data acquisition system. A: active knee extension, P: passive flexion movement.

Table 1. Duration of muscle contraction cycle, peak muscle strength and time to peak muscle strength.

a) 3-min whole exercise

Strength	1-contraction cycle duration (ms) / CV (%) Non-ALL	ALL	Peak muscle strength (kg) [%MVC] / CV (%) Non-ALL	ALL	Time to peak muscle strength (ms) / CV (%) Non-ALL	ALL
Thin	1983 ± 171	2000 ± 124	1.78 ± 0.12 [13.86 ± 0.94]	1.76 ± 0.13 [18.82 ± 1.42]	713 ± 122	424 ± 82
	8.6	6.2	6.8	7.6	17.2	19.2
Medium	1994 ± 150	2002 ± 121	2.63 ± 0.20 [20.53 ± 1.55]	3.10 ± 0.26 [33.22 ± 2.83]	448 ± 93	451 ± 76
	7.5	6.1	7.6	8.5	20.7	16.8
Heavy	1998 ± 129	2002 ± 113	2.10 ± 0.10 [16.39 ± 0.79]	2.88 ± 0.16 [30.86 ± 1.73]	426 ± 54	434 ± 72
	6.5	5.7	4.8	5.6	12.6	16.7
Extra heavy	1999 ± 74	1995 ± 124	3.38 ± 0.18 [26.38 ± 1.39]	4.18 ± 0.27 [44.82 ± 2.84]	465 ± 54	414 ± 55
	3.7	6.2	5.3	6.3	11.5	13.4
Special heavy	2001 ± 130	2003 ± 106	4.37 ± 0.24 [34.05 ± 1.88]	3.86 ± 0.69 [41.37 ± 7.39]	435 ± 65	378 ± 56
	6.5	5.3	5.5	17.9	15.0	14.8
Super heavy	1999 ± 118	2002 ± 120	5.06 ± 0.44 [39.42 ± 3.42]	3.95 ± 0.86 [42.37 ± 9.24]	408 ± 50	293 ± 59
	5.9	6.0	8.7	21.8	12.3	20.1

b) 30 s (steady-state) before the end of exercise

Strength	1-contraction cycle duration (ms) / CV (%) Non-ALL	ALL	Peak muscle strength (kg) [%MVC] / CV (%) Non-ALL	ALL	Time to peak muscle strength (ms) / CV (%) Non-ALL	ALL
Thin	1993 ± 95	2010 ± 130	1.74 ± 0.08 [13.58 ± 0.64]	1.69 ± 0.10 [18.12 ± 1.06]	697 ± 115	391 ± 55
	4.8	6.5	4.7	5.8	16.5	14.2
Medium	1988 ± 175	1995 ± 127	2.45 ± 0.10 [19.09 ± 0.80]	2.81 ± 0.17 [30.12 ± 1.82]	458 ± 138	403 ± 57
	8.8	6.4	4.2	6.1	30.1	14.2
Heavy	2001 ± 189	1997 ± 188	1.98 ± 0.07 [15.47 ± 0.57]	2.67 ± 0.12 [28.64 ± 1.31]	455 ± 68	404 ± 87
	9.4	9.4	3.7	4.6	14.9	21.5
Extra heavy	2001 ± 61	2003 ± 87	3.19 ± 0.06 [24.88 ± 0.47]	3.83 ± 0.14 [41.11 ± 1.49]	449 ± 39	417 ± 59
	3.0	4.3	1.9	3.6	8.8	14.1
Special heavy	2003 ± 58	2013 ± 115	4.02 ± 0.09 [31.34 ± 0.74]	2.87 ± 0.16 [30.80 ± 1.67]	461 ± 35	330 ± 68
	2.9	5.7	2.4	5.4	7.5	20.7
Super heavy	1995 ± 102	2007 ± 135	4.51 ± 0.18 (35.18 ± 1.41]	3.00 ± 0.19 [32.21 ± 2.03]	386 ± 50	277 ± 51
	5.1	6.7	4.0	6.3	13.0	18.3

a) 3-min whole exercise, b) 30-s steady-state before the end of exercise. A stable kicking duty cycle as well as peak muscle contraction strength at 30-s steady-state during exercise may be acceptable for the evaluation of thigh muscle contraction induced leg blood flow increase via the relationship between exercise workload and limb blood flow both ALL and non-ALL.Values are expressed as means± standard deviation (SD). ALL: amputated lower leg, Non-ALL: non-amputated lower leg, %MVC: percentage of maximum voluntary contraction, CV: coefficients of variations.

