Dynamic Balancing Responses in Unilateral Transtibial Amputees Following Transversal Plane Perturbations During Slow Treadmill Walking Differ Considerably for Amputated and Nonamputated Side

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Research

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Abstract

Background

Due to disrupted motor and proprioceptive function lower limb amputation imposes considerable challenges associated with balance and greatly increases risk of falling in case of perturbations during walking. The aim of this study was to investigate dynamic balancing responses in unilateral transtibial amputees when they were subjected to perturbing pushes to the pelvis at the time of foot strike on nonamputated and amputated side during slow walking.

Methods

Fourteen subjects with unilateral transtibial amputation and nine healthy subjects participated in the study. They were subjected to perturbations that were delivered to the pelvis in different directions at the time of foot strike of either left or right leg. Centre of pressure and centre of mass positions, duration of in-stance and stepping periods as well as ground reaction forces were recorded and analysed for significant differences in dynamic balancing responses between healthy subjects and subjects with amputation when subjected to perturbation upon entering stance phases with nonamputated or amputated side.

Results

When perturbations were delivered at the time of foot strike of nonamputated leg subjects with amputation were able to modulate centre of pressure and ground reaction force similarly as healthy subjects. There was a complete lack of in-stance response when perturbations were delivered at the time of foot strike of amputated leg. Instead they used stepping strategy and adjusted placement of nonamputated leg in the ensuing stance phase to increase (forward perturbation) or decrease (backward perturbation) step length or making a cross-step (outward perturbation) which resulted in higher displacement of centre of mass. However, when perturbations were directed inward healthy subjects and subjects with amputation reacted primarily with a stepping response regardless whether healthy, nonamputated or amputated leg was in stance phase.

Conclusions

Results of this study suggest that due to the absence of COP modulation mechanism that is normally supplied by calf muscles people with unilateral transtibial amputation are compelled to choose stepping strategy over in-stance strategy when they are subjected to perturbation on the amputated side. However the stepping response is less efficient than in-stance response which may potentially be significant contributor to frequent falls.

Background
Ability to respond to unexpected perturbation during standing and walking is an essential skill to avoid falling in healthy population and even more so in people with disabilities. Lower limb amputation substantially disrupts motor and proprioceptive functions causing considerable impairments to balance, which greatly increases risk of falling. Approximately half of community-living persons with lower limb amputation fall each year (Miller et al., 2001). Majority of transtibial prostheses used by people with transtibial amputation are still passive devices that can to some extent store and release mechanical energy but are unable to generate it (Windrich et al., 2016). As a result, sensory-motor functions are compromised on one side. Thus, understanding the biomechanical aspects of balance mechanisms used by people with transtibial amputation fitted with passive prosthesis is crucial for deriving appropriate training programs that aim at improving their mobility.

Several studies have investigated balancing responses following unexpected loss of balance during standing in subjects with Unilateral Transtibial Amputation (UTA) (Bolger et al., 2014; Curtze et al., 2010). Findings of these studies suggest that despite different centre of pressure (COP) and stepping responses on nonamputated and the amputated side, UTA subjects control centre of mass (COM) as efficiently as healthy controls which implies equally efficient recovery from perturbation by both groups. However, standing with both feet on the ground enables multitude of strategies to control COM movement that can be shared between both limbs (Hof, 2007). Considerably different situation occurs during walking where, depending on when in gait cycle perturbation occurs, balancing responses may depend on the ability of the stance leg to appropriately modulate COP and GRF under the leg in stance – “in-stance” balancing strategy. In particular, this is the case for very slow walking whereas during faster walking appropriate placement of the ensuing stance is dominant – “stepping” balancing strategy (Matjačić et al., 2019).

Majority of studies investigating balance during walking in UTA subjects used continuous pseudo-random movement of a walking surface (Hak et al., 2013; Beurskens et al. 2014) and in combination of manipulation of visual field (Beurskens et al. 2014). Proactive changes in gait parameters were observed that manifested in decreased step lengths, increased cadence and wider steps which enabled UTA subjects to handle pseudo-random perturbations in similarly efficient fashion as healthy control subjects (Beurskens et al. 2014, Hak et al., 2013). Proactive adaptations of gait parameters were also investigated in experiments where perturbing pushes were delivered to the pelvis in the frontal plane. It was found that when the perturbation timing was unknown a priori no adaptations took place; thus, unexpected perturbation had to be negotiated purely through feedback mechanisms (Major et al., 2018).

Our knowledge on dynamic balancing responses used by UTA subjects following unexpected loss of balance during walking is scarce due to the lack of studies. Previous research mainly focused on the stepping aspects of balance preservation where UTA subjects walked at treadmill speed of 0.8 m/s (Major et al., 2018). Walking speed plays a major role in selection of suitable/appropriate strategy when faced with unexpected loss of balance. Several studies showed that when the walking speed is relatively high (above 0.8 m/s) responses to unexpected perturbation consist primarily in adequate placement of ensuing step(s) (Bruijn and Dieen, 2018; Matjačić et al., 2019). However, when counteracting perturbations at lower walking speeds in-stance strategy plays dominant role (Matjačić et al., 2019).
particular, perturbations to outward direction induce movement of COM to lateral direction with respect to the leg in stance which at very slow walking requires well-organized activity of the stance leg muscles to appropriately modulate COP in sagittal and frontal planes and ground reaction forces (GRF) in all three planes of movement (Matjačić et al., 2019; Matjačić et al., 2020). Clearly, loss of ankle efferent and afferent function must have a pronounced effect on the abilities of UTA subjects to negotiate unexpected loss of balance when perturbation commences while being in the stance phase on the amputated side.

