Vibrations on Mastoid Process Alter the Gait Characteristics During Walking on Different Inclines

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Abstract

Background.~~:~~ ~~Vestibular dysfunction leads to chronic dizziness, falls, and imbalance, which causes significant impacts on quality of life, even a simple walking from one point to another.~~ Eighty-eight percent of the persons with bilateral vestibular dysfunction have reported at least one fall within the past five years. The apparent alternations due to the bilateral vestibular dysfunctions (BVD) are the gait characteristics, such as slower walking speed, prolonged stance phase, and shorter step length. Unexpectedly, due to~~because~~ the prevalence of this BVD is relatively low, attention is not obtained as same as in other vestibular disorders. ~~However~~Moreover, how does walking on different inclines, ~~which is~~ part of daily activity, alter~~s~~ the gait characteristics under the ~~malfunction~~ unreliable~~of~~ ~~the~~ bilateral vestibular systems ~~is still unknown.~~? Previous studies have used vibration-based stimulations (VS) as a perturbation to understand the postural control during walking while the bilateral vestibular systems were perturbed. Therefore, this study attempted to ~~answer the abovementioned question~~extend the knowledge to understand the alternations in spatial-temporal gait ~~using bilateral vibration-based stimulations (VS)~~under perturbed bilateral vestibular systems ~~while~~ while walking on different inclines.

Methods.~~:~~ Nineteen healthy young adults participated in this study. Eight walking conditions were randomly assigned to each participant: 0%, 3%, 6%, and 9% grade of incline with/without VS. ~~The VS was generated by a mechanical vibration using two electromechanical vibrotactile transducers at the frequency of 100Hz and 130% of the amplitude the participants could perceive.~~ The dependent variables were as follows: stance time, double support time, step length, step time, step width, foot clearance, and respective variabilities. All dependent variables were defined by two critical gait events: heel-strike and toe-off. ~~A total of 100 gait cycles (200 steps) were used. The variabilities were defined as the standard deviation of 100 gait cycles.~~ Pre-Hoc paired comparisons with Bonferroni corrections were used to prioritize the dependent. ~~variables under bilateral VS while walking on different inclines.~~ A two-way repeated measure was used to investigate the effect of VS and the effect of inclines (2 with/without VS X 4 types of inclines) on the selected dependent variables from Pre-Hoc analysis. Post-Hoc comparisons were also corrected by the Bonferroni method.

Results.~~:~~ The step length, step time, foot clearance, and foot clearance variability were selected by the Pre-Hoc analysis because the corrected paired t-test demonstrated a significant VS effect ($p < 0.05$) on these gait parameters at least one of four inclines. The significant interaction between the effect of VS and the effect of inclines was found in step length ($p = 0.005$), step time ($p = 0.028$), and foot clearance variability ($p = 0.003$). The results revealed that implementing a VS increased step length~~,~~ and step time ~~, and foot clearance variability~~ when walking on 0%, 3%, and 9% of grade inclines. In particular, the foot clearance variability was only found when walking on 9% of grade inclines.

Conclusion.~~:~~ ~~Additionally, walking on a 9% grade incline increased the step length and time than level walking.~~ The observations in the current study suggested that VS increased the step length, step time, foot clearance, and foot clearance variability while walking on inclines. These results suggested that these gait parameters might be promising targets for future clinical investigations in patients with BVD while walking on different inclines. Importantly, the increases in spatial-temporal gait performance under b~~B~~ilateral VS might be an indicator of gait improvement while walking on different inclines. ~~This study suggested 1) VS altered gait characteristics when walking not only on the level treadmill but also on different inclines, and 2) the inclines could be used as a diagnostic tool for patients with vestibular disorders.~~

Keywords: vestibular stimulation, mastoid vibration, gait, incline

Abstract Word Count: 441~~98~~

Introduction

The vestibular system is crucial to maintaining the dynamic balance in human locomotion (Horak 2009)~~(Horak, 2009)~~ ~~(Horak, 2009)~~~~[13]~~. Specifically, semicircular canals, utricles, and saccule in the labyrinth play essential roles in detecting the rotational and linear acceleration of body movement (Rabbitt 2019)~~(Rabbitt, 2019)~~~~[24]~~, such as the daily gait. A study reports~~ed~~ that persons ~~88% of these patients~~ with ~~bilateral vestibular hypofunction~~bilateral vestibular dysfunctions (BVD) ~~report at least one fall within the past five years~~have an increased 31-fold potential fall risk compared to the average residents in the United States; moreover, these patients with BVD have nine times greater fall risk compared to those health controls with dizziness and unsteadiness during standing and walking (Ward et al. 2013)~~(Ward et al., 2013)~~~~[29]~~. ~~Also, based on this analysis, patients with bilateral vestibular hypofunction have an approximately ten-fold~~

higher potential fall risk than those with other vestibular disorders[29]. One of many factors leading to falls in patients with BVD may be attributed to oscillopsia (Batuecas-Caletrio et al. 2020). (Batuecas-Caletrio et al., 2020). The oscillopsia is an illusion of the surroundings moving caused by an impairment of the vestibular-ocular reflex, which plays a critical role in correcting eye position during alternations in head position so that vision remains on the target (Hain et al. 2018)(Hain et al., 2018). In particular, a study involving 37 patients with BVD reportsed that 81% of these patients havehad moderate to severe oscillopsia severity while walking at different speeds (Guinand et al. 2012).(Guinand et al., 2012). It is worth mentioning in the abovementioned study that ten out of 37 patients with BVD can't evenwere not able to walk at 6 km/h (an average and comfortable walking speed for healthy controls) due to the imbalance caused by the oscillopsia (Guinand et al. 2012). (Guinand et al., 2012). Therefore, the alternations in gait characteristics in patients with BVD during walking may possibly be caused by this illusion. Any deterioration/malfunction in the vestibular system leads to nystagmus, unstable gait, and loss of spatial orientatio

A 2008 National Health Interview Survey estimatesd that a prevalence of 28 per 100,000 or 64,046 US adults has suffered the BVD. n. These symptoms, as mentioned earlier, may eventually lead to falls[29]. A 2008 National Health Interview Survey estimates that a prevalence of 28 per 100,000 or 64,046 US adults has suffered the bilateral vestibular hypofunction.

Moreover, 88% of these patients with bilateral vestibular hypofunction report at least one fall within the past five years[29]. Also, based on this analysis, patients with bilateral vestibular hypofunction have an approximately ten-fold higher potential fall risk than those with other vestibular disorders[29]. Unexpectedly, because the prevalence of this bilateral disorder BVD is relatively low, the attention is not obtained (Kim & Kim 2022)(Kim et al., 2022) [16] as same as other vestibular disorders, such as benign paroxysmal positional vertigo, vestibular neuronitis, and Meniere's disease. Several common clinical diagnoses have been developed to identify the patients with BVD, such as the head impulse test (Halmagyi & Curthoys 1988).(Halmagyi and Curthoys, 1988), dynamic visual acuity (Demer et al. 1994)(Demer et al., 1994), and caloric testing, which Dr. Robert Barany invented. The impulse test is to test the eye movements after examiners quickly and unpredictably move the head to 10 to 15 degrees of neck rotation while subjects sit or stand naturally. In patients with BVD, the vestibular-ocular reflex may be impaired or absent, and the eye movements deviate from the designated target toward the side where the head is rotated when the head impulse test is applied (MacDougall et al. 2009)(MacDougall et al., 2009).. Similar to the head impulse test, dynamic visual acuity is used to determine the accuracy of eye-track ability (vestibular-ocular reflex). At the same time, examiners oscillate the patient's head horizontally or vertically and instruct the patient to read the optotypes on a visual acuity chart. When applying this dynamic visual acuity to patients with BVD, these patients show a decline of two or more lines because the vestibular-ocular reflex may no longer be able to stabilize the gaze on a visual acuity chart (Sargent et al. 1997).(Sargent et al., 1997). The caloric test is to examine the integrity of horizontal semicircular canals and the afferent pathway pouring either cold or warm water into ears. A study reveal~~s~~ed that patients with BVD reduced caloric responses and undergo rotational chair testing (Sargent et al. 1997).~~(Sargent et al., 1997).~~ Although these abovementioned diagnoses have been widely used in clinics, a review state~~s~~d that using these diagnoses for identifying mild BVD remains a diagnostic challenge (Petersen et al. 2013).~~(Peterson et al., 2013).~~ Additionally, a book chapter emphasize~~s~~d that the caloric test has a high sensitivity to unilateral vestibular disorder but is relatively insensitive to BVD (Youmans & HR 2011)~~.(Winn 2011).(Youmans et al., 2011).~~