Figure 4 (averaged data at six different exercise intensities).

There was a significant (r=0.922, P<0.01) positive linear relationship between peak muscle contraction strength and LBF at steady-state in non-ALL. However, the same relationship was not seen (r=0.220, P=ns) in ALL (**Figure 5**). There was no positive linear relationship between peak muscle contraction strength and exercising LVC in non-ALL (r=0.672, P=ns) or ALL (r=0.228, P=ns). The relative muscle strength (%MVC) during 30-s steady state was higher in ALL than non-ALL except at the higher exercise intensities (black and silver bands) (**Figures 2** and **5b**). During the recovery phase after the end of exercise, the rate of decline of blood velocity return to the pre-exercise level was slower in ALL compared to non-ALL. The blood velocity was higher in ALL than non-ALL at 90-120 s after the end of exercise, but the rate of decline in LBF during recovery was similar between non-ALL and ALL (**Figure 4**).

Discussion

The present case report is the first investigation of thigh muscle-resection stump contraction-induced exercising LBF magnitude in a subject with lower leg amputation using PTB by Doppler ultrasound. The finding is that a workload-dependent LBF increase was not seen in ALL compared to non-ALL, although peak muscle contractile effort increased with an increase in muscle contraction intensity.

An amputated limb may potentially limit fitness, with lower muscle strength and/or muscle metabolism in ALL compared to the non-ALL [4,33-35]. Further, older adults with lower limb amputations due to vascular disease have a lower aerobic capacity and limited leg oxygen uptake [36]. Leg oxygen up take is mainly estimated by multiplying LBF (calculated by the product of cross-sectional area and blood velocity) and arteriovenous oxygen differences in the leg. Thus, it is specu-

