Effects of step frequency during running on the magnitude and symmetry of ground reaction forces in individuals with a transfemoral amputation

Toshiki Kobayashi1, Mark W. P. Koh1, Mingyu Hu1, Hiroto Murata2,3, Genki Hisano2,4,5, Daisuke Ichimura2 and Hiroaki Hobara2*

Abstract

Background: Individuals with unilateral transfemoral amputation are prone to developing health conditions such as knee osteoarthritis, caused by additional loading on the intact limb. Such individuals who can run again may be at higher risk due to higher ground reaction forces (GRFs) as well as asymmetric gait patterns. The two aims of this study were to investigate manipulating step frequency as a method to reduce GRFs and its effect on asymmetric gait patterns in individuals with unilateral transfemoral amputation while running.

Methods: This is a cross-sectional study. Nine experienced track and field athletes with unilateral transfemoral amputation were recruited for this study. After calculation of each participant’s preferred step frequency, each individual ran on an instrumented treadmill for 20 s at nine different metronome frequencies ranging from $-20\%$ to $+20\%$ of the preferred frequency in increments of 5\% with the help of a metronome. From the data collected, spatiotemporal parameters, three components of peak GRFs, and the components of GRF impulses were computed. The asymmetry ratio of all parameters was also calculated. Statistical analyses of all data were conducted with appropriate tools based on normality analysis to investigate the main effects of step frequency. For parameters with significant main effects, linear regression analyses were further conducted for each limb.

Results: Significant main effects of step frequency were found in multiple parameters ($P < 0.01$). Both peak GRF and GRF impulse parameters that demonstrated significant main effects tended towards decreasing magnitude with increasing step frequency. Peak vertical GRF in particular demonstrated the most symmetric values between the limbs from $-5\%$ to $0\%$ metronome frequency. All parameters that demonstrated significant effects in asymmetry ratio became more asymmetric with increasing step frequency.

Conclusions: For runners with a unilateral transfemoral amputation, increasing step frequency is a viable method to decrease the magnitude of GRFs. However, with the increase of step frequency, further asymmetry in gait is observed. The relationships between step frequency, GRFs, and the asymmetry ratio in gait may provide insight into the training of runners with unilateral transfemoral amputation for the prevention of injury.

*Correspondence: hobara-hiroaki@aist.go.jp

2 Artificial Intelligence Research Center, National Institute of Advanced Industrial Science and Technology (AIST), Waterfront 3F, 2-3-26, Aomi, Koto-ku, Tokyo 135-0064, Japan

Full list of author information is available at the end of the article

© The Author(s) 2022. Open Access. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Background
Running has been a key method of locomotion for mankind. Although it has become a common competitive sport for some amputee athletes who can run again, many individuals with lower limb amputation still find it challenging. According to a previous study, sixty percent of individuals who suffered combat-related amputations reported a complete inability to run one block with a prosthesis [1]. It has been well documented that individuals with amputation develop a more asymmetric gait pattern, especially while running. The asymmetric gait pattern causes increased ground reaction forces (GRFs) on the intact limb, leading to a higher probability to develop health conditions such as knee osteoarthritis and musculoskeletal injuries [2–4]. The increased GRFs affect the loading rates on the joints which have been associated with the expanded occurrences of these health conditions [5]. Both lateral and medial forces have been correlated with elevated probabilities of developing knee osteoarthritis, although the medial condition is more common than the lateral [6, 7]. Therefore, reduction in GRFs on the intact limb and more symmetric gait pattern may prevent possible future injuries or disease in individuals with lower limb amputation.

Asymmetric gait can be evaluated by analyzing gait cycle, step mechanics, range of motion, and the composing values of GRFs [8]. Individuals with lower limb amputation have an obvious difference in symmetry of gait when compared to able-bodied individuals [9, 10], and gait asymmetry comes with significant increases in load on the intact limb [11]. The prosthetic limb is unable to duplicate the control and mechanism of the intact limb [12] making asymmetric gait unavoidable. The individuals with unilateral transfemoral amputation are likely, consciously or not, to rely more on the intact limb. With the increase of walking velocity or into running, the asymmetry tends to be amplified [5, 13], causing increased loading stress on the intact limb [14, 15]. The increased loading stress can be affected by an increase of factors such as vertical GRFs [16, 17], mediolateral GRFs [6], breaking and propulsive impulses [18], as well as spatiotemporal parameters such as stance time [5]. Anteroposterior GRFs have also been hypothesized to be one of the potential factors in injuries related to running [19].

For an able-bodied individual, increasing cadence or step frequency during gait can decrease GRFs on the lower limb joints [20–24]. Increasing step frequency can also reduce the heart rate of runners as well as oxygen costs when the speed remains constant [25], which can prove beneficial to individuals with transfemoral amputation as they expend significantly more energy compared to those who are able-bodied during gait [26–28]. However, there has been a paucity of studies on the effects of step frequency on GRFs in individuals with amputation [29, 30]. Step frequency manipulation is one of the fundamental locomotor strategies, and there should be an optimum step frequency to minimize GRFs on the intact limb. It has been reported that able-bodied individuals can be trained to vary step frequency at a given running speed resulting in changing GRFs [31, 32]. Thus, retraining of step frequency in individuals with amputation should also be viable [29, 30].

Therefore, the first aim of the present study was to investigate the effects of step frequency on the magnitude of GRFs in the intact and prosthetic limbs of individuals with transfemoral amputation. The second aim was to investigate the effect of step frequency on the symmetry of GRFs and spatiotemporal parameters between intact and prosthetic limbs. The first hypothesis of this study was that increasing step frequency at a given speed will lower the magnitude of GRFs on the intact and prosthetic limbs. The second hypothesis was that the asymmetry of GRFs between the intact and prosthetic limbs will be minimized at the preferred step frequency, although individuals with amputation still run with an asymmetric pattern and loading on the intact limb may not be the minimum.