The aim of this study was to investigate kinematics and kinetics of dynamic balancing responses in UTA subjects following perturbing pushes to the pelvis in various directions in the transverse plane while walking slowly on a treadmill. Our hypothesis was that UTA subjects will show absence of in-stance responses after perturbation occurring at the initial contact of amputated side and will consequently have to counteract the effect of perturbation with substantial delay only when entering ensuing stance with their sound limb.

**Methods**

**Participants**

Fourteen UTA subjects (11 females, 3 males; age: 49.9 ± 12.4 years; body mass: 81.9 ± 15.2 kg; height: 173.7 ± 9.2 cm; left amputations: 9, right amputations: 5) and nine healthy adults (2 females, 7 males; age: 46.7 ± 11.9 years, body mass: 79 ± 15 kg, height: 177.3 ± 7.4 cm) without known neurological, muscular-skeletal problems, participated in this study. The inclusion criteria for UTA subjects were: unilateral transtibial amputation, at least 10 years of experience on using passive transtibial prosthesis, have current prosthesis at least one year, the same medical doctor and prosthetist check for socket fitness and alignment, have no problems with the prosthesis, K3 and K4 (Gailey et al. 2002), have no other muscular-skeletal problems, are able to walk independently without walking aids, and are able to follow instructions. The study was approved by the local ethics committee (number 45/2018) and all participants provided written informed consent.

**Perturbing apparatus**

The Balance Assessment Robot for Treadmill walking (BART), consisting of a wide instrumented treadmill and an actuated pelvic link with pelvis brace, was used to deliver perturbing force impulses at the level of the pelvis in the mediolateral and anteroposterior directions during walking on treadmill. The pelvic link interacts with the participant’s pelvis within 5 actuated degrees of freedom (DoF) – translation in the sagittal, frontal and vertical directions, pelvic rotation and pelvic list; the remaining DoF – pelvic tilt – is passive. Haptic interaction between the actuated pelvic link and the participant’s pelvis was put in place via admittance controller in such a way that the interaction force was as low as possible (transparent mode), allowing the participant to freely move their pelvis during walking (Olenšek et al., 2017). Pelvis movement was measured with the pelvic link and was used to estimate the movement of
centre of mass (COM) in a similar way as in our previous studies (Matjačić et al., 2017; Matjačić et al., 2018); ground reaction forces (GRF) and centre of pressure (COP) signals were obtained by four precise force transducers (K3D120, ME Systeme GmbH) placed underneath the treadmill. Left and right foot strikes were identified from instantaneous COP signals by a custom developed algorithm and were used as triggers to apply perturbations. Extended description of the BART is provided in our previous studies (Olenšek et al., 2016; Matjačić et al., 2017).

**Reactive Balance Assessment Protocol**

Subjects started with a five minute introductory session in order to familiarize themselves with the experimental conditions. They were secured with the pelvic brace, which in case of complete loss of balance would hold the subject erect and immediately stop the treadmill. They were instructed to walk within the central area of the treadmill – current pelvic position and the central treadmill area were visualised on a screen in front of the subject for orientation. Treadmill speed was set to 0.5 m/s and perturbation amplitude was normalised to 10% of each participant’s body weight. The perturbation duration was set to 150 ms as in our previous studies (Matjačić et al., 2017; Matjačić et al., 2018). When no perturbing force impulses were delivered during the experiment the pelvic link of the BART was in transparent mode. At the beginning of the experiment each participant walked for approximately three minutes with no perturbations being delivered to assess their unperturbed gait; this initial period was followed by approximately 7 minutes of perturbed walking. During the perturbed walking force impulses were triggered at either the left or right foot contact in one of four perturbation directions: forward, backward, inward or outward. Fig. 1 illustrates a top view of the experimental setup with indicated perturbation directions. All subjects were subjected to seven repetitions for each perturbation direction on both body sides. The time between two consecutive perturbations was randomly chosen from 6 s to 9 s interval. Altogether each subject was subjected to 56 perturbations that were block-randomised.