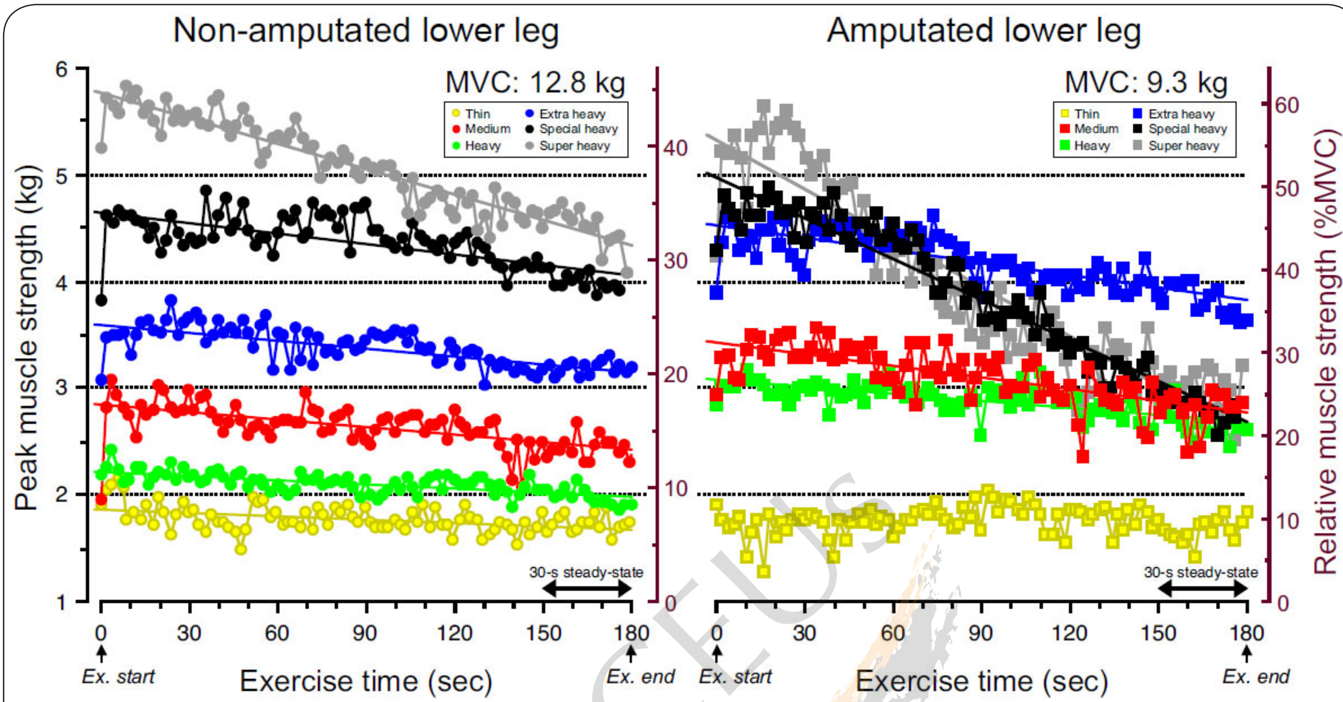

Figure 2. Magnitude in peak muscle strength during 3-min dynamic knee extensor exercise for six different rubber resistance bands.
The peak muscle strength due to each muscle contraction declined from onset (Ex. start) to the end of exercise (Ex. end). This may indicate difficulty in performing stable and repeated voluntary kicking for 3 min at the target muscle strength. The steady-state exercise was defined as the 30-s period before the end of exercise. The peak muscle strength for each kick was inversely related (P<0.001, solid line) to the exercise duration in both legs except for low intensity muscle contractions (yellow band) in the amputated lower leg. The relationship between net (average)-peak muscle strength and net (average)-leg blood flow at 30-s steady-state exercise was determined, as shown in **Figure 5**. The maximum voluntary contraction (MVC) was different between the non-amputated (12.8 kg) and amputated lower legs (9.3 kg), and therefore the scale for the relative muscle strength (%MVC, defined as peak muscle strength/MVC×100, %) is also shown as a vertical axis on the right side.

lated that reduced active muscle/mass may cause a lower oxygen need in the muscle, with subsequent remodeling and diameter change in the artery.

Therefore, the evaluation of LBF in relation to exercise may be useful for rehabilitation programs, increasing the general knowledge on oxygen supply and energy metabolism as well as on central and peripheral hemodynamics for patients with amputations which may potentially be inactive resection-stump muscles and/or demonstrate lower exercise tolerance. The intervention in this case study sheds new light on the ALL-thigh muscle contraction induced-hyperemia observed during repeated dynamic exercise, which may potentially be a key factor for leg muscle blood flow. These findings are discussed below.

Magnitude of LBF at pre-exercise and during exercise

First, the measurement of femoral artery blood velocity can be performed at pre-exercise and during rhythmic thigh resection stump (knee extensor) exercise using PTB with a high time resolution Doppler ultrasound, as shown in **Figure 3** and **Supplemental Figure A**. There was a clear difference in LBF between ALL and non-ALL during exercise compared to pre-exercise in **Figure 4e**.

Our result of lower LBF in ALL (234 ml/min) compared to non-ALL (379 ml/min) at pre-exercise may partially be related to 1) the loss of blood volume content in ALL as well as 2) reduced inflow via smaller diameter following atrophy of the thigh muscle resection-stump due to reduced circumference of the thigh in ALL.

We have no calculation for the LBF value per leg muscle mass/volume and/or no measures of popliteal artery blood flow in the non-ALL. However, in terms of general diameters and blood velocity in healthy legs, the femoral artery has a diameter of about 10-7 mm and a peak (systolic) blood velocity of about 80 cm/sec, and the popliteal artery has a diameter of about 7-5 mm and a peak (systolic) blood velocity of about 60 cm/sec [37]. Since approximately 20-40% of blood flow volume in the femoral artery may correspond to the popliteal blood flow volume, the blood flow value in the present non-ALL may be estimated to be in the range 76-152 ml/min in the popliteal artery and 227-303 ml/min in the thigh parts. Thus, in the present case, LBF (234 ml/min) in the thigh