Methods
Participants
This cross-sectional study shared a participant pool with a previous study [30]. Nine individuals with transfemoral amputation with experience in the long jump or 100-m sprint were recruited. They also were members of track and field teams and had been sprint training for more than 5 years. The mean of their best recorded times for the 100-m spring in the past year was 17.59 ± 2.15 s. Their demographic characteristics are summarized in Table 1. Four participants wore the 1E90 Sprinter (categories 2 to 4, Ottobock, Duderstadt, Germany) and five participants wore the 1E91 Runner (categories 2 to 5, Ottobock, Duderstadt, Germany). All participants attached rubber soles to their running-specific prostheses. The Institutional Review Board (Environmental and Safety Headquarters, Safety Management Division, National Institute of Advanced Industrial Science and Technology) ethically approved the study, and we conducted it following the guidelines given in the Declaration of Helsinki (1983).
Table 1 Demographic characteristics

| Subject | Preferred $f_{\text{step}}$ (Hz) | Trial speed (m/s) | Age (years) | Sex | Total mass (kg) | Height (m) | Amputated limb | Time since amputation (years) | Prosthetic knee | RSP model |
|---------|-------------------------------|-------------------|-------------|-----|----------------|------------|----------------|-------------------------------|----------------|-----------|
| 1       | 2.53                          | 2.11              | 36          | M   | 59.8           | 1.61       | Right          | 17.9                         | 3S80           | Runner 1E91 (cat.3) |
| 2       | 2.65                          | 2.00              | 31          | M   | 59.7           | 1.65       | Right          | 3.0                          | Cheetah Knee   | Runner 1E91 (cat.2) |
| 3       | 2.68                          | 2.14              | 20          | F   | 45.2           | 1.62       | Left           | 3.5                          | 3S80           | Runner 1E90 (cat.3) |
| 4       | 2.70                          | 2.86              | 27          | M   | 70.4           | 1.75       | Right          | 6.2                          | 3S80           | Runner 1E91 (cat.4) |
| 5       | 2.73                          | 2.31              | 23          | M   | 56.3           | 1.68       | Left           | 20.0                         | 3S80           | Runner 1E90 (cat.3) |
| 6       | 2.85                          | 2.25              | 34          | M   | 58.7           | 1.61       | Left           | 21.0                         | 3S80           | Runner 1E91 (cat.5) |
| 7       | 2.88                          | 2.39              | 20          | F   | 56.5           | 1.56       | Right          | 5.7                          | 3S80           | Runner 1E91 (cat.3) |
| 8       | 2.93                          | 2.75              | 17          | M   | 86.0           | 1.77       | Right          | 3.5                          | 3S80           | Runner 1E90 (cat.4) |
| 9       | 3.00                          | 1.94              | 21          | F   | 46.7           | 1.49       | Right          | 100                          | 3S80           | Runner 1E90 (cat.2) |
| Mean    | 2.77                          | 2.31              | 25.44       |     | 59.91          | 1.64       |                |                               |                |            |
| (SD)    | (0.14)                        | (0.30)            | (6.45)      |     | (11.6)         | (0.08)     |                |                               |                |            |

$f_{\text{step}}$: Step frequency; RSP: Running specific prosthesis; Total mass = Body mass + Prosthetic mass
Informed written consent was obtained from all participants (or guardians, in participant’s 8 situation) prior to the experiment.

Experimental procedures
An instrumented split-belt treadmill (FTMH-1244WA, Tec Gihan, Kyoto, Japan) was used in this study (Fig. 1). Participants were first instructed to walk and run for more than 5 min on the instrumented treadmill before the experiment in order to familiarize themselves with the equipment [33]. An estimation of each participant’s maximum speed was done by taking 100 m and dividing it by their personal best time for the 100-m sprint [34]. The treadmill was set for 40% of each individual’s estimated maximum speed, and participants were instructed to run as normal for 20 s to determine each participant’s preferred step frequency (Table 1). In order to obtain a wide step frequency range, 40% of their estimated maximum speed was elected to be the running speed, as it was sufficiently low enough. The preferred step frequency was calculated by using 14 sequential steps taken during the middle of the trial. The inverse of the time from contact to the contralateral contact was used as the definition for the step frequency [30]. Preceding studies used a range of step frequency between $-30\%$ and $+30\%$ at a determined running speed [35–37]. Therefore, our participants were asked to run while matching a digital metronome beat at nine different step frequencies (metronome frequency): preferred (0%), four above (+5%, +10%, +15%, and +20%), and four below (−5%, −10%, −15%, and −20%) the preferred step frequency. The participants were experienced runners using running-specific prostheses. They were given an adequate practice period and instructed to follow a target metronome beat as accurately as possible at each step frequency, before asking them to conduct one running trial for 20 s at each determined step frequency (with randomized order). They were also given rest periods between trials of 1 to 3 min to reduce the effects of fatigue. In this study, the mean values of the running speed (40% of participants’ maximum speed) and preferred step frequency were $2.31 \pm 0.30$ m/s and $2.77 \pm 0.14$ Hz, respectively (Table 1).

Data collection
Two force platforms (TF-40120-CL and TF-40120-CR; Tec Gihan, Kyoto, Japan) integrated into the instrumented treadmill collected all components of GRF data (sampling at 1 kHz). The GRFs were further filtered by a fourth-order zero-lag low-pass Butterworth filter with a cut-off frequency at 25 Hz, according to the findings of a previous study [38]. Additionally, a 40-N threshold was applied for further vertical GRF analysis [39–42] to determine foot contact timing.

Data analyses
In this study, data were calculated by averaging seven consecutive steps. As dependent variables, we determined spatiotemporal parameters (Additional file 1: Fig. S1 and Additional file 6: Material: equations for temporal parameters), such as contact time (the time that the applied force exceeded the threshold on the force plate) and aerial time (the time interval between the end of the contact period of one foot and the beginning of the contact period of the opposite foot) at each metronome frequency in each limb. We also determined swing time (time interval between foot-off to ipsilateral foot contact), step time (time interval between successive heel contacts), realized step frequency (the inverse of the time from touchdown to contralateral touchdown), and step length (the belt distance traveled between successive contact periods of opposite feet).