Based ~~on~~ upon the objective of public health in the World Health Organization (WHO)~~,~~ to assess and monitor the health of communities and populations at risk, it is essential to develop an objective measurement to identify ~~the progress of bilateral vestibular disorders~~BVD. ~~Furthermore, this objective measurement could reduce the potential risks of falls.~~ Therefore, in the past decades, increasing studies have attempted to understand the balance control mechanism in different kinds of vestibular ~~hypofunction~~[8, 12, 17, 23]dysfunctions ~~(Chen et al. 2021)(Chen et al., 202~~(Chen et al. 2021; Herssens et al. 2021; Kingma et al. 2019; McCrum et al. 2019).~~1(Herssens et al. 2021), Herssens et al., 2021, Kingma et al., 2022, McCrum et al., 2019)~~ ~~by~~ using different measures, such as ~~gait characteristics~~the galvanic vestibular stimulation. A review (Dlugaiczyk et al. 2019)~~(Dlugaiczyk et al., 2019)~~ indicate~~s~~d that galvanic stimulation ~~can~~could be used for diagnosing different types of vestibular deficits by perturbing the vestibular system. Also, this galvanic stimulation can be used for rehabilitation in balance control while turning the amplitude of stimulations to a noisy (sub-threshold, persons can't perceive) level. However, this galvanic vestibular stimulation has its inevitable side effects, such as increases in anxiety levels (Pasquier et al. 2019) ~~(Pasquier et al., 2019)~~ and mild uncomfortable sensations (Utz et al. 2011).~~(Utz et al., 2011).~~ These side effects may hinder the true outcomes of motor adjustments due to the unreliable vestibular system. ~~Importantly,~~Therefore, there is a need to ~~this is also one of the main goals of this study to~~ find an effective measure to identify ~~bilateral vestibular diseases~~the alternations in gait characteristics under perturbed bilateral vestibular function. Applying~~Using~~ bilateral supra-threshold vibration-based ~~vestibular~~ stimulation (persons can perceive) on the mastoid process is one of the options to perturb the vestibular system and further investigate the role of the vestibular system for balance control in standing and walking (Chien et al. 2016; Lin et al. 2022; Lu et al. 2022).~~(Chien et al., 2016, Lin et al., 2022, and Lu et al., 2022)during walking. Also, previous studies~~[9, 19] ~~have explored the effects of mastoid vibration on the vestibular system during standing and level walking on a treadmill.~~ In other words,~~,~~ by ~~showing~~ measuring the increased sway variability and margins of stability induced by vibration-induced stimulations~~-~~, the ~~with no reports of side effects~~different types of vestibular deficits may potentially be identified by the alternations in gait performance (Chien et al. 2016; Lu et al. 2022).~~(Lu et al., 2022, Chien et al., 2017).~~ Additionally, using this vibration-induced stimulation has a similar effect as the galvanic vestibular stimulation without uncomfortable sensations (Lin et al. 2022).~~(Lin et al., 2022).~~ .

The gait characteristics have been used to identify the vestibular function (Chae et al. 2021; Herssens et al. 2021)(Chae et al., 2021, Herssens et al., 2021)[7, 12]. For example, a longitudinal observation indicates that immediately after having unilateral vestibular neuritis, the step width in these patients is significantly greater than in controls (Chae et al. 2021)(Chae et al., 2021)[7]. After eight weeks of recovery, significant increases in walking speed, stride length, and swing phase and significant decreases in stance phase and step width are observed. These results suggest that the feasibility of use in gait characteristics to identify the differences between controls and patients with unilateral vestibular neuritis and also to monitor the progress of recovery. Similarly, when comparing the gait characteristics among healthy controls, patients with unilateral vestibular loss, and patients with bilateral vestibular loss demonstrated shorter stride lengths during walking straight for 10 meters. , the results reveal that the stride length in both patients with bilateral and unilateral vestibular loss is significantly smaller than healthy controls. Similarly, a study finds found higher cadence and lower step time in patients with bilateral vestibulopathy than in controls (Herssens et al. 2021)(Herssens et al., 2021)[12].

The gait variability measure, which has been widely used to identify the potential fall risk in the older population (sample size: 52) (Hausdorff et al. 2001), , Hausdorff et al., 2001) and the different types of neurological disorders, such as patients with Parkinson's disease (sample size: 51) (Ma et al. 2022) , Ma et al., 2022) and patients with stroke (sample size: 16), (Zukowski et al. 2019)Zukowski et al., 2019). , Indeed, the gait variability measure identifies the differences in spatial-temporal gait variabilities between healthy young controls and patients with bilateral vestibulopathy (McCrum et al. 2019).(McCrum et al., 2019). However, this gait variability measure fails failed to determine the differences between healthy older controls and patients with bilateral vestibulopathy (12 older adults, age: 71.5 years and 44 patients with bilateral vestibulopathy, age: 57.6 years), (McCrum et al. 2019)McCrum, et al., 2019)[23] in cadence variability, step time variability, step length stability, step width variability, and double support phase variability (McCrum et al. 2019)(McCrum et al., 2019)[23]. These insignificant results may be attributed to the limited number of measured steps or gait cycles. Or the differences in age and sample sizes between healthy older controls and patients with vestibulopathy. Or the gait variability itself is not sensitive enough to detect the effect of deteriorated vestibular system on locomotion. To answer the abovementioned studiesFurthermore, (Lu et al. 2022)Lu et al. (2022) useuses the gait variability measure to and find identify the differences in locomotion gait variabilities when the vestibular system is perturbed either unilaterally or bilaterally. The results reveal that applying either bilateral or unilateral vestibular vibrations increasesincrease the step width variability, which is a sign of relative gait instability, compared to no vestibular stimulation condition, confirming the feasibility of use in variability measure for the vestibular dysfunctions (Lu et al. 2022)(Lu et al., 2022)[20]. However, similar to the abovementioned studies, using gait variability measure also fails to identify the differences between unilateral and bilateral vestibular manipulation[20].

Walking on different inclines is part of daily activities. Importantly, walking on different inclines might induce alternations in self-referenced coordinates with respect to global coordinates that could influence vestibular input due to the different perceptions of gravity (Cromwell 2003)(Cromwell, 2003)[10]. Specifically, when walking on the inclines, the vector of a sum of translational acceleration and gravitational acceleration is different from the level walking, resulting in alterations in otolith information (Dai et al. 1994)(Dai et al., 1994)[11]. Thus, do these alternations in the inclines alterations affect the gait or gait variability of patients with vestibular disorderswhile the bilateral vestibular system becomes unreliable? Or can these differences in gait or gait variabilities attributed to the different inclines be used for differentiating the variety of vestibular disorders?

To answer all the abovementioned important research questions, this study attempted to understand the fundamental alternations in gait characteristics under unreliable bilateral vestibular systems while walking on different inclines in healthy young adults. applies vibration-based stimulations (VS) on bilateral vestibular systems to investigate the gait alternations during walking on different inclines. This study hypothesized that 1) applying the VS on bilateral systems caused the increases in stance time, double support time, step time, and step width but decreased the step length and foot clearance, and 2) applying the VS increased the gait variability in all gait characteristics.