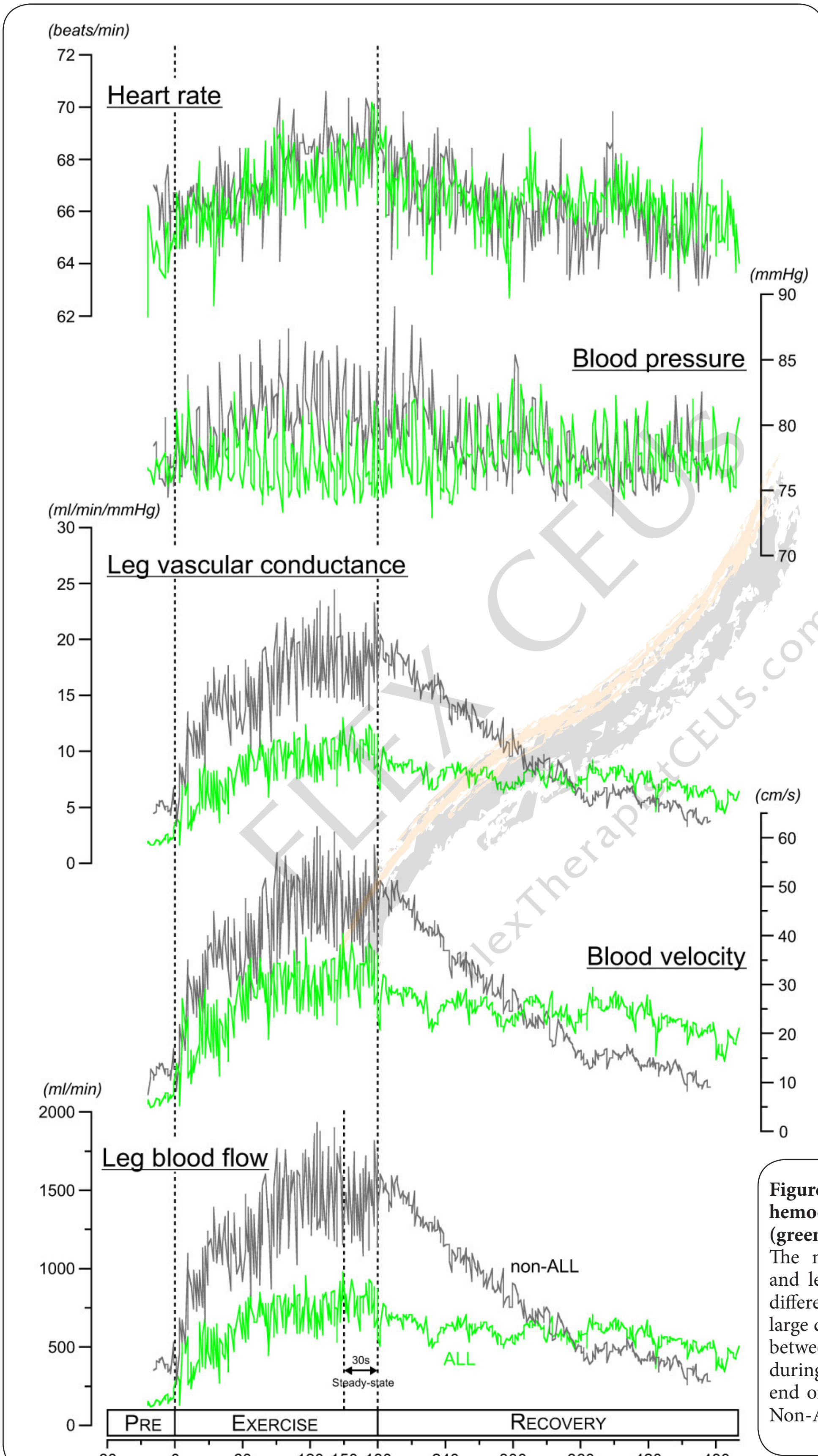

Figure 3. Overall time-course of hemodynamics magnitude for the heavy (green) rubber band (raw data).
The magnitudes of both leg blood flow and leg vascular conductance were clearly different during exercise and recovery. A large difference in changes in leg blood flow between ALL and non-ALL was clearly seen during exercise and immediately after the end of exercise. ALL: amputated lower leg, Non-ALL: non-amputated lower leg.