**Measurements and Data Analysis**

The COM, COP and GRF signals were first segmented into strides. Gait cycle was defined as the period between two consecutive foot strikes of the same leg. Gait cycles immediately after the onset of perturbation and gait cycles where no perturbation occurred were considered for further analysis of the perturbed and the unperturbed experimental conditions respectively. The data of the selected gait cycles were segmented into the “in-stance” periods (from right foot strike to the next left foot strike or from left foot strike to the next right foot strike – from 0% to approx. 60% of gait cycle) and into the “stepping” periods (from left foot strike to the next right foot strike or from right foot strike to the next left foot strike – from approx. 60% to 100% of gait cycle) (Zadravec et al. 2020). In both periods and separately for unperturbed and perturbed experimental conditions they were normalized to the duration of each period to allow visual comparison between different sub-phases of the in-stance and stepping periods of the gait cycles.
Data were grouped into three groups: i) control group (responses to perturbations delivered at left foot strike in the group of control subjects), ii) nonamputated (responses to perturbations delivered at the foot strike of nonamputated leg in the group of UTA subjects) and iii) amputated group (responses to perturbations delivered at the foot strike of amputated leg in the group of UTA subjects). For each subject COM, COP, GRF and durations of in-stance and stepping periods ($T_{\text{in-stance}}$ and $T_{\text{stepping}}$ respectively) were averaged across seven repetitions for each perturbation direction and unperturbed walking. If any of the seven repetitions markedly differed it was excluded from further analysis, however, for each combination of experimental conditions at least five repetitions were averaged. Peak excursions were obtained from averaged COM and COP for each subject and for each perturbation direction as well as for unperturbed walking. $\Delta$COM was calculated as the difference between COM peak excursion of perturbed walking and COM peak excursion of unperturbed walking (sagittal plane – $\Delta$COM$_{\text{AP}}$; frontal plane – $\Delta$COM$_{\text{ML}}$). Likewise, $\Delta$COP was calculated as the difference between COP peak excursion of perturbed walking and COP peak excursion of unperturbed walking (sagittal plane – $\Delta$COP$_{\text{AP}}$; frontal plane – $\Delta$COP$_{\text{ML}}$). $\Delta$COM was determined once in the gait cycle while $\Delta$COP was determined separately for the in-stance and for the stepping period of gait cycles. Similarly, for each perturbation direction separately we calculated the deviation of the durations of in-stance and stepping periods ($\Delta T_{\text{in-stance}}$ and $\Delta T_{\text{stepping}}$ respectively) of perturbed walking from unperturbed walking.

We have further calculated time integrals of perturbed and unperturbed GRF for each perturbation direction separately for the in-stance and for the stepping periods. Finally, these integrals were normalized to body mass of each subjects and subtracted to yield force impulses for both planes (sagittal plane - $\Delta$GRF$_{\text{AP}}$; frontal plane – $\Delta$GRF$_{\text{ML}}$) which acted against perturbation in both periods of the gait cycle. $\Delta$COM can be viewed as a controlled variable while $\Delta$GRF and to some extent also $\Delta$COP (when the human body can be considered as an inverted pendulum) can be viewed as control variables; thus separately determining $\Delta$GRF and $\Delta$COP for the in-stance and stepping periods provides information on the relative share of dynamic balancing responses coming from the in-stance strategy and the stepping strategy.

**Statistical Analysis**

The normal distribution of data was tested using a Kolmogorov-Smirnov test. For each combination of perturbation direction and group (control group, nonamputated group and amputated group) paired samples t-test was conducted to compare the $T_{\text{in-stance}}$ and $T_{\text{stepping}}$ of perturbed walking to $T_{\text{in-stance}}$ and $T_{\text{stepping}}$ perturbed conditions respectively. One-way analysis of variance (ANOVA) was conducted to compare the effect of group factor on $T_{\text{in-stance}}$ and $T_{\text{stepping}}$ of unperturbed walking in control, nonamputated and amputated groups. Likewise, for each perturbation direction separately one-way ANOVA was conducted to compare the effect of group factor on $\Delta T_{\text{in-stance}}$ and $\Delta T_{\text{stepping}}$ in control, nonamputated and amputated groups. Finally, for each combination of perturbation direction and in-stance or stepping periods one-way ANOVA was conducted to compare the effect of group factor on
ΔCOM, ΔCOP and ΔGRF in control, nonamputated and amputated groups. The Bonferroni method was used in post-hoc comparisons. The level of statistical significance was set to 5%. Data processing and data analysis were performed in MATLAB R2018b (The MathWorks, Inc.).

Results

Temporal parameters of unperturbed walking

Figure 2 shows durations of in-stance ($T_{\text{in-stance}}$) and stepping ($T_{\text{stepping}}$) periods for unperturbed walking for the three selected groups. $T_{\text{in-stance}}$ was smallest in amputated group followed by nonamputated group and was largest in control group. There was a statistically significant effect of group factor on $T_{\text{in-stance}}$ ($F(2,34) = 21.27; p < 0.001$) for the three groups and post-hoc analysis showed statistically significant differences in all pair-wise comparisons between groups. The group factor had statistically significant effect also on $T_{\text{stepping}}$ ($F(2,34) = 12.4; p < 0.001$), however only $T_{\text{stepping}}$ of control group with respect to nonamputated and amputated groups was statistically different in the post-hoc comparison.

Responses to forward perturbations

Figure 3 shows representative responses for each of the three groups. The responses to forward perturbations were similar for the control and the nonamputated groups. They were characterized with anterior displacement of $\text{COP}_{\text{AP}}$ under the stance leg accompanied by an increase in $\text{GRF}_{\text{AP}}$ in backward direction which acted to decelerate $\text{COM}_{\text{AP}}$ movement. Such in-stance activity during the in-stance period to a large extent contained the effect of perturbation. On the other hand a complete lack of in-stance response was noticed when perturbations were delivered upon entering the stance phase with amputated leg. Only when the nonamputated leg entered the ensuing stance phase we observed substantially more anterior foot placement compared to unperturbed walking as indicated by appropriately modulated anterior position of $\text{COP}_{\text{AP}}$. $\text{GRF}_{\text{AP}}$ was modified to account for increased anterior position of $\text{COP}_{\text{AP}}$ with respect to $\text{COM}_{\text{AP}}$, which acted to decelerate $\text{COM}_{\text{AP}}$. Using this stepping response following a perturbation at the foot strike of amputated side resulted in a substantially higher peak excursions of $\text{COM}_{\text{AP}}$ as compared to the one following the perturbation commencing at the foot strike of nonamputated side.