We also analyzed peak vertical GRF (vGRF), peak braking GRF, peak propulsive GRF, peak medial GRF, and peak lateral GRF across a range of metronome frequencies in both intact and prosthetic limbs. Additionally, the GRF impulses (GRIs) were calculated as time integrals of the GRFs over the stance phase. The medial, lateral, braking, and propulsive impulses were computed as time
integrals of all negative and positive values of the mediolateral and anteroposterior GRFs. Net mediolateral and net anteroposterior impulses were calculated as the sum of the medial and lateral, and braking and propulsive impulses. Moreover, vertical GRI was calculated as time integrals of the vGRF. All GRF variables were normalized to the participant’s total mass (body mass + prosthetic mass) and represented by a unit of body weight (BW). Thus, the GRIs relative to BW have a unit of BW-second (BW·s).

Based on a previous study [43], the asymmetry ratio of spatiotemporal and peak GRFs and GRIs were calculated. For individuals with unilateral transfemoral amputation, the asymmetry ratio was calculated as the prosthetic limb’s data divided by the intact limb’s data.

\[
Asymmetry\ ratio = \frac{Prosthetic\ limb’s\ data}{Intact\ limb’s\ data}
\]

Therefore, 1.0 will denote perfect symmetry, and any deviation whether higher than 1.0 (prosthetic limb dominant) or lower than 1.0 (intact limb dominant) indicates asymmetry.

**Statistical analyses**

We performed the Shapiro–Wilk test to confirm normality of data. If the data for each limb followed a normal distribution, we used one-way repeated measures ANOVA to investigate the main effects of step frequency in each limb. In contrast, if the data did not follow a normal distribution, the Friedman test was used. In both cases, we further used best-fit linear regression analyses when significant main effect was observed in each limb. In the analysis, coefficients of determination (\(R^2\)), correlation coefficient and 95% confidence intervals (95% IC) were calculated. Treating the limb data as unpaired in both analyses represents a conservative approach and has been implemented in previous studies [44–46]. Statistical significance was set at 0.05 for ANOVA, Friedman test, and regression analyses. Statistically significant results at exact values less than 0.001 were represented as \(P < 0.001\) by convention [47]. SPSS for Windows Version 26 (IBM, Armonk, NY, USA) was used for all the statistical analyses.

**Results**

**Percent error of realized step frequency to metronome frequency**

Percent error (%) of the realized step frequency from the metronome frequency is presented in Fig. 2. The mean error was within 4% of the metronome frequency. In the prosthetic limb, the error tended to be more negative at a higher metronome frequency and more positive at a lower metronome frequency. In the intact limb, the error tended to be more positive at either a higher or lower metronome frequency.

**Mean ground reaction forces**

Mean GRF (\(N=9\)) across the mean realized step frequencies at each metronome frequency in mediolateral, anteroposterior, and vertical directions are presented in Fig. 3. For all GRF measurements (mediolateral, anteroposterior, and vertical), the magnitude at all stages of stance tends to decrease as step frequency increases. Overall, the prosthetic limb GRF measurements showed more regular and uniform changes across metronome frequency when compared to the intact limb as well.

**Spatiotemporal parameters**

Mean values and linear regressions with \(R^2\) values of each spatiotemporal parameter across the mean realized step frequencies at each metronome frequency are presented in Fig. 4. All the spatiotemporal parameters demonstrated significant main effects of step frequency, which are indicated by filled red (prosthetic limb) and blue (intact limb) circles in Fig. 4. Except for the contact time of the prosthetic limb (A), all parameters displayed statistical significances (\(P<0.05\)) in linear regression analyses and exhibited a negative linear association with realized step frequency. The contact time (A) for the prosthetic limb was not statistically significant (\(R^2=0.019\)). The contact time (A) in the intact limb (\(R^2=0.737, P=0.003\)) had a decreasing trend in relationship with increasing realized step frequency. Aerial time (B) for both the prosthetic limb (\(R^2=0.969, P<0.001\)) and the intact limb (\(R^2=0.918,\)
P < 0.001) exhibited a visible curved decrease with increasing realized step frequency, although still significant with linear association. Swing time (C) (prosthetic limb $R^2 = 0.986$, P < 0.001; intact limb $R^2 = 0.946$, P < 0.001), step time (D) (prosthetic limb $R^2 = 0.990$, P < 0.001; intact limb $R^2 = 0.987$, P < 0.001), and step length (E) (prosthetic limb $R^2 = 0.989$, P < 0.001; intact limb $R^2 = 0.986$, P < 0.001), all displayed nearly complete linear relationships with realized step frequency and decreased with increasing realized step frequency. Correlation coefficients, mean (SD), median (IQR), and 95% confidence intervals (95% CI) of the
spatiotemporal parameters are summarized in Additional file 2: Table S1.

**Ground reaction force parameters**

Mean values and linear regressions with $R^2$ values of each ground reaction force parameter across the mean realized step frequencies at each metronome frequency are presented in Fig. 5. All the ground reaction force parameters, except for the peak lateral GRF of the prosthetic limb (shown by the unfilled red circles in Fig. 5), demonstrated significant main effects, which are indicated by filled red (prosthetic limb) and blue (intact limb) circles. All parameters, except for the peak lateral GRF of the prosthetic limb, exhibited statistical significances with realized step frequency ($P < 0.05$) in linear regression analyses. The parameters: peak vertical GRF (vGRF) (A) (prosthetic limb $R^2 = 0.941$, $P < 0.001$; intact limb $R^2 = 0.831$, $P < 0.001$), peak propulsive GRF
(C) (prosthetic limb $R^2 = 0.839$, $P < 0.001$; intact limb $R^2 = 0.947$, $P < 0.001$), and peak medial GRF (D) (prosthetic limb $R^2 = 0.959$, $P = 0.001$; intact limb $R^2 = 0.928$, $P < 0.001$), displayed a decreasing linear relationship with the increase in realized step frequency. For peak braking GRF (B) (prosthetic limb $R^2 = 0.954$, $P < 0.001$; intact limb $R^2 = 0.926$, $P < 0.001$) and peak lateral GRF (E) of the intact limb ($R^2 = 0.597$, $P = 0.015$), the relationship had an increasing trend. For peak vGRF (A), the prosthetic limb exhibited higher vGRFs at realized step frequency of $-20\%$ metronome frequency than the intact limb and lower vGRFs at realized step frequency of $+20\%$ metronome frequency. The magnitude of peak braking GRF (B) tended to be smaller with increasing realized
step frequency in both limbs. Also, the magnitude of difference in peak braking GRF between limbs was smaller at relatively higher step frequency and showed the clearest convergence towards symmetry among the GRF parameters. Both limbs exhibited almost linear trends. For both peak propulsive GRF (C) and peak medial GRF (D) parameters, the prosthetic limb had a lower magnitude of values than the intact limb across realized step frequencies, but both limbs appeared to be decreasing at a similar rate as realized step frequency increases. For the peak lateral GRF (E) parameter, only the intact limb demonstrated statistical significance with a positive trend towards zero. It should also be noted that for all significant parameters, the magnitude of GRFs decreased in relation to increasing realized step frequency. Correlation coefficients, mean (SD), median (IQR), and 95% confidence intervals (95% CI) of the GRF parameters are summarized in Additional file 3: Table S2.