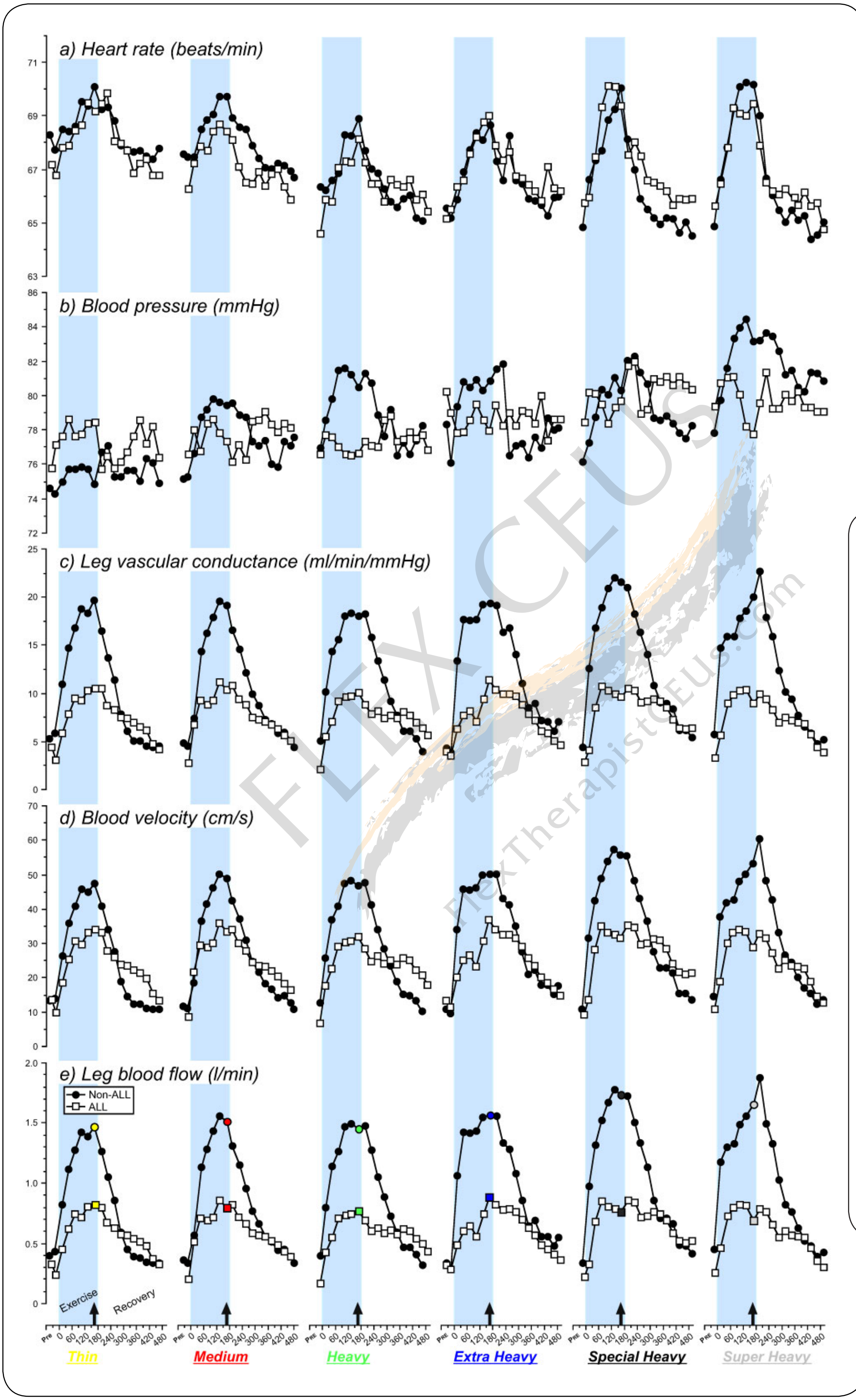

Figure 4. Time-courses of hemodynamics at all intensities.
The magnitudes of hemodynamics parameters **a**) heart rate, **b**) blood pressure, **c**) leg vascular conductance, **d**) blood velocity in the femoral artery and **e**) leg blood flow in the femoral artery were described. Measurement was defined at pre-exercise, during the whole exercise and recovery period. The data were determined by averaging (net) every 30 s. The net-leg blood flow value for the last 30-s of steady-state exercise (↑) was used for the correlation between leg blood flow and peak muscle strength/ percentage of maximum voluntary contraction as exercise intensity in Figure 5. Circles: non-amputated lower leg (non-ALL). Squares: amputated lower leg (ALL). The plotted data (↑) in various colors correspond to the resistance band colors used, as in **Figure 5**.