Figure 4 shows group mean values and standard deviations of outcome measures for all three groups. Paired samples t-test showed that for all three groups the $T_{\text{in-stance}}$ for perturbed walking was significantly smaller than $T_{\text{in-stance}}$ for the unperturbed walking. However one-way ANOVA showed there was no statistically significant effect of group factor on $\Delta T_{\text{in-stance}}$ ($F(2,34) = 0.9755; p = 0.3873$). On the other hand, $T_{\text{stepping}}$ for unperturbed and for perturbed walking conditions was significantly different only for the amputated group and there was no statistically significant effect of group factor on $\Delta T_{\text{stepping}}$ ($F(2,34) = 2.3135; p = 0.1143$).
One-way ANOVA also showed significant effect of group factor on $\Delta \text{COM}_{\text{AP}}$ ($F(2,34) = 17.0551; p < 0.001$). Post-hoc comparison further showed that $\Delta \text{COM}_{\text{AP}}$ for amputated group was significantly higher than $\Delta \text{COM}_{\text{AP}}$ for control and for nonamputated groups. There was no significant effect of group factor on $\Delta \text{COM}_{\text{ML}}$ ($F(2,34) = 0.0718; p = 0.9308$).

Further analysis showed that in the in-stance period there was a significant effect of group factor on $\Delta \text{COP}_{\text{AP}}$ ($F(2,34) = 14.0771; p < 0.001$). Post-hoc comparison showed that $\Delta \text{COP}_{\text{AP}}$ for amputated group was significantly smaller than $\Delta \text{COP}_{\text{AP}}$ for control and for nonamputated groups. Also in the in-stance period there was a significant effect of group factor also on $\Delta \text{GRF}_{\text{AP}}$ ($F(2,34) = 50.6491; p < 0.001$) where post-hoc comparison further showed that $\Delta \text{GRF}_{\text{AP}}$ for amputated group was significantly different when compared to $\Delta \text{GRF}_{\text{AP}}$ for control or nonamputated groups.

Results were similar also for the stepping period. One-way ANOVA showed significant effect of group factor on $\Delta \text{COP}_{\text{AP}}$ ($F(2,34) = 115.4337; p < 0.001$) and post-hoc analysis again showed significant differences in all pair-wise comparisons. In the stepping period there was also a significant effect of group factor on $\Delta \text{GRF}_{\text{AP}}$ ($F(2,34) = 46.1769; p < 0.001$) and post-hoc comparison showed significant differences between all groups.

**Responses to backward perturbations**

Figure 5 shows representative responses for the three groups. The responses to backward perturbations for the nonamputated group were similar to those obtained for the control group. The response was characterized with posterior COP$_{\text{AP}}$ displacement under the stance leg accompanied by an increase in forward-directed GRF$_{\text{AP}}$ which acted to accelerate COM$_{\text{AP}}$ forward. Perturbation was predominantly contained by the described in-stance activity of the stance leg (intact leg for control group and nonamputated leg for nonamputated group) during the in-stance period. In an amputated group a complete lack of in-stance response was noticed at first. Only when the nonamputated leg entered the ensuing stance phase, which was substantially more backward compared to unperturbed walking, the resulting posterior position of COP$_{\text{AP}}$ in relation to COM$_{\text{AP}}$ modified GRF$_{\text{AP}}$ which acted to accelerate COM$_{\text{AP}}$ forward. This stepping response that was used utilized by the amputated group resulted in a substantially higher $\Delta \text{COM}_{\text{AP}}$ as compared to the one for the nonamputated group.

Figure 6 shows group mean values and standard deviations of outcome measures for all three groups. Paired samples t-test showed that for all three groups $T_{\text{in-stance}}$ for perturbed walking was significantly larger than $T_{\text{in-stance}}$ periods for unperturbed walking. There was also statistically significant effect of group factor on $\Delta T_{\text{in-stance}}$ ($F(2,34) = 4.1728; p = 0.0240$) and post-hoc comparisons showed that only $\Delta T_{\text{in-stance}}$ for amputation group was significantly different from $\Delta T_{\text{in-stance}}$ for nonamputated condition. $T_{\text{stepping}}$ for unperturbed and perturbed walking conditions were statistically different for the nonamputated and the amputated groups but not for the control group. Furthermore, there was a
statistically significant effect of group factor on \( \Delta T_{\text{stepping}} \) \( (F(2,34) = 5.0523; p = 0.0120) \) and post-hoc comparison showed statistically significant difference only between the control group and the nonamputated group.

One-way ANOVA also showed significant effect of group factor on \( \Delta \text{COM}_{\text{AP}} \) \( (F(2,34) = 23.3613; p < 0.001) \) for the three groups. Post-hoc analysis further showed that \( \Delta \text{COM}_{\text{AP}} \) for amputated group was significantly different from \( \Delta \text{COM}_{\text{AP}} \) for control and for nonamputated groups. On the other hand there was no significant effect of group factor noted on \( \Delta \text{COM}_{\text{ML}} \) \( (F(2,34) = 0.6734; p = 0.5166) \).

In addition, in the in-stance period there was a significant effect of group factor on \( \Delta \text{COP}_{\text{AP}} \) \( (F(2,34) = 6.5896; p = 0.0038) \) and further post-hoc comparison showed that only \( \Delta \text{COP}_{\text{AP}} \) for amputated group was significantly different from \( \Delta \text{COP}_{\text{AP}} \) for the control and for the nonamputated groups. It has also been found that in the in-stance period the group factor had statistically significant effect on \( \Delta \text{GRF}_{\text{AP}} \) \( (F(2,34) = 36.4913; p < 0.001) \) and post-hoc comparison showed that \( \Delta \text{GRF}_{\text{AP}} \) for the amputated group was significantly smaller than \( \Delta \text{GRF}_{\text{AP}} \) for the control and for the nonamputated groups.