**Ground reaction force impulse parameters**

Mean values and linear regressions with R² values of each ground reaction force impulse (GRI) parameter across the mean realized step frequencies at each metronome frequency are presented in Fig. 6. All the GRI parameters, except for the net b-p (braking–propulsive) GRI (D) of the prosthetic and intact limbs (shown by unfilled red and blue circles in Fig. 6), demonstrated statistical main effects, which are indicated by filled red (prosthetic limb) and blue (intact limb) circles. All parameters that demonstrated significant main effects exhibited statistical significances with realized step frequency (P < 0.05) in linear regression analyses. Except for braking GRI (B) and lateral GRI (F), all significant parameters had a negative linear association as realized step frequency increased. The braking GRI (B) (prosthetic limb R² = 0.859, P < 0.001; intact limb R² = 0.942, P < 0.001) and lateral GRI (F) (prosthetic limb R² = 0.777, P = 0.002; intact limb R² = 0.744, P = 0.003) had positive linear association towards zero with increasing realized step frequency. The magnitude of braking GRI (B) also is lower for the prosthetic limb at all increments of realized step frequency compared to the intact limb. For vertical GRI (vGRI) (A), the prosthetic limb (R² = 0.982, P < 0.001) had a higher magnitude when compared to the intact limb (R² = 0.993, P < 0.001) as realized step frequency increased. The values of the propulsive GRI (C) over increasing realized step frequency did not show remarkable differences between the prosthetic limb and the intact limb. The prosthetic limb (R² = 0.886, P < 0.001) showed a higher value at realized step frequency of -20% metronome frequency, and intact limb (R² = 0.950, P < 0.001) had close values to the prosthetic limb at realized step frequency of +20% metronome frequency. The values of the prosthetic and intact limbs for medial GRI (E) were similar. The linear association of the prosthetic limb (R² = 0.960, P < 0.001) and the intact limb (R² = 0.956, P < 0.001) were also similar. For the lateral GRI (F), both limbs had a positive association with realized step frequency with the prosthetic limb (R² = 0.777, P = 0.002) showing close value to the intact limb (R² = 0.744, P = 0.003) as realized step frequency increased. The net m-l (medial–lateral) GRI (G) values for the prosthetic limb (R² = 0.920, P < 0.001) are similar to the intact limb (R² = 0.929, P < 0.001) at all increments of realized step frequency and both linear models decreased at a similar rate. Correlation coefficients, mean (SD), median (IQR), and 95% confidence intervals (95% CI) of the GRI parameters are summarized in Additional file 4: Table S3.

**Asymmetry ratio of the parameters**

The parameters that demonstrated statistical main effects in the asymmetry ratio are presented in Fig. 7. Due to the calculation of this parameter given in the methods section, 1.0 denotes perfect symmetry. The asymmetry ratio of seven parameters demonstrated significant main effects, which are indicated by filled circles in Fig. 7. Except for swing time (B), step frequency (D), and peak vGRF (F), other parameters appeared to become prosthetic limb dominant at higher metronome frequencies. For swing time (B) and step frequency (D) the intact limb became more dominant as metronome frequency increased. Peak vGRF (F) favored the prosthetic limb at –20% metronome frequency, but favored the intact limb at +20% metronome frequency, with the most symmetric value between -10% and +5%. Correlation coefficients, mean (SD), median (IQR), and 95% confidence intervals (95% CI) of the asymmetry ratio of the parameters are summarized in Additional file 5: Table S4.

**Discussion**

The first aim of this study was to investigate the effects of step frequency on the magnitude of GRFs in the intact and prosthetic limbs of individuals with transfemoral amputation. Our results showed that increasing step frequency had significant main effects in the reduction of the magnitude of GRFs in both limbs, except peak lateral GRF in the prosthetic limb (Fig. 5). This was in line with our first hypothesis that increasing step frequency at a given speed would lower the magnitude of GRFs on the intact and prosthetic limbs. This relationship could be
Fig. 6 Mean ground reaction force impulse (GRI) parameters and linear regressions of both limbs across mean realized step frequencies at each metronome frequency. In every plot, the horizontal axis represents realized step frequency. Each plot represents a different parameter. Parameters are represented in respective vertical axis as: vertical GRI (BW) (A), braking GRI (BW) (B), propulsive GRI (BW) (C), net braking-propulsive GRI (BW) (D), medial GRI (BW) (E), lateral GRI (BW) (F), and net medial–lateral GRI (BW) (G). Each circle indicates the mean of all the participant's data at each increment of metronome frequency from −20% to +20%. Blue and red plots indicate intact and prosthetic limb, respectively. Significant main effects are indicated by filled circles, while main effects that were not significant are indicated by unfilled circles. Relevant $R^2$ and $P$ values for the linear regressions are also displayed in matching color to the intact or prosthetic limb. $P < 0.001$ indicates $P$ values which were at or smaller than 4 decimal places.
Fig. 7 Asymmetry ratio of parameters that demonstrated significant main effects. Plot A is contact time, plot B is swing time, plot C is step time, plot D is step frequency, plot E is step length, plot F is peak vertical GRF, and plot G is vertical GRI. In every plot, the horizontal axis represents metronome frequency from $-20\%$ to $+20\%$, with the vertical axis representing the ratio between limbs by dividing the mean of prosthetic limb data by the mean of intact limb data for each parameter, resulting in 1.0 representing perfect symmetry. Note that $+$ means more toward the prosthetic side and vice versa.
explained by natural bouncing gait during running [48]. As step frequency increases during constant velocity, the stiffness of the leg increases, resulting in the reduction of the vertical displacement of the center of mass [35]. The lowered displacement of the center of mass reduces the GRFs the body undertakes upon contact with the ground. Our results are also consistent with previous studies that investigated the effects of increased step frequency on gait parameters in able-bodied individuals [20–24]. Therefore, current results indicate that increasing step frequency has similar effects on GRFs in both individuals with and without amputation.