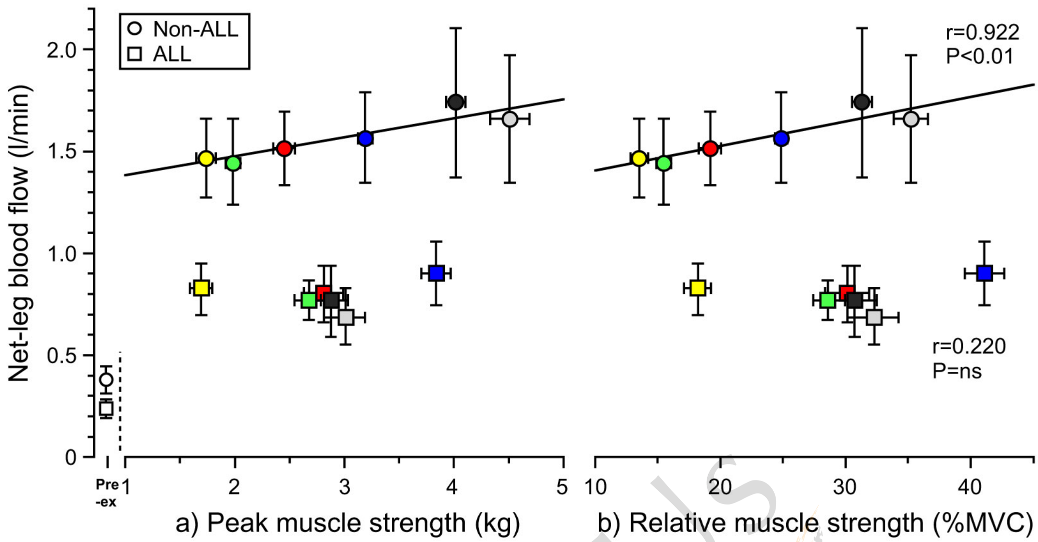

Figure 5. Relationship between net-leg blood flow at steady-state exercise and net-peak muscle contraction strength as well as relative muscle strength (%MVC).
At steady-state exercise, there was a significant (r=0.922, P<0.01) positive linear relationship between exercising net-leg blood flow and the net values of a) peak muscle strength or b) relative muscle strength (percentage of maximum voluntary contraction, %MVC) in non-ALL. However, the same relationship was not seen (r=0.220, P=ns) in ALL. The net value for leg blood flow (corresponding to the value as ↑ in **Figure 4**) and the net value for peak muscle strength (**Figure 2**) were determined by averaging the raw data between 150 s and 180 s during the 30-s steady-state before the end of exercise. The relative muscle strength (percentage of maximum voluntary contraction; % MVC) was defined as peak muscle strength/MVC×100, %). Circle: non-amputated lower leg (non-ALL). Squares: amputated lower leg (ALL). The plotted data in various colors correspond to the resistance band colors - yellow (thin), red (medium), green (heavy), blue (extra heavy), black (special heavy) and silver (super heavy) –and match the colors in **Figure 4e**. The values are expressed as means ± standard deviation (SD).

resection-stump in ALL might be reduced as it is close to the lower value of above mentioned normal range in healthy legs. In addition, relatively reduced blood inflow to the atrophic thigh muscle resection-stump may also have been a factor.

A previous study demonstrated that vessel diameter in the feeding conduit femoral artery was closely related to quadriceps muscle mass and thigh volume measured by computer tomography, and was also correlated to peak pulmonary oxygen uptake during cycle ergometer exercise [38]. This correlation potentially indicates that a large muscle mass/volume and thigh muscle group strength can maintain the muscle metabolic function (inclusive exercise tolerance) with enlargement of vessel diameter through blood flow feeding.

In the present case, the femoral artery diameter was approximately 1 mm smaller in ALL (7.16 mm) than non-ALL (8.12 mm), which may indicate a reduction in conduit artery size due to disuse and/or thigh muscle atrophy after the subject's amputation 15 years previously. This is in agreement with the approximate reduction of 12% in maximum thigh circumference in ALL compared to non-ALL supported by previous studies [34,35,39]. The diameter in ALL may be around the lower value of the normal range in healthy legs [37].

Theoretically, a 1 mm reduction in diameter may result in a 22% reduction of LBF if the blood velocity is the same. However, LBF was reduced by 38% in ALL compared with non-ALL, suggesting a greater influence on blood velocity in the smaller ALL vessel (9.7 cm/s) than non-ALL (12.2 cm/s) as shown in **Figures 4d** and **4e**. This lower value of blood velocity in ALL may be due to the atrophic thigh and loss of blood inflow to the lower leg.

The time course of blood flow alterations during exercise may be influenced by remodeling of the arterial structure or restricted motor control as seen in musculoskeletal disorders like disuse syndrome and cerebrovascular disorders [40-42].

Interestingly, the time-course in LBF after 60-s exercise clearly demonstrated a large difference in the time-dependence of LBF magnitude (rather than blood velocity) in all sessions, which may be coordinated by the enhanced vasodilation although the magnitude was similar between ALL and non-ALL at the onset of exercise due to muscle pumping [43] (**Figures 4d** and **4e**).

Basically, the knee extensor model uses only the knee extensors of the thigh quadriceps muscle group, and it therefore may require less activity of the lower leg and consequently not increase feeding inflow into the lower leg in non-ALL. It is expected that the changes in (delta) LBF may be similar between ALL and non-ALL if only the thigh parts are active.

However, this dissociation of changes in (delta) LBF magnitude between ALL and non-ALL was definitely larger during exercise than at pre-exercise (**Figure 4e**), which may indicate a limitation of LBF increase and vasodilation in the thigh stump during exercise in spite of the comparatively higher muscle contraction strength (%MVC) in ALL than non-ALL (**Figure 5b**). Regarding to muscle workload, it may consider that the muscle contraction strength with the resistance bands might have been sufficient stress in ALL as well as non-ALL because of the similarity of magnitude of heart rate during exercise representing the systemic load (**Figure 4a**).