Results were similar also in the stepping period. One-way ANOVA showed significant effect of group factor on \( \Delta \text{COP}_{\text{AP}} \) \( (F(2,34) = 30.1795; p < 0.001) \) and post-hoc analysis again showed that \( \Delta \text{COP}_{\text{AP}} \) for amputated group was significantly different when compared to \( \Delta \text{COP}_{\text{AP}} \) for control and for nonamputated groups. In the stepping period there was also a significant effect of group factor on \( \Delta \text{GRF}_{\text{AP}} \) \( (F(2,34) = 46.1283; p < 0.001) \) and post-hoc comparison showed significant differences between \( \Delta \text{GRF}_{\text{AP}} \) for the amputated group and \( \Delta \text{GRF}_{\text{AP}} \) for the control and for the nonamputated groups.

**Responses to inward perturbations**

Figure 7 shows representative responses for the three groups. The responses to inward perturbations were similar across all groups. The main strategy was to bring the swinging leg substantially more laterally thus making a substantially wider first step following the perturbation. Once the swinging leg entered the stance phase of a gait cycle, which was substantially more laterally compared to unperturbed walking, the established lateral position of \( \text{COP}_{\text{ML}} \) in relation to \( \text{COM}_{\text{ML}} \) modified lateral \( \text{GRF}_{\text{ML}} \) which acted to decelerate \( \text{COM}_{\text{ML}} \) movement.

Figure 8 shows group mean values and standard deviations of outcome measures for all three groups. Paired samples t-test showed that for all groups \( T_{\text{in-stance}} \) for perturbed walking was significantly smaller than \( T_{\text{in-stance}} \) period for unperturbed walking. Furthermore, statistically significant effect of group factor on \( \Delta T_{\text{in-stance}} \) \( (F(2,34) = 4.1728; p = 0.0240) \) was noted and post-hoc comparison showed that \( \Delta T_{\text{in-stance}} \) for the nonamputated group differed significantly from \( \Delta T_{\text{in-stance}} \) for the control group as well as from \( \Delta T_{\text{in-stance}} \) for the amputated group. \( T_{\text{stepping}} \) for perturbed and for unperturbed walking conditions were statistically different only for the control group but not also for the other two groups. Further
analysis also showed that the group factor had statistically significant effect on $\Delta T_{\text{stepping}}$ ($F(2,34) = 6.8371; p = 0.0030$) and post-hoc comparison showed significant differences between nonamputated group and the remaining two groups.

Statistical analysis also showed that the group factor had statistically significantly effect on $\Delta \text{COM}_{\text{AP}}$ ($F(2,34) = 3.6640; p = 0.0362$) but not on $\Delta \text{COM}_{\text{ML}}$ ($F(2,34) = 0.4607; p = 0.6347$). Subsequent post-hoc comparison of $\Delta \text{COM}_{\text{AP}}$ showed no statistically significant differences between groups.

In the in-stance period group factor did not significantly affect $\Delta \text{COP}_{\text{ML}}$ ($F(2,34) = 0.2143; p = 0.8082$) but it did have significant effect on $\Delta \text{GRF}_{\text{ML}}$ ($F(2,34) = 4.8890; p = 0.0136$). Post-hoc analysis of $\Delta \text{GRF}_{\text{ML}}$ showed statistically significant difference between control and nonamputated groups. Similar was ascertained for the stepping period where no significant effect of group factor on $\Delta \text{COP}_{\text{ML}}$ ($F(2,34) = 0.9457; p = 0.3984$) was found but group factor did have significant effect on $\Delta \text{GRF}_{\text{ML}}$ ($F(2,34) = 5.3994; p = 0.0092$). Subsequent post-hoc comparison of $\Delta \text{GRF}_{\text{ML}}$ again showed statistically significant difference between control and nonamputated groups.

**Responses to outward perturbations**

Figure 9 shows representative responses for the three groups. The responses to outward perturbations for nonamputated group were similar to those for control group. The response was characterized with COP$_{\text{AP}}$ displacement under the stance leg toward the toes and increase in posterior GRF$_{\text{AP}}$ that temporarily decelerated COM$_{\text{AP}}$. COP$_{\text{ML}}$ was displaced toward the outer edge of the foot while an impulse-like increase in lateral GRF$_{\text{ML}}$ was produced that acted to decelerate COP$_{\text{ML}}$ movement. Perturbation was contained completely by the described in-stance activity. On the other hand, we noticed a complete lack of in-stance response in amputated group. Here the main strategy was first to bring the nonamputated leg substantially more laterally thus making a “cross-step”. Once the nonamputated leg entered the stance phase of a gait cycle, which was substantially more laterally compared to unperturbed walking, the established lateral position of COP$_{\text{ML}}$ in relation to COM$_{\text{ML}}$ modified lateral GRF$_{\text{ML}}$ which acted to decelerate COM$_{\text{ML}}$ movement. This stepping response for amputated group resulted in a substantially higher maximal excursions of COM$_{\text{ML}}$ in the frontal plane as compared to the nonamputated group.