Materials & Methods

Participants:

Nineteen healthy young adults participated in this study (10 males and nine females; 24.42 ± 2.11 years old; walking speed: 1.41 ± 0.22 m/s; body mass: 60.68 ± 11.42 kg; height: 1.66 ± 0.08 m). We excluded participants if they had any neurological disorders, neuropathy due to diabetes, joint injuries, or unanticipated falls in the prior year. Importantly, these participants were also excluded if they got a score above zero on the dizziness handicap inventory, indicating no the potential deficits in the vestibular system. Also, these participants verbally declared that they had no deficits in the vestibular systems at or before the day of data collection. Moreover, these participants never experienced any type of vestibular stimulationstimulations. If participants agreed to attend the data collection, they needed to sign an informed consent before the data collection. This study obeyed the guideline and regulations of the University of Nebraska Medical Center Institutional Review Board that approved this study (IRB# 379-17-EP).

Experimental Materials:

The gait characteristics were captured by a Qualisys motion capture system. This system contained eight high-speed infra-red digital cameras (Qualisys AB, Gothenburg, Sweden) and used the Qualisys Tracker Manager (QTM) software (Qualisys AB) to record the three-dimensional gait data at 100Hz. Four retro-reflective markers were placed on the toe (second metatarsophalangeal joint) and the heel of both legs. Eight inclined treadmill walking conditions

(0%, 3%, 6%, and 9% grade of inclination with/without bilateral vestibular perturbation) were randomly assigned to all participants. Also, the RTM 600 treadmill in the current study (Biodex RTM 600, Biomex Medical System, Inc, Shirley, New York, USA. Fig. 1) included a 50.8 x 160 cm Teflon-impregnated running deck and incorporated a shock-absorbing surface. The RTM 600 treadmill offered a speed range of 0-10 mph (0 – 4.47 m/s) with 0.1 mph (0.045 m/s) speed increments. This treadmill also could be configured for 0 to 15% grade (0 to approximately 8.5 degrees) inclines with 1% grade increments. A handrail was provided for participants to maintain their balance if they felt unsteady during walking. Importantly, a safety lanyard and a red safety button were provided for participants and experimenters to stop the treadmill when encountering any potential risks. The safety lanyard was attached to the participants' waists to prevent any sudden falls.

The different types of vestibular simulations were generated by a mechanical vibration using two electromechanical vibrotactile transducers (EMS2 tactors, Engineering Acoustics, FL, USA. Fig. 1). These two transducers were attached inside a customized swim cap using double-sided adhesive strips and can be adjusted to place on the mastoid processes bilaterally. These transducers were designed for mounting with a cushion and could produce high displacement levels that allow the vibration to be easily sensed even through layers of padding. The maximum peak-to-peak displacement was 2 mm. The height and weight of the tactors were 19.05 mm and 24g. The diameter of this transducer was 49.26 mm (Fig. 1). The frequency was set at 100Hz. Also, the amplitude of supra-threshold vibrations was set at 130% of the amplitude that the participants could perceive (Lu et al. 2022)(Lu et al., 2022)[20]. This frequency of bilateral VS on the mastoid process was selected as 100Hz because this frequency is strong enough to trigger the nystagmus and needs compensatory responses from the vestibular system in healthy young adults (Perez 2003),(Perez, 2003), patients with vestibular neuritis (Nuti & Mandala 2005)(Nuti and Mandala, 2005) and in patients with otosclerosis (Manzari & Modugno 2008)(Manzari and Modugno, 2008). The frequency of amplitude of the mastoid vibrations was controlled by software (TAction Creator, Engineering Acoustics, FL, USA) by sending the designed signal from the laptop to the controller through Bluetooth technology. The minimum perceived amplitude was detected by adjusting the amplitude of vibration through TAction CreatorCreater commercial software until participants could perceive it during quick standing. This abovementioned procedure was performed three times to get the average minimum perceived amplitude. The vibrations were administrated to participants on both mastoid processes simultaneously. The vibration activation was an impulse typeimpulse-type, indicating a 0.5 s activation period and 0.5 s de-activation period. The rationale for using this impulse-type vibration was to reduce the saturation of the vestibular sensation (Chien et al. 2016)(Chien et al., 2016)[9].

Experimental Protocol:

A total of eight walking trials were randomly assigned to participants in one visit. These walking conditions were walking on 0%, 3%, 6%, and 9% grade of inclines with/without bilateral mastoid vibrations. Each walking trial lasted three minutes. Before the beginning of the data collection, the preferred walking speed was determined for each participant. First, participants needed to stand on the sidebar of the treadmill; then, the belt was accelerated to 0.8 m/s. Next, participants stepped on the treadmill belt, holding the handrail to prevent tripping (Fig. 2). After 30 seconds, participants were encouraged to walk naturally without holding the handrail. Then, experimenters asked each participant~~s as~~ “is this walking speed similar to the walking speed when you walk on the street?”. The walking speed was repeatedly assessed after 10 seconds (±0.1 m/s) until participants admitted, “this is my comfortable walking speed.” After the preferred walking speed was determined, participants needed to walk for five minutes to familiarize the treadmill on 0% grade incline. After the familiarization, participants needed to take a two-minute mandatory rest break to catch their breath. Then, eight walking conditions were randomly provided to these participants. Also, participants needed to take a two-minute mandatory rest between trials to eliminate the training effect from different inclines and vibrations (Lu et al. 2022)~~(Lu et al., 2022)[20]~~. Additionally, at the end of each trial, participants were verbally asked whether they had any discomfort sensation, such as nausea, vomiting, or dizziness induced by the inclines or the mastoid vibrations. If they felt any abovementioned discomfort sensations~~sensation~~, the data collection was terminated.

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Data Analysis:

The spatial-temporal gait parameters were used as follows: stand time (% of the gait cycle), double support time (% of the gait cycle), step length (mm), step time (ms), step width (mm), ~~maximum heel elevation~~foot clearance (mm) and their respective variabilities. ~~The variability was defined as the standard deviation of 100 gait cycles (200 steps).~~ The 100 gait cycles were selected from the 31st stride to the 130th stride to eliminate the large step-to-step fluctuations due to the speed up and slowing down at the beginning/end of the walking. One heel-strike was defined as the farthest position that a heel marker could reach in the anterior-posterior direction within one gait cycle, whereas the one toe-off was defined as the minimum position that a toe marker could make in the anterior-posterior direction within one gait cycle (Ihlen et al. 2012) ~~(Ihlen et al., 2012).~~ The foot clearance was defined as the maximum vertical height that a heel maker could reach from the ground in one gait cycle (Mariani et al. 2012)~~(Mariani et al., 2012)[22]~~. The ~~duration~~ step time of one gait cycle was defined as the time between the one heel strike and the subsequent heel strike in the ~~ipsilateral~~ contralateral leg. The stance phase was defined as the percentage of the gait cycle from one heel-strike to one toe-off in the same leg. The double support time was defined as the percentage of the gait cycle that both feet stayed~~were staying~~ on the ground during walking. The step length was defined as the traveling distance of the treadmill belt in the anterior-posterior direction from one heel strike to another contralateral heel strike. ~~The step time was the time between two consecutive heel strikes from different legs.~~ Also, the step width was calculated by the distance between two continuous heel strikes in the medial-lateral direction. ~~The foot clearance was defined as the maximum vertical height that a heel maker could reach from the ground in one gait cycle[22].~~ The gait variability was defined as the standard deviation within 100 gait cycles for each dependent variable. The current study selected the standard deviation as a gait variability measure because using the coefficient of variation tended to amplify the values if the mean values were close to zero (Gouelle et al. 2018). ~~(Gouelle et al., 2018).~~