Furthermore, the increase in net-LBF at 30-s steady-state exercise in non-ALL was closely related (r=0.992) to the peak muscle strength. However, no significant increase in exercising net-LBF to incremental workload in ALL was seen, although peak muscle contractile effort (both absolute- and relative-peak muscle contraction strength) increased with increasing muscle contraction intensity using the six different resistance bands (**Figure 5**).

It is generally acknowledged that an increase in exercising LBF evaluated by the muscle contraction-relaxation and/or beat-to-beat cycle is directly proportional to the steady-state workload performed in relation to the interplay between cardiovascular regulation and muscle energy metabolism [**9-11**,**13**]. The femoral arterial blood velocity and blood flow increase linearly with incremental exercise intensities of work rate (for instance, peak muscle force) during steady-state rhythmic thigh muscle contractions [**11**]. This implies that an enhanced vasodilatation is elicited, in relation to the increased average muscle force exerted at higher workloads, to meet the elevated metabolic activity. Therefore, the present finding is in agreement with our previous findings that net-LBF in non-ALL might depend on the peak muscle contraction strength at the steady-state, but was not seen in ALL as shown in **Figure 5**. This contradiction potentially suggests that exercising LBF in ALL may be less influenced by the thigh-stump muscle contraction intensity.

The mechanism behind the absence of a workload-dependent increase in LBF in ALL is not clear. However, a possible explanation may be the disparity in behavior of thigh knee extensor exercise (quantitative variable of electromyogram) between ALL and non-ALL. A previous study reported differences in peak muscle activity measured by surface electromyography during the gait cycle for both vastus medialis and biceps femoris muscles between ALL with prosthesis and non-ALL [**44**]. Voluntary muscle contraction strength in ALL with the PTB prosthesis seems to exhibit muscle contraction effort (i.e., difficulty with muscle contraction in the functional knee extension with the prosthesis) in consideration of the potential muscle weakness indicated by the decreased thigh circumference or the lower MVC. Consequently, the functional difference in voluntary muscle contraction manner in kicking may be supported by the data of a shorter time to peak muscle strength in ALL compared to non-ALL (**Table 1**). There may also be insufficient muscle vasodilation in the periphery during knee extensor exercise.

Another possible explanation may be the lack of the original biomechanical effect in ALL, i.e., the muscle-pump venous return from the lower leg with changes in arteriovenous pressure gradient and/or hydrostatic pressure at the onset and during muscle contraction [**45-48**]. Swinging the lower leg with an extended rubber band in non-ALL may induce more or less activation of the muscles in the lower leg, including alterations in hydrostatic pressure and/or activation of the tibialis anterior, even if the quadriceps muscle group is especially active during repeated knee extensions [**49**,**50**]. However, in ALL there are fewer effects on circulatory adjustment from the lower leg. In addition, the fluctuations in blood pressure during exercise were more significant in ALL than in non-ALL (**Figure 4b**). There may be significant difference in exercise pressor reflex through the autonomic regulation, central command, and mechanical muscle contraction between ALL and (seemingly healthy) non-ALL in relation to exercise.

Methodological considerations and limitations

Fluctuations in both LBF and blood velocity during exercise

Our previous studies demonstrated rapid changes in the time courses of blood velocity profiles in the conduit femoral artery, with muscle contraction and/or muscle relaxation superimposed cardiac cycle during exercise in different states of muscle contraction time/frequency and workload [**9**,**11**,**12**,**51**]. In dynamic exercise at 30 contractions per minute (1-s contraction-1-s relaxation cycle), the continuous blood velocity curve during repeated muscle contractions fluctuated rapidly due to the muscle force curve, which indicated that the muscle contraction restricted LBF (temporal reduced blood velocity), and consequently muscle relaxation may induce an increase in LBF (higher blood velocity) [**11**,**13**,**51**].

In the present study, fluctuations in LBF/blood velocity due to muscle contraction-relaxation were seen in both ALL and non-ALL (**Figure 3** and **Supplemental Figure A, B**). However, it may be that the ranges of amplitude (fluctuation) of both blood velocity and LBF were smaller in ALL than non-ALL at low intensity exercise (thin, medium and heavy). A possible explanation may be an insufficiency of functional mechanical compression for restricted LBF in the thigh muscle in ALL compared to non-ALL, even if the peak muscle strength is similar between legs (**Figure 5**). The steeper decline of peak muscle strength at each kicking cycle during 3-min exercise may suggest a weakness in muscle contraction strength in ALL compared to non-ALL for high intensity (special and super heavy) exercise (**Figure 2**).