Figure 10 shows group mean values and standard deviations of outcome measures for all three groups. Paired samples t-test showed that for all groups $T_{\in\text{stance}}$ for perturbed walking were significantly larger than $T_{\in\text{stance}}$ for the unperturbed walking. There was a statistically significant effect of group factor on $\Delta T_{\in\text{stance}}$ ($F(2,34) = 16.4577; p < 0.001$) and post-hoc comparisons showed that $\Delta T_{\in\text{stance}}$ for amputated group was significantly different than $\Delta T_{\in\text{stance}}$ for the control and for the nonamputated groups. On the other hand, $T_{\text{stepping}}$ for perturbed and for unperturbed walking conditions were
statistically different for nonamputated and amputated groups but not also for the control group. Group factor did not have significant effect on $\Delta T_{\text{stepping}}$ ($F(2,34) = 1.0512; p = 0.1143$).

One-way ANOVA also showed significant effect of group factor on $\Delta \text{COM}_{\text{AP}}$ ($F(2,34) = 3.0192; p < 0.001$) and but no significant differences were found in post-hoc analysis. Similarly, group factor had significant effect also on $\Delta \text{COM}_{\text{ML}}$ ($F(2,34) = 38.5987; p < 0.001$) and subsequent post-hoc comparison showed that $\Delta \text{COM}_{\text{ML}}$ for amputated group was significantly higher than $\Delta \text{COM}_{\text{ML}}$ for the control group as well as for the nonamputated group.

Further analysis showed that in the in-stance period there was a significant effect of group factor on $\Delta \text{COP}_{\text{ML}}$ ($F(2,34) = 40.1967; p < 0.001$) and post-hoc comparison showed that $\Delta \text{COP}_{\text{ML}}$ for amputated group was significantly smaller than $\Delta \text{COP}_{\text{ML}}$ for control and for nonamputated groups. It has also been found that in the in-stance period the group factor had significant effect on $\Delta \text{GRF}_{\text{ML}}$ ($F(2,34) = 81.5651; p < 0.001$) and further post-hoc analysis showed that $\Delta \text{GRF}_{\text{ML}}$ for amputated group was significantly different than $\Delta \text{GRF}_{\text{ML}}$ for control and for nonamputated group.

Results were similar also for the stepping period where the effect of group factor on $\Delta \text{COP}_{\text{ML}}$ was significant ($F(2,34) = 45.3744; p < 0.001$). Post-hoc comparison again showed that $\Delta \text{COP}_{\text{ML}}$ for amputated group was significantly larger than $\Delta \text{COP}_{\text{ML}}$ for control and for nonamputated groups. Finally, statistical analysis also showed that in the stepping period the effect of group factor on $\Delta \text{GRF}_{\text{ML}}$ was statistically significant ($F(2,34) = 34.0405; p < 0.001$). Post-hoc analysis again showed that $\Delta \text{GRF}_{\text{ML}}$ for amputated group was statistically larger than $\Delta \text{GRF}_{\text{ML}}$ for control and for nonamputated groups.

**Discussion**

In this study we investigated dynamic balancing responses to perturbations applied to the pelvis in the sagittal and frontal planes during slow treadmill walking in a group of healthy subjects and a group of high-functioning UTA subjects. The results showed that UTA subjects, when subjected to perturbation upon entering stance phase with their nonamputated side, and control group modulate COP and GRF in a similar way which indicates both groups used similar in-stance strategies. However, when perturbations were delivered at the foot strike on amputated side a complete lack of in-stance strategies was observed and the balancing response started only when the nonamputated leg entered the ensuing stance which was in agreement with our hypothesis.

**Dynamic balancing responses**

Assessing the dynamic balancing responses show that at tested speed of walking the control subjects used in-stance balancing strategy when subjected to perturbation at the time of foot strike in any direction. This reflected in modulation of COP and GRF under the stance leg which has already been addressed in the literature (Hof 2007; Matjačić et al., 2019). Acting against perturbation during the stance
phase is efficient as it quickly minimizes deviation of COM and restores stability (Hof et al., 2010, Matjačić et al., 2019). Likewise, when perturbed at the beginning of the stance phase on nonamputated side UTA subjects were also able to appropriately modulate COP and GRF under their nonamputated leg. However, when perturbations occurred when entering the stance phase on the amputated side UTA subjects did not show such modulation under their prosthetic leg. Instead, they placed their nonamputated leg in the ensuing stance so as to make a longer step (forward perturbation), a shorter step (backward perturbation) or a cross-step (outward perturbation) which indicates their response mainly consisted of the stepping strategy. Delaying the corrective action resulted in higher COM displacement, which is in agreement with findings from other perturbation studies conducted in healthy and post-stroke subjects (Haarman et al., 2017; Matjačić et al., 2018, Zadravec et al. 2020). However, when perturbations were directed inward both control and UTA subjects reacted primarily with a stepping response by making the next step wider, regardless whether healthy, nonamputated or amputated leg was in stance phase (Vlutters et al. 2018; Bruijn and Dieen, 2018; Matjačić et al., 2019).

We found only one study that investigated dynamic balancing responses following unexpected perturbing pushes in the frontal plane applied to the pelvis of UTA subjects (Major et al., 2018). In that study subjects walked at speed of 0.8 m/s and developed stepping responses regardless whether the nonamputated or amputated leg was in stance phase at the time of perturbation. This may be explained by (Matjačić et al., 2019) where it has been shown that balancing responses, when evoked by force impulses to the pelvis of healthy subject at walking speeds of 0.8 m/s and higher, predominantly consist of stepping responses while the contribution from in-stance responses is small (Matjačić et al., 2019). Our study suggests that when UTA subjects counteract discrete perturbation during slow walking they are subjected to greater risk for gait instability and for a potential fall if perturbation is delivered while entering stance phase on their amputated side than if it is delivered while entering stance phase on their nonamputated side.