Statistical Analysis:

A Shapiro-Wilk Normality Test was used to identify the normality of each spatial-temporal gait parameter and their respective variabilities. The alpha value was set at 0.05. If the alpha value for the Shapiro-Wilk test was greater than 0.05, indicating the data was normally distributed. ~~d, a two-way repeated measure was used (2 types of vibrations x 4 different inclines) for each dependent variable.~~ In the current study, a Pre-Hoc comparison was used for a couple of reasons as follows: 1) using a Pre-Hoc comparison may lower the numbers of multiple comparisons and further reduce the statistical type-I errors, and 2) using a Pre-Hoc comparison can accentuate that only meaningful/interesting hypotheses were included (Midway et al. 2020) ~~(Midway et al., 2020).~~ Thus, in the current study, a paired t-test with Bonferroni correction was used to compare the effect of bilateral VS on each gait parameter while walking on 0%, 3%, 6%, and 9% grades of inclines. If any significant effect of VS was found on each gait characteristic in any incline, this specific gait characteristic was included, and a two-way repeated measure was used (2 types of vibrations x 4 different inclines) for this selected dependent variable. Post-Hoc multiple comparisons were also corrected by the Bonferroni method. All statistical analysis was performed using SPSS 20 (IBM, Armonk, New York, USA). The way that SPSS calculated the Pre- and Post-Hoc comparisons using the Bonferroni method was to take the uncorrected p-value and multiply it by the number of comparisons made (https://www.ibm.com/support/pages/calculation-bonferroni-adjusted-p-values, last viewed on December 10, 2022). Here, there were a total of twenty-eight multiple comparisons for each selected gait characteristic. Therefore, to obtain the corrected p-value, the uncorrected p-value needed to be multiplied by twenty-eight. If the corrected p-value is less than 0.05, the significance level was reached. If the alpha value for the Shapiro-Wilk test was less than 0.05, indicating the data was not normally distributed, a Friedman test was used. Wilcoxon Signed Rank Test was used for post hoc comparisons for each dependent value. This study used the current sample size based on a previous publication to investigate the effect of mastoid vibrations on the net center of pressure (Chien et al. 2016)~~(Chien et al., 2016)[20]~~. In this abovementioned study, recruiting 20 healthy young could obtain observed power of approximately 1. Additionally, this study used G∗ power (URL: http://www.gpower.hhu.de/) to calculate the power. The η2 = 0.09 was used because this specific partial eta squared value was between 0.059 for the moderate effect size and 0.138 for the large effect size based on the partial eta squared method (Lu et al., 2022)~~[20]~~. By this calculation, ~~the~~ 80% of statistical power, which represented a reliable balance between the alpha and beta risk, could be reached by recruiting 18 healthy young adults for the repeated measure. In this study, the partial eta squared was used to measure the observed power~~[20]~~.

Results

Normality Tests ~~and Effect Size~~:

The alpha values of the Shapiro-Wilk Test for each spatial-temporal gait parameter and individual variability were greater than 0.05, indicating that the data were normally distributed. Therefore, a two-way repeated measure was used for ~~each~~ selected spatial-temporal gait parameters~~parameter~~ and ~~respective~~ selected variabilities. ~~The Partial Eta Squared values were 0.219 for stand time, 0.219 for double support time, 0.207 for step length, and 0.153 for step time. These values indicated that the effect size was large based on a previous study, indicating that at least 0.138 for large effect size, 0.059 for moderate effect size, and 0.01 for small effect size[20].~~

Pre Hoc Tests:

Pre Hoc comparisons revealed that a significance level was reached (VS vs. No VS) in step length (p = 0.004 for 0% grade walking, p = 0.013 for 3% grade walking, p = 0.002 for 9% grade walking), step time (p = 0.027 for 0% grade walking, p = 0.028 for 3% grade walking, p = 0.028 for 9% grade walking), step time variability (p = 0.05 for 3% grade walking), foot clearance (p = 0.008 for 9% grade walking), and foot clearance variability (p = 0.022 for 0% grade walking, p = 0.003 for 3% grade walking, p = 0.001 for 9% grade walking) (Table 1).

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Post Hoc Tests:

Interaction Between the Effect of Inclines and Mastoid Vibrations on Each Spatial-Temporal Gait Parameters and Variabilities ~~(Table 1, Fig. 3)~~:

A significant interaction was found in ~~stance time ($F_{3,54}$ = 5.035, p = 0.004), double support time ($F_{3,54}$ = 5.050, p = 0.004),~~ step length ($F_{3,54}$ = 4.701, p = 0.005), and ~~and~~ step time ($F_{3,54}$ = 3.283, p = 0.028) (Fig. 3). ~~,~~ Post hoc comparisons indicated that ~~significant less stance time (p = 0.028), significant less double support time (p = 0.029),~~ significantly~~significant~~ greater step length (p = 0.004, p = 0.014, and p = 0.00~~01~~34), and significantly~~significant~~ greater step time (p = 0.027, p = 0.0~~01~~028, and p = 0.028), when walking with bilateral mastoid vibrations than when walking without vibrations on 0%, 3% and 9% grade of incline respectively. ~~For both step length and step time, the significantly greater values induced by bilateral mastoid vibrations could be observed when walking on the level ($p < 0.001$, $p < 0.001$) and on the 3% grade of incline ($p < 0.001$, $p < 0.001$).~~ However, when walking on the 6% grade of incline, the effect of mastoid vibration could not be found in all spatial-temporal gait parameters. The only significant interaction between the effect of inclines and mastoid vibrations on gait variability was the foot clearance variability ($F_{3, 54} = 5.207$, $p = 0.003$). Moreover, the post hoc comparisons demonstrated that when applying the bilateral mastoid vibrations, the foot clearance variability was significantly greater during walking on ~~0% (p = 0.008),~~ a 3% (p = 0.003~~1~~)~~,~~ ~~6% (p = 0.003),~~ and 9% (p < 0.001) grade of inclines compared to walking without vibrations.

------------------ Insert Figure 3 Here -----------------------------

Effect of Mastoid Vibration on Each Spatial-Temporal Gait Parameters and Variabilities ~~(Table 1, 2)~~:

A significant effect of ~~mastoid vibration~~ VS was found in step length ($F_{1,18} = 19.551$, $p < 0.001$), step time ($F_{1,18} = 16.760$, $p = 0.001$), ~~maximum heel elevation~~foot clearance ($F_{1,18} = 23.081$, $p < 0.001$)~~, stance time variability ($F_{1,18} = 5.500$, $p = 0.031$)~~, ~~step length variability ($F_{1,18} = 6.104$, $p = 0.024$),~~ step time variability ($F_{1,18} = 6.110$, $p = 0.024$), and ~~maximum elevation~~foot clearance variability ($F_{1,18} = 38.485$, $p < 0.001$). Marginal means indicated that implementing a bilateral mastoid vibration significantly increased step length, step time, foot clearance~~, stance time variability~~, ~~step length variability,~~ step time variability, and foot clearance variability.

--------------- Insert Table 2 Here ---------------------------

Effect of Inclines on Each Spatial-Temporal Gait Parameters and Variabilities ~~(Table 1, 2)~~:

A significant effect of inclines was found in step length ($F_{3,54} = 6.620$, $p = 0.001$), step time ($F_{3,54} = 4.345$, $p = 0.008$), ~~maximum heel elevation~~foot clearance ($F_{3,54} =$ 58.312~~58.312~~, $p < 0.001$)~~, step width variability ($F_{3,18} = 4.56$, $p = 0.006$)~~, and foot clearance variability ($F_{3,54} =$ ~~58.312,~~ 45.606, $p < 0.001$). Marginal means demonstrated significantly greater step length, step time, and foot clearance when walking on a 9% grade of incline than when walking on a 0% grade of incline. ~~Moreover, for max heel elevation, marginal means showed significant differences between each incline, and the foot clearance increased significantly with the increasing grade of inclines.~~

Effect Size:~~Interaction Between the Effect of Inclines and Mastoid Vibrations on Each Spatial-Temporal Gait Parameters and Variabilities (Table 1, Fig. 3):~~

The Partial Eta Squared values were 0.207 for step length and 0.153 for step time. These values indicated that the effect size was large based on a previous study, meaning that at least 0.138 for a large effect size, 0.059 for a moderate effect size, and 0.01 for a small effect size.