The second aim of this study was to investigate the effect of step frequency on the symmetry of GRFs and spatiotemporal parameters between intact and prosthetic limbs. As shown in Fig. 7F, the peak vertical GRF asymmetry ratio was closest to 1.0 between −5% and 0% of preferred step frequency. In addition, some parameters, such as contact time (Fig. 7A) and vGRI (Fig. 7G) showed minimized asymmetry between −10% and −5% of the preferred step frequency. These results partially support our second hypothesis that the asymmetry of GRFs between the intact and prosthetic limbs would be minimized at the preferred step frequency. Previous studies that found asymmetric gait being more pronounced in individuals with amputation [9, 10] mainly focused on differences between the intact and prosthetic limb, but did not consider the effects of step frequency. Due to the nature of bouncing gait, the vertical displacement of the center of mass is affected by the stiffness of the leg [35]. However, the prosthetic limb’s stiffness cannot be modified due to the absence of biological ankle and knee joints. This could cause the additional asymmetry as there is a need for the intact limb to compensate for additional forces generated by the unadaptable stiffness of the prosthetic limb. Future studies on asymmetric gait in individuals with amputation should consider step frequency, as our study has found that this also has an impact on asymmetric gait. However, the mechanism of how step frequency affects each gait parameter is not clear and further investigation is warranted.

A metronome was used to control the step frequency in this study. The realized step frequency was assumed to be constant between the prosthetic and intact limb, but our results showed otherwise. Realized step frequency of the prosthetic and intact limbs did not increase at a similar rate over increasing metronome frequency. The error analyses of realized step frequency showed that both intact and prosthetic limbs generally had more percent errors at higher or lower metronome frequencies (Fig. 2). This means that the realized step frequency of both limbs did not increase in exact 5% increments. Therefore, the lack of adaptation to each step frequency may have affected the results of our study, and this is one of the limitations of our study [30].

Regarding the spatiotemporal parameters, contact time (Fig. 4A) tended to be constant across realized step frequencies, but aerial time (Fig. 4B) decreased constantly as realized step frequency increased in both limbs. This suggests that the individuals with amputation coped with increased step frequency by reducing swing time (Fig. 4C), but maintained contact time for balance and stability. As swing time decreased over increasing realized step frequency, step time and step length also decreased because running speed was kept constant.

For ground reaction force impulses (GRIs), all statistically significant parameters displayed a trend of change towards zero as realized step frequency increased (Fig. 6). One explanation is that the ground reaction forces decreased as realized step frequency increased. This may be due to the lowered magnitude of impulse per step for acceleration required to maintain speed, as the total amount of impulse can be spread among the larger number of steps taken (Fig. 6C). Our results for running propulsive GRI seem to disagree with prior studies on walking that intact leg impulses to be higher than prosthetic leg impulses [18, 43, 49]. This may have been affected by the relationship between leg stiffness and step frequency as discussed earlier. A previous study suggested that increased GRI on the intact side is a larger factor for injury and degenerative disease in individuals with transfemoral amputation, while increased peak GRF is a larger factor in individuals with transtibial amputation [50]. This leads us to the clinical applications for the reductions of GRFs or GRIs in individuals with amputation. Knee osteoarthritis is much more prevalent in the population of individuals with amputation [51, 52], and studies have shown higher GRFs [53] or GRIs [50] to be a possible cause. A study that was done in able-bodied individuals also found correlations between higher GRFs and stress fractures [22]. Although there may be a variety of factors such as prosthetic components [54, 55], physical conditions [56, 57], and other gait parameters such as velocity and motor control [54, 58] that can affect GRFs and GRIs on individuals with amputation, our results indicate that increasing step frequency is a viable strategy to reduce GRFs or GRIs. Therefore, reduction of GRFs or GRIs by increasing step frequency may have implications to reduce potential injury risk during running in individuals with amputation. Future studies should investigate the optimal relationship between running speed and step frequency to minimize injury risk through comprehensive kinetic and kinematic analyses.

The instrumented treadmill was used to control the running speed while varying step frequency. Therefore, we are not able to generalize our study’s data to
overground running. While there are few studies on the generalization of treadmill running to overground running in amputees, studies on able-bodied individuals found no statistical differences between the majority of kinetic and kinematic parameters in the two modalities of running conditions [59–61]. In our study, we allowed five minutes or more for the individuals with amputation to habituate to treadmill running, according to prior studies [62–64]. While some studies found differences in the use of instrumented treadmills between individuals with and without amputation such as variability in gait asymmetry and increased energy costs [59, 65, 66], measurements using instrumented treadmills appeared to be valid for gait analyses [59, 67].

Several other limitations should be taken into account when interpreting our results. We had nine participants (Table 1) in our study which limited the statistical power, but this is common for studies on running in individuals with amputation. Previous studies on running in individuals with amputation had similar small sample sizes due to the difficulty of recruiting participants that meet study criteria [13, 20, 26, 29]. The participants with unilateral transfemoral amputation recruited for the study were also experienced members of track and field teams. However, some recruits were more experienced compared to others. Care should be taken before applying our results to the general population of individuals with amputation.

Conclusions
It is found that increasing step frequency (from −20% to +20% of preferred step frequency) on individuals with amputation decreases the magnitude of GRFs, similar to prior studies on able-bodied individuals. Further, minimized gait asymmetry can be observed in peak vertical GRF between −5% and 0% of the preferred step frequency, and in contact time and vertical ground reaction impulse between −10% and −5% of the preferred step frequency during running. However, individuals with amputation were not able to match the frequency of the intact and prosthetic limbs at higher step frequencies. Therefore, while increased step frequency can reduce GRFs overall, it introduces further asymmetry in individuals with amputation. Further study is needed to investigate the optimal step frequency for the prevention of injury while running.