The outcomes of this study show the importance of calf muscles in efficiently counteracting perturbations directed in forward, backward and outward directions during slow walking. It is interesting to observe that even though UTA subjects have in general well-preserved hip abductor muscles (Molina-Rueda et al., 2014), which have been shown to be the primary contributors to in-stance balancing response following outward perturbation (Matjačić et al. 2018), there was a complete lack of modulation of lateral GRF on the amputated side during the stance phase. In our previous study we have shown that inertial strategy (modulation of lateral component of GRF) and braking strategy (modulation of posterior component of GRF) are closely coupled when healthy subjects counteract outward perturbation that is delivered in double stance while walking very slowly (Matjačić et al., 2020). It has been further shown that this coupling is necessary to efficiently control angular momenta in frontal and sagittal planes. Since the possibilities to efficiently modulate COP under the passive transtibial prosthesis are almost none, braking the movement in the plane of progression, which is normally in domain of calf muscles (Honeine et al. 2014), is not possible. Also missing is a concomitant lateral displacement of COP, which has been shown in several previous studies to be essential in counteracting perturbation in the frontal plane (Hof et al., 2010; Hof and Duysens, 2018, Matjačić et al. 2019). Thus, the results of this study provide further
experimental evidence on close interplay between the inertial and braking balancing strategies that constitute in-stance balancing response following an outward perturbation during very slow walking as shown in our recent study (Matjačić et al., 2020).

Our study also shows that even though treadmill speed was set to be equal for all subjects, stride time was significantly shorter in UTA subjects than in control subjects meaning that cadence was higher and steps were shorter. This is in agreement with findings of other studies (Hak et al., 2013, Breuskens et al., 2014; Sheehan et al., 2015; Major et al., 2018) and may be related to the need of being capable to react faster to prospective perturbation with ensuing step. Since the perturbations in our study occurred at the beginning of the stance phase, adopting shorter and faster steps enabled the group of UTA subjects to react faster with the ensuing step of the nonamputated leg.

Clinical relevance

The perturbing paradigm used in this study bear close similarity with other perturbing modalities. For example, accelerating treadmill belt in forward direction for a period of time at the beginning of the gait cycle is regarded to mimic a slip (Patel and Bhatt, 2014) which is similar to pushing the pelvis of a walking subject in backward direction (Hof and Duysens, 2018). Conversely, accelerating treadmill belt backward for a period of time is regarded to mimic a trip (Joshi et al. 2018) which is similar to pushing the pelvis of a walking subject in forward direction (Hof and Duysens, 2018). Perturbing pushes to the pelvis of walking subject in the frontal plane mimic situations where persons bump into each other in the crowd. In this context results of this study suggest that inferior performance in counteracting perturbing push when applied at the beginning of stance phase on amputated side may also be an indication of inferior responses to real slips, trips and bumps in everyday walking.

Development of active prosthesis focused mainly on the aspects of improving propulsion on the impaired limb but did not explicitly consider dynamic balancing aspects, particularly not in the context of coping with unexpected perturbations (Windrich et al., 2016). Few studies investigated effects of stiffness properties of the prosthetic leg on balance in the sagittal (Major et al., 2016) and frontal plane (Kim and Collins, 2017). They showed that appropriate control of stiffness in the artificial ankle joint may improve balance in terms of reduced variability and asymmetry in spatio-temporal parameters during unperturbed walking at self-selected speed. The results of our study suggest that the developers and producers of active transtibial prosthesis should consider incorporating in their designs also functionality that could efficiently address the need of appropriate control of COP that would enable UTA subject to effectively cope with unexpected loss of balance during slow walking.

Methodological considerations and limitations of the study

In this study we have selected a relatively low walking speed of 0.5 m/s for assessment of dynamic balancing responses. We have shown in our previous study that when walking on a treadmill with
walking speed ranging from 0.4 to 0.6 m/s, in-stance balancing strategies play dominant role when counteracting perturbations (Matjačić et al., 2019). Another aspect related to the choice of suitable walking speed is that starting and stopping of walking as well as changing directions are all manoeuvres that are typically performed at reduced walking speed (Weerdesteyn et al., 2008).

We also focused on balancing responses to only one level of perturbation amplitude, i.e. 10% of body weight. Previous studies (Hof et al., 2010; Matjačić et al., 2019) have shown that such perturbation amplitude is strong enough to elicit substantial imbalance during walking without exciting leg pivoting or arm and trunk movement. The choice of triggering perturbations at the beginning of stance phase was motivated by the observation that it elicits the use of the in-stance strategy to the largest extent (Matjačić et al., 2019). While the results of this study would be different if another set of experimental parameters was used it is our opinion that the set used appropriately challenged the group of UTA subjects.

The BART was controlled such that the interaction forces between the walking subject and the pelvis link were as low as possible. We have assessed the effect of interaction forces in a previous study and found that the influence of these forces on COP and GRF in the sagittal and frontal planes as well as on the EMGs of major lower limb muscles during unperturbed walking with walking speed ranging from 0.4 to 0.8 m/s was negligible (Olenšek et al., 2017). In another study we have demonstrated that interaction between the balance assessment robot and the pelvis of a walking subject is purely passive, meaning that there is no exchange of energy between walking subject and the BART except for the period when a perturbing push is delivered (Matjačić et al., 2017). Thus, we may consider that the method used to deliver perturbations to the pelvis of walking subjects had negligible effects on the presented results.