A significant interaction was found in stance time ($F_{3, 54}$ = 5.035, *p* = 0.004), double support time ($F_{3, 54}$ = 5.050, *p* = 0.004), step length ($F_{3, 54}$ = 4.701, *p* = 0.005), and step time ($F_{3, 54}$ = 3.283, *p* = 0.028). Post hoc comparisons indicated that significant less stance time (*p* = 0.028), significant less double support time (*p* = 0.029), significant greater step length (*p* = 0.001), and significant greater step time (*p* = 0.001), when walking with bilateral mastoid vibrations than when walking without vibrations on 9% grade of incline. For both step length and step time, the significantly greater values induced by bilateral mastoid vibrations could be observed when walking on the level (*p* < 0.001, *p* < 0.001) and on the 3% grade of incline (*p* < 0.001, *p* < 0.001). However, when walking on the 6% grade of incline, the effect of mastoid vibration could not be found in all spatial-temporal gait parameters. The only significant interaction between the effect of inclines and mastoid vibrations on gait variability was the foot clearance variability ($F_{3, 54}$ = 5.207, *p* = 0.003). Moreover, the post hoc comparisons demonstrated that when applying the bilateral mastoid vibrations, the foot clearance was significantly greater during walking on 0% (p = 0.008), 3% (p = 0.001), 6% (p = 0.003), and 9% (p < 0.001) grade of inclines compared to walking without vibrations.

Discussion

In this study, we attempted to understand the effect of bilateral VS on gait characteristics during walking on different inclines. This study expected to observe the temporal gait parameters increments but however, the spatial gait characteristics decrements. The results partially agreed with the hypotheses that greater step length and step time were observed at the same time, and shorter stance time and double support time were obtained. Additionally, the effect of bilateral vestibular VS significantly increased the stance time variability, step length variability, step time variabilityfoot clearance, and foot clearance variability.

Bilateral VS increased the step length and step time, but not the step width.

Surprisingly, an increase in the step length was observed, specifically, approximately a 3% increment of step length on level, 3%, and 9% incline walking when the bilateral VS was applied in comparison with no VS conditions. Also, this finding diverged from previous studies that patients with vestibular disorders demonstrated smaller step lengths than controls (Borel et al. 2004; Marchetti et al. 2008)(Chien et al. 2016; Marchetti et al. 2008)(Borel et al., 2004, Marchetti et al., 2008)[4, 21]. This study provided two a possible rationales for explaining this greater step length under bilateral VS as follows. : 1) the effect of acute vestibular stimulation was different than the effect of chronic vestibular on gait adjustments; 2) the effect of bilateral vestibular stimulation increased the walking speed. For rationale #1, Angunsri et al., 2011 investigated tactile stimulation's effect on different vestibular disorders by measuring the duration of stance, swing, and double support phases[1]. Their results found that the tactile stimulation had different impacts on patients with different types of vestibular disorders, inferring that 1) either increases or decreases in step length was attributed to the dysfunction of the vestibular system, and 2) patients with different types of vestibular disorders might demonstrate different types of temporal gait pattern when other sensory systems also became reliable. In other words, these different types of temporal gait patterns could be used to differentiate the different types of vestibular disorders when other sensory systems were perturbed. Thus, in the current study, the increases in step length could be an indicator for identifying the patients with acute vestibular disorder rather than chronic vestibular disorder[4, 21]. Another possible explanation[14, 15] was that Aapplying bilateral vestibular stimulation induced an illusion that leaded participants to sense their body's center of mass being located forward, termed "pressing for forward" (Ivanenko et al. 2000). (Ivanenko et al., 2000). (Kavounoudias et al. 1999)Kavounoudias et al. (1999) Kavounoudias et al. (1999) conducted an interesting study that applied single or multiple vibration-based stimulations on neck muscles during standing and found as follows: 1) while applying a single vibration-based stimulation on one side of the neck muscle, the body moved toward the contralateral side, and 2) while the co-vibrations were applied on adjacent neck muscles orthogonally, the body moved diagonally toward to the contralateral side. Furthermore, (Ivanenko et al. 2000)Ivanenko et al. (2000) Ivanenko et al. (2000) applied the co-vibration on the neck muscles -- splenius tendons on both sides symmetrically and found that walking speed increases when walking on a position feedback treadmill (treadmill belt speed is controlled by human walking speed). Both abovementioned studies suggested that applying vibrations on neck muscle induced the illusion. Expressly, (Ivanenko et al. 2000)Ivanenko et al. (2000)Ivanenko et al. (2000) indicated that this illusion might be highly associated with the vibrations that impacted the vestibular system for controlling the body orientation. In other words, when bilateral vestibular systems were perturbed by the VS, participants might feel a forward falling sensation and had no choice but to increase their step length to counterbalance this "press for forward" sensation in the current study. At the same time, because the constant treadmill speed was given to a participant throughout every condition, the step time was forced to be increased with increasing step length to maintain dynamic balance. This result inexplicitly confirmed the illusion induced by VS, like the abovementioned studies, particularly in the anterior-posterior direction. This study speculated that this "pressing for forward" illusion might be associated with nystagmus induced by the bilateral VS (Manzari & Modugno 2008; Nuti & Mandala 2005; Perez 2003)(Nuti and Mandala, 2005, Perez, 2003, Manzari and Modugno, 2008); however, the eye movement was not recorded during walking on different inclines in the current study. This speculation needs to be confirmed in future studies.

It is also worth mentioning that the increase in spatial-temporal gait parameters induced by noisy bilateral galvanic vestibular stimulation has been thought of as an improvement in gait in healthy controls and patients with bilateral vestibulopathy (Iwasaki et al. 2018). (Iwasaki et al., 2018). Based on this observation, the supra-threshold Bilateral VS in the current study also had a similar effect on enhancing the gait performance while walking on 0%, 3%, and 9% grades of inclines. On the one hand, applying sub-threshold vestibular stimulation referred to the phenomenon whereby the presence of vibration enhanced the perception of weak sensory stimuli by reducing the threshold of the vestibular system for rehabilitation (Iwasaki et al. 2018; Wuehr et al. 2017; Wuehr et al. 2022)(Iwasaki et al., 2018, Wuehr et al., 2017, Wuehr et al., 2022). On the other hand, applying supra-threshold vestibular stimulation generally has been thought to play a role in perturbing the vestibular system (Chien et al. 2016; Lu et al. 2022). (Chien et al., 2016, Lu et al., 2022). However, in the current study, it was not the case, and applying bilateral VS played a critical key in improving the gait performance while walking on different inclines. "If you want to find the secrets of the universe, think in terms of energy, frequency, and vibration," by Nikola Tesla. Thus, the “benefit” of gait improvement in different locomotor tasks in young adults might depend on various combinations of frequencies and amplitudes. Also, significant increases in spatial-temporal gait parameters were observed while walking on different inclines, inferring that walking inclines might also be a benefit of the gait performance.

increased the step length to increase walking speed. This speculation was supported by Iwasaki et al. (2018) that applying noisy galvanic vestibular stimulation increased the walking speed in both healthy controls and patients with bilateral vestibulopathy[14]. Also, because participants walked on the treadmill in the current study, the treadmill belt and treadmill speed were fixed for each trial; thus, significant increases in step time were also observed. These results also verified that using vibration-based vestibular had a similar effect on gait characteristics compared to electric-based vestibular stimulation.