Abbreviations
BW: Body weight; \( f_{\text{step}} \): Step frequency; GRF: Ground reaction force; GRI: Ground reaction force impulse; Net b-p GRI: Net braking-propulsive ground reaction force impulse; Net m-l GRI: Net medial–lateral ground reaction force impulse; RSP: Running specific prosthesis; vGRF: Vertical ground reaction force; vGRI: Vertical ground reaction force impulse.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12984-022-01012-8.

Additional file 1: Figure S1. Schematic definition of spatiotemporal parameters. A. Illustration of a sprinter with unilateral transfemoral amputation during the contact and aerial phases for the intact (blue) and prosthetic (red) limbs. Toontact, Taerial, Tstep and Tswing indicate contact, aerial, step and swing time, respectively. B. Corresponding vGRF data for the intact and prosthetic limbs in a representative two steps while running at preferred step frequency (in 2.11 m/s) recorded from one participant. The vGRF data were normalized to the BW.

Additional file 2: Table S1. Descriptive statistics for spatiotemporal parameters. Correlation coefficient, mean (SD), median (IQR), and 95% confidence intervals (95%CI) in both limbs at all metronome frequencies are presented. Since calculation of 95%CI are based on normal distribution, we made several cells blank at a particular frequency condition, where the data were not normally-distributed. *p<0.05, **p<0.01.

Additional file 3: Table S2. Descriptive statistics for Peak GRF. Correlation coefficient, mean (SD), median (IQR), and 95% confidence intervals (95%CI) in both limbs at all metronome frequencies are presented. Since calculation of 95%CI are based on normal distribution, we made several cells blank at a particular frequency condition, where the data were not normally-distributed. *p<0.05, **p<0.01.

Additional file 4: Table S3. Descriptive statistics for Asymmetry ratio. Correlation coefficient, mean (SD), median (IQR), and 95% confidence intervals (95%CI) in both limbs at all metronome frequencies are presented. Since calculation of 95%CI are based on normal distribution, we made several cells blank at a particular frequency condition, where the data were not normally-distributed. **p<0.01.

Additional file 5: Table S4. Descriptive statistics for GRI. Correlation coefficient, mean (SD), median (IQR), and 95% confidence intervals (95%CI) in both limbs at all metronome frequencies are presented. Since calculation of 95%CI are based on normal distribution, we made several cells blank at a particular frequency condition, where the data were not normally-distributed. **p<0.01.

Additional file 6. Equations for temporal parameters.

Acknowledgements
Not applicable.

Authors’ contributions
This project was conceived by TK and HH. Data collection were conducted by HM, GH, HH. Data analyses were conducted by TK, MK, HM, HH. The manuscript was drafted by TK and MK. Critical review and edits of the manuscript were done by MH, HM, GH, DI and HH. TK and HH supervised the study. All authors read and approved the final manuscript.

Funding
This work was partly supported by JSPS KAKENHI, Grant Number 26702027 and 19K11338.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
The Institutional Review Board (Environmental and Safety Headquarters, Safety Management Division, National Institute of Advanced Industrial Science and Technology) ethically approved the study, and we conducted it following the guidelines given in the Declaration of Helsinki (1983). Informed written consent was obtained from all participants (or guardians, in participant’s 4 situation) prior to the experiment.
Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Department of Biomedical Engineering, Faculty of Engineering, The Hong Kong Polytechnic University, Hong Kong, China.
2 Artificial Intelligence Research Center, National Institute of Advanced Industrial Science and Technology (AIST), Waterfront 3F, 2-3-26, Aomi, Koto-ku, Tokyo 135-0064, Japan.
3 Department of Mechanical Engineering, Tokyo University of Science, Chiba, Japan.
4 Department of Systems and Control Engineering, Tokyo Institute of Technology, Tokyo, Japan.
5 Research Fellow of Japan Society for the Promotion of Science (JSPS), Tokyo, Japan.

Received: 9 August 2021   Accepted: 14 March 2022
Published online: 23 March 2022