In both legs, the muscle contraction cycle was stable (1.983-2.001 sec in non-ALL and 1.995-2.003 sec in ALL) at the target muscle contraction frequency, with low coefficients of variation (<8.7%) (**Table 1**). However, the peak muscle strength consistently declined toward the end of exercise in ALL as well as non-ALL (**Figure 2**). In the present subject, this suggested difficulty in performing similar repeated knee extensions at the target contraction intensity for 3 min in both legs, and consequently the range of motion in the knee decreased from onset to the end of exercise. This may be due to shortening of the time to peak muscle strength in ALL compared to non-ALL.

Thigh muscle contraction cycle, peak muscle strength and their variations

The muscle contraction frequency and kicking muscle contraction strength are major factors for the evaluation of LBF at a target intensity [11]. **Table 1** shows the stable muscle contraction cycle with the coefficients of variation (<8.7%) for 3-min exercise, as well as during the last 30-s of exercise, which are in an acceptable range between ALL and non-ALL. The variations in peak muscle strength during kicking cycles are low except for the high intensity exercise, i.e.,"special" and "super heavy", in ALL. However, the data samples during the last 30 s before the end of exercise remain within an acceptable range of variations for evaluation of the relationship between LBF and peak muscle strength.

Muscle contraction strength vs. rubber resistance band

The mean value of peak muscle strength during exercise was higher for medium (red) than heavy (green) resistance bands, as shown in **Figure 2** and **Table 1**. This contradiction may be due to the smaller difference (0.4 kg) in tension in between medium and heavy resistance bands in the moderate exercise intensity in both ALL and non-ALL [52].

%MVC between ALL and non-ALL

In ALL, the weight of the lower leg corresponds to the PTB prosthesis. Therefore, the thigh muscle strength determined by voluntary thigh muscle contraction may be functionally different between ALL and non-ALL. Voluntary thigh muscle contractions in non-ALL may partially involve the lower leg moving, but in ALL solely swing the PTB prosthesis. Therefore, the %MVC (relative muscle contraction strength) may have been relatively higher for "extra heavy" in ALL than non-ALL in **Figure 5**.

Perspective

Information for prosthesis types other than PTB, e.g., total surface bearing, may be required. Hip flexion as a simple exercise model and/or isometric/isotonic muscle contractions in the thigh resection-stump with measurement of muscle activity using surface electromyography may also yield additional information. The limb muscle contraction intensity is major factor for exercise prescription (muscle resistance strength and/or endurance training) and physical therapy, and can reveal disparities in magnitude at different exercise intensities with circulatory regulation between ALL and non-ALL that may be significant. The time-course of LBF alterations may be influenced by remodeling of the arterial structure in an amputated lower limb. Continuous resistance training in the thigh muscle-stump before remodeling of the arterial structure occurs may improve the muscle contraction-induced blood flow increase. Furthermore, for lower leg amputees, the determination of LBF in relation to leg exercise may be useful information for rehabilitation programs, increasing the general knowledge on oxygen supply and energy metabolism as well as on central and peripheral circulatory adjustment.

Conclusion

Leg amputations may be necessitated by diabetes mellitus and peripheral arterial disease, potentially requiring aerobic exercise training for the prevention of exacerbated cardiovascular events and/or disuse disorders. The present intervention using continuous measurement of blood velocity/flow in the amputated limb conduit artery during repeated/dynamic knee extensor exercise offers new insights into muscle blood flow regulation in the ALL, with potential benefits for exercise therapy for thigh muscle-stump contraction induced LBF hyperemia.

Furthermore, our finding may help in the design and prescription of localized exercise therapy for amputated limbs. An exercise model with the fitted prosthesis may improve evaluation of the relationship between muscle contraction strength and muscle blood flow response. As leg oxygen uptake is evaluated by the product of LBF and arteriovenous oxygen difference, the determination of exercising LBF in relation to muscle contraction intensity may provide essential information about cardiovascular adjustment during repeated exercise and its evaluation in relation to integrated applied physiology and/or physical exercise therapy.

List of abbreviations

LBF: Leg blood flow
ALL: Amputated lower leg
Non-ALL: non-amputated lower leg
PTB: Patella tendon bearing
MVC: Maximum voluntary contraction
LVC: Leg vascular conductance