Unexpectedly, the VS didn’t affect the step width at all. This observation was supported by previous studies (Herssens et al. 2021)(Hessen et al., 2021)[12]. (Herssens et al. 2021). Herssens et al.et al. (2021)(2021)[12] also showed reported no significant differences in step width during overground walking in patients with bilateral vestibulopathy. This phenomenon could be explained by the “offset” hypothesis. This hypothesis was observed by Séverac Cauquil et al. (1998)(1998)[26] that there was no center of pressure (CoP) deviation in the frontal plane when placing galvanic vestibular stimulators on both sides of the mastoid process during standing. Additionally, this “offset the movement in the medial-lateral and then pressing the forward the movement in the anterior-posterior direction” was supported by Chien et al.’ study (2016)(2016) during walking. In their study, the effect of bilateral vestibular stimulation didn’t increase the net center of pressure sway and respective variability in the medial-lateral (Chien et al. 2016)(Chien et al., 2016)[9]. Therefore, this study speculated that bilateral mastoid vibration would cancel the perceived “tilt of surface” feeling if both vestibular systems were perturbed simultaneously, resulting in no change in step width but increased deviation in the anterior-posterior direction. To verify this speculation, future studies need to be performed to investigate the movements in the trunk and head.

VS increased the temporal gait variability but not the spatial gait variability

In the current study, the marginal means revealed the significant VS effect on step time variability but not the step length and width variability. The step time variability increased by 9.6% on level walking, 14% on the 3%, 6.2% on the 6%, and 6.7% on the 9% grade of incline walking. Why did the VS only increase the temporal gait variability but not the spatial gait variability? First of all, increases in gait variability have been thought of as an indicator for continuously identifying self-orientation with the environment in a step-to-step fashion. In the current study, providing the bilateral mastoid vibration might generate the rhythmical cue for the participant to follow; however, the rhythm of the treadmill was not match up to the rhythm of vibrations. These conflicts in the temporal domain might lead the greater step-to-step fluctuations in step time to adapt this novel locomotor behavior. However, while walking on the constant-speed treadmill, the step length might be relatively easy to be controlled. A similar observation was found that controlling the step-to-step fluctuations in the temporal domain but not the spatial domain might be the key to adapting the novel locomotor behaviors (Gregory et al. 2021). ~~(Gregory et al., 2021).~~

VS increases the foot clearance and respective variability

Another interesting finding was that the VS increased ~~the~~ foot clearance. ~~regardless of walking on level, 3%, 6%, or 9% of the grade of inclines.~~ To our best knowledge, this study was the first ~~study~~ to investigate ~~the~~ foot clearance while~~during~~ walking on ~~different~~ the inclines~~s~~ with/without VS. Foot~~The foot~~ clearance has been suggested as a precise end-point~~precise-end-point~~ control task during the sway phase (Winter 1992)~~(Winter, 1992)~~[30]. Importantly, a review concluded that a greater mean of foot clearance and greater foot clearance variability was found in older adults than in young adults and in fallers than in non-fallers. This review further suggested that the increases in foot clearance and respective variability were the safety mechanisms for preventing falling. In the current study, the increases in foot clearance and respective variability could be the results of conflicted-sensory systems. ~~In this specific case, the perturbed vestibular system might force participants to slow down the walking speed; however, the treadmill belt speed still ran at the fixed speed consistently.~~ For safety issues, the demands in control of foot clearance become critical by two rationales: 1) for preventing tripping under sensory-conflicted conditions, and 2) for allowing a greater degree of freedom to control foot clearance in the step-to-step fashion. A similar observation was found in Ivanenko et al.'s study (2000) ~~(2000)~~; participants elevated the average thigh-shank-foot loop while the vibrations were activated on bilateral neck muscle. Again, Ivanenko et al.~~'s~~ (2000) study suggested this elevation of kinematics was evoked by illusion. Therefore, the third rationale for raising the foot clearance and respective variability was to counterbalance the VS-induced illusion.

Why didn't the VS affect the step length and step time while walking on the 6% grade incline?

Unexpectedly, the VS ~~didn't~~did not affect the step length and step time while walking on the 6% grade incline. ~~If~~When inspecting ~~taking a careful look at~~ the step length and step time (Table 2), the step length had a sharp increase while walking without VS in both step length and step time from a 3% grade incline to 6% grade incline, implying the possible alternations in gait pattern when walking from 3% to 6% grade of inclines. The rationale might be that, on the one hand, the locomotor tasks became a little challenging as the grade of incline increased. Thus, participants had to sharply increase their step length and step time to adapt to this new locomotor task even though no bilateral VS was applied. On the other hand, walking with bilateral VS itself was already challenging; therefore, there were no apparent changes from 3% to 6% grade of inclines. Thus, the step length and time under bilateral VS were similar to the ones under no bilateral VS while walking on 6% grade of incline. This phenomenon was also observed by Earhart and Bastian that healthy young participants used different control mechanisms when they stepped on steeper grades of a wedge (Earhart & Bastian 2000). ~~(Earhart and Bastian, 2000).~~ Similarly, another study, which investigated the first step on an inclined surface, suggested the above observation that the control mechanisms were changed to prepare the limb for an elevated heel contact with increased propulsive force when the grade of inclines reached a certain level (Prentice et al. 2004). ~~(Prentice et al., 2004).~~ In short, this study proposed an explanation for this phenomenon: between 3% to 6% grade incline might be a transient phase from one control mechanism to another for gait.

~~**Walking on the inclined treadmill could be used for future vestibular diagnosis**~~

~~The previous study found an increase in step length when walking uphill at 8% compared to level walking in both young and elderly healthy subjects[28]. Also, Swanenburg's study[27] also found that patients with chronic vestibular hypofunction demonstrated greater step length and faster walking speed than healthy individuals when walking uphill but not overground, which further confirms the observations in the current study under VS. One of the working hypotheses behind these observations might be that walking uphill increases the potential energy of the center of mass and transfers the increased power into the forward direction based on the energy shift of different gait phases[6]. Then, increased forward kinetic energy gave a larger forward prompt in step length during the process from the swing phase to initial contact. Therefore, the vestibular system needed to adjust the vector sum of translational acceleration and gravitational acceleration based on different inclines. In the current study, under VS, the only significant increase in step length while walking on 9% of a grade than walking on level, suggesting that greater inclines might be more useful for future vestibular diagnosis.~~

~~**VS increased the step length variability but not the step width variability**~~

~~In the current study, the marginal means revealed the significant VS effect on step length variability but not the step width variability. The step length variability increased 9.7% on level~~

walking, 13% on 3% of the grade, 7.2% on 6% of the grade, and 8% on 9% of grade incline walking. Measuring the step length variability and the step width variability could be used to explain the level of active control[3, 25, 31]. The active control hypothesis indicated that due to the human body anatomy structure[25] or walking direction[31], the medial-lateral direction, in general, required a greater level of active control (greater gait variability) than the control in the anterior-posterior direction during normal walking. Interestingly, such an effect of VS was only found in the anterior-posterior direction in the current study, indicating they required greater active control in the anterior-posterior direction. This observation could be explained by the abovementioned hypothesis -- "offset the movement in the medial-lateral and then pressing the forward the movement in the anterior-posterior direction." This observation might be crucial for developing sensor-motor training in patients with bilateral vestibular disorders, indicating regardless of the different inclines, applying bilateral VS might train patients to active control the dynamic balance in the anterior-posterior direction, where the falls most occur.