References
1. Eskridge SL, Clouser MC, McCabe CT, Watrous JR, Galarneau MR. Self-reported functional status in U.S. service members after combat-related amputation. Am J Phys Med Rehabil. 2019;98(7):631–5.
2. Nolan L, Lees A. The functional demands on the intact limb during walking for active trans-femoral and trans-tibial amputees. Prosthet Orthot Int. 2000;24(2):117–25.
3. Royer T, Koenig M. Joint loading and bone mineral density in persons with unilateral, trans-tibial amputation. Clin Biomech (Bristol, Avon). 2008;23(10):1119–25.
4. Hobara H, Sakata H, Amma R, Hisano G, Hashizume S, Baum BS, et al. Loading rates in unilateral transfemoral amputees with running-specific prostheses across a range of speeds. Clin Biomech (Bristol, Avon). 2020;75:104999.
5. Nolan L, Wilt A, Dudasinski K, Lees A, Lake M, Wychowanski M. Adjustments in gait symmetry with walking speed in trans-femoral and trans-tibial amputees. Gait Posture. 2003;17(2):142–51.
6. Miller RH, Krupenevich RL, Pruziner AL, Wolf EJ, Schnall BL. Medial knee joint contact force in the intact limb during walking in recently ambulatory service members with unilateral limb loss: a cross-sectional study. PeerJ. 2017;5:e2960.
7. Wise BL, Niu J, Yang M, Lane NE, Harvey W, Felson DT, et al. Patterns of compartment involvement in tibiofemoral osteoarthritis in men and women in and whites and African Americans. Arthritis Care Res (Hoboken, NJ). 2012;64(6):847–52.
8. Roerdink M, Roeles S, van der Pas SC, Bosboom O, Beek PJ. Evaluating joint contact force can be reduced by specific prosthesis. Gait Posture. 2017;56:65–7.
9. Soares DP, de Castro MP, Mendes EA, Machado L. Principal component analysis in ground reaction forces and center of pressure gait waveform during walking of individuals with unilateral transfemoral amputation. Prosthet Orthot Int. 2016;40(6):729–38.
10. Rutkowska-Kucharska A, Kowal M, Winiarski S. Relationship between asymmetry of gait and muscle tone in patients after unilateral transfemoral amputation. Appl Bionics Biomech. 2018;2018.
11. Casto MP, Soares D, Mendes E, Machado L. Plantar pressures and ground reaction forces during walking of individuals with unilateral transfemoral amputation. PM R. 2014;6(8):698–707.
12. Koelwijn AD, van den Bogert AJ. Joint contact forces can be reduced by improving joint moment symmetry in below-knee amputee gait simulations. Gait Posture. 2016;49:219–25.
13. Burkett B, Sneathers J, Barker T. Walking and running inter-limb asymmetry for Paralympic tran-femoral amputees, a biomechanical analysis. Prosthet Orthot Int. 2003;27(1):36–47.
14. Sakata H, Hashizume S, Amma R, Hisano G, Murata H, Takemura H, et al. Anterior-posterior ground reaction forces across a range of running speeds in unilateral transfemoral amputees. Sports Biomech. 2020;1–12.
15. Sano Y, Makimoto H, Hashizume S, Murai A, Kobayashi Y, Takemura H, et al. Leg stiffness during sprinting in trans-femoral amputees with running-specific prosthesis. Gait Posture. 2017;56:65–7.
16. Cook TM, Farrell KP, Carey IA, Gibbs JM, Wiger GE. Effects of restricted knee flexion and walking speed on the vertical ground reaction force during gait. J Orthop Sports Phys Ther. 1997;25(4):238–44.
17. Hobara H, Baum BS, Kwon HJ, Linberg A, Wolf EJ, Miller RH, et al. Amputee locomotion: lower extremity loading using running-specific prostheses. Gait Posture. 2014;39(1):386–90.
18. Schaarachmich M, Lipfert SW, Meier-Gratz C, Scholle HC, Seyfarth A. Functional gait asymmetry of unilateral transfemoral amputees. Hum Mov Sci. 2012;31(4):907–17.
19. Chan MS, Huang SL, Shih Y, Chen CH, Shianny TY. Shear cushions reduce the impact loading rate during walking and running. Sports Biomech. 2013;11(1):334–42.
20. Bonacci J, Hall M, Fox A, Saunders N, Shipsides T, Vicenzino B. The influence of cadence and shoes on patellofemoral joint kinetics in runners with patellofemoral pain. J Sci Med Sport. 2018;21(6):574–8.
21. Schubert AG, Kempf J, Heiderscheidt BC. Inference of stride frequency and length on running mechanics: a systematic review. Sports Health. 2014;6(3):210–7.
22. van der Worp H, Vrielink JW, Bredeweg SW. Do runners who suffer injuries have higher vertical ground reaction forces than those who remain injury-free? A systematic review and meta-analysis. Br J Sports Med. 2016;50(8):450–7.
23. Willy RW, Willson JD, Clewkos K, Baggaaley M, Murray N. The effects of body-borne loads and cadence manipulation on patellofemoral and tibiocomfemoral joint kinetics during running. J Biomech. 2016;49(16):4028–33.
24. Hobara H, Sato T, Sakaguchi M, Sato T, Nakazawa K. Step frequency and lower extremity loading during running. Int J Sports Med. 2012;33(4):310–3.
25. van Oeveren BT, de Ruter CJ, Beek PJ, van Deen JH. Optimal stride frequencies in running at different speeds. PLoS ONE. 2017;12(10):e0184273.
26. Mengelkoch LJ, Kahle JT, Highsmith MJ. Energy costs and performance of transfemoral amputees and non-amputees during walking and running: a pilot study. Prosthet Orthot Int. 2017;41(5):484–91.
27. Czermiecki JM, Morganroth DC. Metabolic energy expenditure of ambulation in lower extremity amputees: what have we learned and what are the next steps? Disabil Rehabil. 2017;39(2):143–51.
28. Zellke KE, Collins SH, Adamczyk PG, Segal AD, Kluft GK, Morganroth DC, et al. Systematic variation of prosthetic foot spring affects center-of-mass mechanics and metabolic cost during walking. IEEE Trans Neural Syst Rehabil Eng. 2011;19(4):411–9.
29. Oudenhoven LM, Boes JM, Hak L, Faber GS, Houdijk H. Regulation of step frequency in transfemoral amputee endurance athletes using a running-specific prosthesis. J Biomech. 2017;51:42–8.
30. Hobara H, Sakata H, Namiki Y, Hisano G, Hashizume S, Usui F. Effect of step frequency on leg stiffness during running in unilateral transfemoral amputees. Sci Rep. 2020;10(1):15965.
31. Hafer JF, Brown AM, deMille P, Hillstrom HJ, Garber CE. The effect of a cadence retraining protocol on running biomechanics and efficiency: a pilot study. J Sports Sci. 2015;33(7):724–31.
32. Wang J, Luo Z, Dai R, Fu W. Effects of 12-week cadence retraining on impact peak, load rates and lower extremity biomechanics in running. PeerJ. 2020;8:e9813.
33. Zeni JA Jr, Higginson JS. Gait parameters and stride-to-stride variability during familiarization to walking on a split-belt treadmill. Clin Biomech (Bristol, Avon). 2010;25(4):383–6.
34. Hobara H, Sakata H, Hashizume S, Kobayashi Y. Leg stiffness in unilateral transfemoral amputees across a range of running speeds. J Biomech. 2019;84:67–72.
35. Farley CT, Gonzalez O. Leg stiffness and stride frequency in human running. J Biomech. 1996;29(2):181–6.
36. Monte A, Mugolo V, Nardello F, Zamparo P. Sprint running: how changes in step frequency affect running mechanics and leg spring behaviour at maximal speed. J Sports Sci. 2017;35(4):339–45.
37. Morin JB, Samozino P, Zameziati K, Belli A. Effects of altered stride frequency and contact time on leg-spring behavior in human running. J Biomech. 2007;40(15):3341–8.
38. Kram R, Griffin TM, Donelan JM, Chang YH. Force treadmill for measuring vertical and horizontal ground reaction forces. J Appl Physiol (1985). 1998;85(2):764–9.
39. Clark KP, Weyand PG. Are running speeds maximized with simple-spring stance mechanics? J Appl Physiol (1985). 2014;117(6):604–15.
40. Grabowski AM, McGowan CP, McDermott WJ, Beale MT, Kram R, Herr HM. Running-specific prostheses limit ground-force during sprinting. Biol Lett. 2010;6(2):201–4.