Only high-functioning UTA subjects that were experienced and self-confident walkers have been included in this study. We may thus expect that balancing responses in less experienced prosthesis users would have been worse.

**Conclusion**

The results of this study show how important calf muscles are for negotiating forward-, backward- and outward-directed perturbations at the slow walking speed efficiently. Since people after transtibial amputation lack this important resource, dynamic balancing response following the perturbation occurring at the heel strike of the amputated side begins with a substantial delay. Thus, the consequential stepping response is much less efficient compared to the in-stance response following the perturbation occurring at the heel strike on the nonamputated side which points out one of the potential causes of frequent falls among UTA population.

The findings of this study suggest that to improve their balancing responses to unexpected balance perturbation people fitted with passive transtibial prostheses should undergo perturbation-based balance training during clinical rehabilitation. Likewise, the results of this study are relevant for the developers of active powered-prostheses, which should consider incorporating in their designs proper algorithms that
would be able to identify destabilizing event during walking and modulate COP and GRF under the prosthetic leg during stance phase accordingly.

**Abbreviations**

UTA – Unilateral Transtibial Amputation

COP – Centre of Pressure

COM – Centre of Mass

GRF – Ground Reaction Force

DoF – Degrees of Freedom

ΔCOM – Difference in peak values of COM between perturbed and unperturbed walking

ΔCOM\textsubscript{AP} – Difference in peak values of anteroposterior component of COM direction between perturbed and unperturbed walking

ΔCOM\textsubscript{ML} – Difference in peak values of mediolateral component of COM direction between perturbed and unperturbed walking

ΔCOP – Difference in peak values of COP between perturbed and unperturbed walking

ΔCOP\textsubscript{AP} – Difference in peak values of anteroposterior component of COP between perturbed and unperturbed walking

ΔCOP\textsubscript{ML} – Difference in peak values of mediolateral component of COP between perturbed and unperturbed walking

T\textsubscript{in-stance} – Duration of in-stance period.

T\textsubscript{stepping} – Duration of stepping period.

ΔT\textsubscript{in-stance} – Difference in duration of in-stance period between perturbed and unperturbed walking

ΔT\textsubscript{stepping} – Difference in duration of stepping period between perturbed and unperturbed walking

ΔGRF – Difference in GRF integrals between perturbed and unperturbed walking

ΔGRF\textsubscript{AP} – Difference in anteroposterior component of GRF integrals between perturbed and unperturbed walking
ΔGRF<sub>ML</sub> – Difference in mediolateral component of GRF integrals between perturbed and unperturbed walking

Declarations

Ethics approval and consent to participate

The study was approved by the Republic of Slovenia National Ethics Committee, application number 45/2018. All participants gave written informed consent.

Consent for application

Not applicable.

Availability of data and materials

The data used in this study may be available by the corresponding author upon a reasonable request to any qualified researcher.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

MZ, HB and ZM contributed to the concept, research design and interpretation of the experimental results. HB recruited individuals with amputations that met inclusion criterial. MZ and AO performed perturbation-based measurements. MZ contributed to the signal processing, data analysis and prepared figures. ZM drafted the initial manuscript. AO wrote the final version of the manuscript. All authors critically revised the manuscript and approved the final version.

Availability of data and materials
The data used in this study may be available by the corresponding author upon a reasonable request to any qualified researcher.

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Figures
Experimental setup for assessing balance responses after perturbations applied to pelvis. Perturbations were applied in forward, backward, inward, outward directions and were triggered at either left or right foot strike.
Figure 2

Group mean values and standard deviations for the duration of in-stance and stepping periods of unperturbed walking for all three groups. P-values are given where statistically significant effect of group factor and significant differences between groups in Bonferroni post-hoc paired comparisons were found.

Figure 3

Kinematics and kinetics following forward perturbation. Mean values and standard deviations are shown for group representatives.
Figure 4

Outcome measures of temporal parameters, kinematics and kinetics following forward perturbation. Group mean values and standard deviations are shown for all three groups. P-values are given where statistically significant effect of group factor and significant differences between groups in Bonferroni post-hoc paired comparisons were found.

Figure 5
Kinematics and kinetics following backward perturbation. Mean values and standard deviations are shown for group representatives.

Figure 6

Outcome measures of temporal parameters, kinematics and kinetics following backward perturbation. Group mean values and standard deviations are shown for all three groups. P-values are given where statistically significant effect of group factor and significant differences between groups in Bonferroni post-hoc paired comparisons were found.
Figure 7

Kinematics and kinetics following inward perturbation. Mean values and standard deviations are shown for the group representatives.

Figure 8
Outcome measures of temporal parameters, kinematics and kinetics following inward perturbation. Group mean values and standard deviations are shown for all three groups. P-values are given where statistically significant effect of group factor and significant differences between groups in Bonferroni post-hoc paired comparisons were found.

**Figure 9**

Kinematics and kinetics following outward perturbation. Mean values and standard deviations are shown for the group representatives.
Figure 10

Outcome measures of temporal parameters, kinematics and kinetics following outward perturbation. Group mean values and standard deviations are shown for all three groups. P-values are given where statistically significant effect of group factor and significant differences between groups in Bonferroni post-hoc paired comparisons were found.