VS decreased the stance time and double support time when walking on 9% of grade incline

For temporal gait characteristics, the effect of VS decreased the stance time and double support time when walking on 9% of grade incline. It has been shown that reducing the vestibular function was associated with longer stance time[2] and longer double support time[21] for maintaining the dynamic balance control. In other words, these patients with vestibular disorders needed to increase the staying time of both feet on the ground to control the center of mass variation[5]. From a rehabilitation point of view, how to reduce the stance time and double support time became an urgent issue[18]. These current results inferred two critical improvement-to-be in gait characteristics: 1) increasing the inclines to 9% of the grade and above shortened both stance time and double support time, and 2) applying VS also reduced both stance time and double support. These results suggested that either applying VS or increasing the grade of incline would enhance the effectiveness of rehabilitation.

Conclusions

In conclusion, walking with VS increased step length, step time, and foot clearance. Also, VS increased both spatial andonly the temporal variabilitybut not the spatial gait variability. Surprisingly, while walking on a certain grade of incline, the effect of VS on spatial-temporal gait parameters might be offset due to the transient phase from one control mechanism to another control mechanism. This study suggested that step length, step time, foot clearance, step time variability, and foot clearance variability might be the primary parameters for future clinic diagnoses for patients with BVD while walking on different inclines. Also, the increases in spatial-temporal gait parameters might be a positive indicator for gait improvement while the bilateral VS was applied during different inclines walking. However, these increases were only found in the anterior-posterior direction instead of the medial-lateral. Thus, people with bilateral

~~vestibular dysfunction might adopt different gait strategies when walking uphill, with more active control in the anterior-posterior direction. This study also found increases in step length, step time, foot clearance, step width variability, and foot clearance variability at a 9% grade of incline than at 0%. This result suggested that changing gait parameters during uphill walking at a certain gradient might be helpful in diagnosing vestibular disorders. Patients with possible vestibular dysfunction might be confirmed by investigating their gait performance. Further studies are needed to explore specific gait changes during uphill in patients with different vestibular disorders. At the 9% grade of incline, less stance time and double support time were found with VS than without, indicating a new training method to improve dynamic balance in patients with vestibular disorders. In addition, changes in gait performance when walking on slopes in patients with bilateral vestibular dysfunction could reflect disease progression, deterioration, or treatment effects.~~

~~In the current study, VS was applied for motor control and not for motor learning. Whether VS enhances the dynamic balance over time is still unknown. Further study needs to explore the effects of VS on gait characteristics in a longtail study. Moreover, the generalization of our results was limited to healthy subjects and laboratory environments. Uphill comes unexpectedly in real life, and the distracting outside environment could bring more challenges, especially for patients with vestibular disorders.~~

Limitations

The apparent limits of this study were the lack of healthy older and pathological groups. To our best knowledge, this exploratory study was the first study to investigate the effect of perturbed vestibular systems on gait characteristics while walking on different inclines. Many gait characteristics were investigated in this study to prioritize the importance of each gait characteristic. Therefore, only healthy young adults were recruited for the study for clear descriptions and to prevent potential statistical problems. Further research could compare the walking performance of healthy subjects with VS and patients with vestibulopathy in different settings. Another apparent limit was a small sample size. To solve this problem, the Pre-Hoc tests were applied to prioritize the importance of each gait characteristic. Five out of twelve gait characteristics were selected for future statistical analysis for compensating statistical procedure shortcomings. Although the sample size was relatively small in the current study, the effect size demonstrated a large effect. These outcomes of large effect size also were similar to previous studies, which recruited twenty healthy young adults and investigated more than ten gait parameters (Chien et al. 2016; Lu et al. 2022). ~~(Chien et al., 2016, Lu et al., 2022).~~ Additionally, the statistical power was calculated by GPower, and the 80% of statistical power, which represented a reliable balance between the alpha and beta risk, could be reached by recruiting 18 healthy young adults for the repeated measure. Another limitation was that the eye tracker device was not used in the current study. Thus, the abnormal eye movement induced by bilateral VS could not be confirmed. This limitation needs to be performed in future research. ~~Last but not~~

~~least, the study used a small sample size but powered enough for a large effect size. More subjects are needed to increase the robustness of our study results.~~

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Conflict of interest statement:

Jung H Chien is an independent researcher and is not employed by any companies. Yuxiao Sun and Dongqi Zhu are physical therapy students in University of Nebraska Medical Center. Huiyan Song is an employee of West China Hospital in China. All authors declare that they have no competing interests.~~The authors reported no conflicts of interest.~~

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Authors' contributions:

YS, DZ, HS, and JC had an idea~~ideal~~ to generate this manuscript during the literature review. YS, DZ, HS, and JC wrote the main text. YS, DZ, and JC designed the experiment, and JC experimented~~carried out the experiment~~. YS processed and analyzed the data. All authors reviewed and agreed on the content of this manuscript.

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Figure Captions:

Figure 1. The diagram of experimental design. (A) Treadmill. (B) A vibration system (a controlled unit, two vibrators, and a portable battery); (C) Step length. Four retro-reflective markers were placed on the toe (second metatarsophalangeal joint) and the heel of both legs. One heel-strike was defined as the farthest position that a heel marker could reach in the anterior-posterior direction within one gait cycle, whereas the one toe-off was defined as the minimum position that a toe marker could make in the anterior-posterior direction within one gait cycle. The step time of one gait cycle was defined as the time between the one heel strike and the subsequent heel strike in the contralateral leg. The stance phase was defined as the percentage of the gait cycle from one heel-strike to one toe-off in the same leg. The double support time was defined as the percentage of the gait cycle that both feet stayed on the ground during walking. The step length was defined as the traveling distance of the treadmill belt in the anterior-posterior direction from one heel strike to another contralateral heel strike. Also, the step width was calculated by the distance between two continuous heel strikes in the medial-lateral direction.

Figure 2. The experimental protocol. Participants were randomly assigned to walk on 0%, 3%, 6%, or 9% with/without bilateral vibration-based vestibular stimulation.

Figure 3. The results of the step length and the step time with/without bilateral vibration-based vestibular stimulation when walking on different inclines. Empty Box: without vestibular stimulation, Diagonal Stripes Box: with vestibular stimulation. X: mean values, *: $p < 0.05$, **: $p < 0.01$.

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Table 1. Pre-Hoc Test. This table demonstrated the effect of bilateral vestibular stimulation on each gait characteristic while walking on 0%, 3%, 6%, and 9% grades of inclines. This Pre-Hoc tests were used to identify the priorities from multiple dependent variables. A paired t-test with Bonferroni correction was used to compare the effect of bilateral VS on each gait parameter while walking on 0%, 3%, 6%, and 9% grades of inclines. The gait characteristics were as follows: stance time (StanceTime, %), stance time variability (StanceTimeV, %), double support time (DoubleSupport, %), double support time variability (DoubleSupportV, %), step length (StepLength, mm), step length variability (StepLengthV, mm), step time (StepTime, ms), step time variability (StepTimeV, ms), step width (Stepwidth, mm), step width variability (StepwidthV, mm), foot clearance (Footclearance, mm), foot clearance variability (FootclearanceV, mm). No: Walking on the treadmill without bilateral vestibular stimulation, VS: walking on the treadmill with bilateral vestibular stimulation. NS: the p-value is larger than 0.05, indicating not significant.