41. Weyand PG, Bundle MW, McGowan CP, Grabowski A, Brown MB, Kram R, et al. The fastest runner on artificial legs: different limbs, similar function? J Appl Physiol (1985). 2009;107(3):903–11.

42. Weyand PG, Sandell RF, Prime DN, Bundle MW. The biological limits to running speed are imposed from the ground up. J Appl Physiol (1985). 2010;108(4):950–61.

43. Silverman AK, Fey NP, Portillo A, Walden JG, Bosker G, Neptune RR. Compensatory mechanisms in below-knee amputee gait in response to increasing steady-state walking speeds. Gait Posture. 2008;28(4):602–9.

44. Amma R, Hisano G, Murata H, Major MJ, Takemura H, Hobara H. Inter-limb weight transfer strategy during walking after unilateral transfemoral amputation. Sci Rep. 2021;11(1):4793.

45. Howard C, Wallace C, Stokic DS. Stride length-cadence relationship is disrupted in below-knee prosthesis users. Gait Posture. 2013;38(4):880–7.

46. Kobayashi T, Hisano G, Namiki Y, Hashizume S, Hobara H. Walking characteristics of runners with a transfemoral or knee-disarticulation prosthesis. Clin Biomech (Bristol, Avon). 2020;80:105132.

47. Zhu W. p < 0.05, < 0.01, < 0.001, < 0.0001, < 0.00001, < 0.000001, or < 0.0000001. J Sport Health Sci. 2016;5(1):77–9.

48. Farley CT, Ferris DP. Biomechanics of walking and running: center of mass movements to muscle action. Exerc Sport Sci Rev. 1998;26:253–85.

49. Zmitrewicz RJ, Neptune RR, Walden JG, Rogers WE, Bosker GW. The effect of foot and ankle prosthetic components on braking and propulsive impulses during transfalbal amputee gait. Arch Phys Med Rehabil. 2006;87(10):1334–9.

50. Cutti AG, Verni G, Migliore GL, Amoresano A, Raggi M. Reference values for gait temporal and loading symmetry of lower-limb amputees can help in refocusing rehabilitation targets. J Neuroeng Rehabil. 2018;15(Suppl 1):61.

51. Russell Esposito E, Wilken JM. Biomechanical risk factors for knee osteoarthritis when using passive and powered ankle-foot prostheses. Clin Biomech (Bristol, Avon). 2014;29(10):1186–92.

52. Struyf PA, van Heugten CM, Hitters MW, Smets R. The prevalence of osteoarthritis of the intact hip and knee among traumatic leg amputees. Arch Phys Med Rehabil. 2009;90(3):440–6.

53. Silverman AK, Neptune RR. Three-dimensional knee joint contact forces during walking in unilateral transfemoral amputees. J Biomech. 2014;47(11):2556–62.

54. Beck CN, Taboga P, Grabowski AM. How do prosthetic stiffness, height and running speed affect the biomechanics of athletes with bilateral transfemoral amputations? J R Soc Interface. 2017;14(131).

55. Zhang T, Bai X, Liu F, Fan Y. Effect of prosthetic alignment on gait and biomechanical loading in individuals with transfemoral amputation: a preliminary study. Gait Posture. 2019;71:219–26.

56. Taheri A, Karimi MT. Evaluation of the gait performance of above-knee amputees while walking with 3R20 and 3R15 knee joints. J Res Med Sci. 2012;17(3):258–63.

57. Fey NP, Neptune RR. 3D intersegmental knee loading in below-knee amputees across steady-state walking speeds. Clin Biomech (Bristol, Avon). 2012;27(4):409–14.

58. Abouhossein A, Awad MI, Maqbool HF, Crisp C, Stewart TD, Messenger N, et al. Foot trajectories and loading rates in a transfemoral amputee for six different commercial prosthetic knees: an indication of adaptability. Med Eng Phys. 2019;68:46–56.

59. Kluitenborg B, Bredeweg SW, Zijlstra S, Zijlstra W, Buist I. Comparison of vertical ground reaction forces during overground and treadmill running. A validation study. BMC Musculoskelet Disord. 2012;13:235.

60. Oliveira AS, Gazzi L, Ketabi S, Farina D, Kersting UG. Modular control of treadmill vs overground running. PLoS ONE. 2016;11(4):e0153307.

61. Riley PO, Dicharry J, Franz J, Della Croce U, Wilder RP, Kerrigan DC. A kinematics and kinetic comparison of overground and treadmill running. Med Sci Sports Exerc. 2008;40(6):1093–100.

62. Riley PO, Paolino G, Della Croce U, Paylo KW, Kerrigan DC. A kinematic and kinetic comparison of overground and treadmill walking in healthy subjects. Gait Posture. 2007;26(1):17–24.

63. Selgrade RP, Toney ME, Chang YH. Two biomechanical strategies for locomotor adaptation to split-belt treadmill walking in subjects with and without transfemoral amputation. J Biomech. 2017;53:136–43.

64. Wall JC, Charteris J. The process of habituation to treadmill walking at different velocities. Ergonomics. 1980;23(5):425–35.

65. Starholm IM, Gjovaag T, Mengshoel AM. Energy expenditure of transfemoral amputees walking on a horizontal and tilted treadmill simulating different outdoor walking conditions. Prosthet Orthot Int. 2010;34(2):184–94.

66. Trabalisie M, Porcacchia P, Averna T, Brunelli S. Energy cost of walking measurements in subjects with lower limb amputations: a comparison study between floor and treadmill test. Gait Posture. 2008;27(1):70–5.

67. Bagesteiro LB, Gould DH, Evans D. A vertical ground reaction force–measuring treadmill for the analysis of prosthetic limbs. Revista Brasileira de Engenharia Biomédica. 2011;27(1):3–11.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.