StanceTime(%)	**0% of Grade**	**3% of Grade**	**6% of Grade**	**9% of Grade**	**StanceTimeV(%)**	**0% of Grade**	**3% of Grade**	**6% of Grade**	**9% of Grade**
No	63.56 (1.27)	63.49 (1.13)	63.62 (1.15)	63.62 (1.03)	**No**	1.62 (0.39)	1.65 (0.33)	1.75 (0.44)	1.78 (0.65)
VS	63.57 (1.20)	63.61 (1.32)	63.37 (1.39)	63.01 (1.95)	**VS**	1.75 (0.39)	1.79 (0.41)	1.87 (0.57)	1.86 (0.69)
Pre hoc (p value)	NS	NS	NS	NS	**Pre Hoc (p value)**	NS	NS	NS	NS
Doublesupport(%)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	**DoubleSupportV(%)**	0% of Grade	3% of Grade	6% of Grade	9% of Grade
No	25.12 (2.54)	24.99 (2.27)	25.23 (2.31)	25.25 (2.08)	**No**	1.44 (0.39)	1.41 (0.35)	1.49 (0.37)	1.49 (0.49)
Vs	25.13 (2.41)	25.23 (2.65)	24.75 (2.77)	24.48 (2.62)	**VS**	1.44 (0.38)	1.47 (0.38)	1.55 (0.59)	1.52 (0.57)
Pre hoc (p value)	NS	NS	NS	NS	**Pre Hoc (p value)**	NS	NS	NS	NS
Steplength (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	**StepLength V (mm)**	0% of Grade	3% of Grade	6% of Grade	9% of Grade
No	537.27 (57.27)	536.82 (54.56)	546.55 (57.88)	547.88 (61.44)	**No**	134.73 (30.83)	129.95 (34.73)	141.94 (40.07)	141.77 (45.11)
Vs	551.02 (65.72)	554.57 (65.94)	552.42 (65.84)	559.15 (68.91)	**VS**	147.78 (26.66)	146.87 (31.52)	152.22 (35.69)	153.18 (46.22)
Pre hoc (p value)	0.004	0.013	NS	0.002	**Pre Hoc (p value)**	NS	NS	NS	NS
Steptime (ms)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	**StepTimeV (ms)**	0% of Grade	3% of Grade	6% of Grade	9% of Grade
No	619.25 (55.81)	617.46 (49.98)	626.74 (59.73)	628.25 (58.61)	**No**	15.89 (4.40)	15.29 (4.88)	17.02 (5.44)	17.23 (6.31)
Vs	631.39 (57.41)	635.24 (59.53)	632.09 (59.36)	639.64 (58.15)	**VS**	17.41 (4.35)	17.49 (4.75)	18.07 (4.89)	18.37 (6.27)
Pre hoc (p value)	0.027	0.028	NS	0.028	**Pre Hoc (p value)**	NS	0.05	NS	NS
Stepwidth (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	**StepWidth V (mm)**	0% of Grade	3% of Grade	6% of Grade	9% of Grade
No	537.27	536.82	546.55	547.88	**No**	14.88	13.89	16.15	15.51

	(57.27)	(54.56)	(57.88)	(61.44)		(2.78)	(3.71)	(2.79)	(3.38)
Vs	551.02 (65.72)	554.57 (65.94)	552.42 (65.84)	559.15 (68.91)	**VS**	15.05 (2.83)	15.21 (2.94)	15.86 (3.27)	15.60 (3.32)
Pre hoc (p value)	NS	NS	NS	NS	**Pre Hoc (p value)**	NS	NS	NS	NS
Footclearance (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	**FootClearanceV (mm)**	0% of Grade	3% of Grade	6% of Grade	9% of Grade
No	231.98 (30.79)	243.32 (33.02)	253.18 (32.94)	260.40 (34.52)	**No**	35.96 (20.12)	39.32 (20.05)	44.75 (23.52)	51.98 (28.11)
Vs	235.01 (32.22)	246.48 (30.59)	256.58 (32.66)	264.25 (34.94)	**VS**	38.92 (18.97)	43.96 (21.28)	49.89 (25.43)	61.22 (27.55)
Pre hoc (p value)	NS	NS	NS	0.008	**Pre Hoc (p value)**	0.022	0.003	NS	0.001

Table 2. This table demonstrated the effect of bilateral vestibular stimulation on selected gait characteristic from Pre-Hoc while walking on 0%, 3%, 6%, and 9% grades of inclines. Step length (StepLength, mm), step time (StepTime, ms), step time variability (StepTimeV, ms), foot clearance (Footclearance, mm), foot clearance variability (FootclearanceV, mm). No: Walking on the treadmill without bilateral vestibular stimulation, VS: walking on the treadmill with bilateral vestibular stimulation. NS: the p-value is larger than 0.05, indicating not significant. NA: the interaction was didn't reach the significance level. The Post-Hoc can't be performed. Therefore, NA represented not available.

StepLength (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	The effect of inclines	The effect of MV	Interaction
No	537.27 (57.27)	536.82 (54.56)	546.55 (57.88)	547.88 (61.44)	p = 0.001	p < 0.001	p = 0.005
VS	551.02 (65.72)	554.57 (65.94)	552.42 (65.84)	559.15 (68.91)	-		
PostHoc (p-value)	p = 0.004	p = 0.014	NS	p = 0.034	-	-	-
StepTime (ms)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	The effect of inclines	The effect of MV	Interaction
No	619.25 (55.81)	617.46 (49.98)	626.74 (59.73)	628.25 (58.61)	p = 0.008	p = 0.001	p = 0.028
VS	631.39 (57.41)	635.24 (59.53)	632.09 (59.36)	639.64 (58.15)	-		
PostHoc (p-value)	p = 0.027	p = 0.028	NS	p = 0.028	-	-	-
StepTimeV (ms)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	The effect of inclines	The effect of MV	Interaction
No	15.89 (4.40)	15.29 (4.88)	17.02 (5.44)	17.23 (6.31)	NS	p = 0.024	NS
VS	17.41 (4.35)	17.49 (4.75)	18.07 (4.89)	18.37 (6.27)	-		
PostHoc (p-value)	NA	NA	NA	NA	-	-	-
FootClearance (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	The effect of inclines	The effect of MV	Interaction
No	231.98 (30.79)	243.32 (33.02)	253.18 (32.94)	260.40 (34.52)	p < 0.001	p < 0.001	NS
VS	235.01 (32.22)	246.48 (30.59)	256.58 (32.66)	264.25 (34.94)			
PostHoc (p-value)	NA	NA	NA	NA	-	-	-
FootClearanceV (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	The effect of inclines	The effect of MV	Interaction
No	35.96 (20.12)	39.32 (20.05)	44.75 (23.52)	51.98 (28.11)	p < 0.001	p < 0.001	p = 0.003

~~VS~~	~~38.92 (18.97)~~	~~43.96 (21.28)~~	~~49.89 (25.43)~~	~~61.22 (27.55)~~	-		
~~PostHoc (p-value)~~	~~NS~~	~~p = 0.003~~	~~NS~~	~~p = 0.001~~	-	-	-

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Figure Captions:

Figure 1. The diagram of experimental design. (A) Example session showing walking on a treadmill wearing a vibration system. (B) Vibration system (a controlled unit, two vibrators, and a portable battery); (C) Step length. Four retro-reflective markers were placed on the toe (second metatarsophalangeal joint) and the heel of both legs. One heel-strike was defined as the farthest position that a heel marker could reach in the anterior-posterior direction within one gait cycle, whereas the one toe-off was defined as the minimum position that a toe marker could make in the anterior-posterior direction within one gait cycle. The step time of one gait cycle was defined as the time between the one heel strike and the subsequent heel strike in the contralateral leg. The stance phase was defined as the percentage of the gait cycle from one heel-strike to one toe-off in the same leg. The double support time was defined as the percentage of the gait cycle that both feet stayed on the ground during walking. The step length was defined as the traveling distance of the treadmill belt in the anterior-posterior direction from one heel strike to another contralateral heel strike. Also, the step width was calculated by the distance between two continuous heel strikes in the medial-lateral direction.

Figure 2. The experimental protocol. Participants were randomly assigned to walk on 0%, 3%, 6%, or 9% with/without bilateral vibration-based vestibular stimulation.

Figure 3. The results of the step length and the step time with/without bilateral vibration-based vestibular stimulation when walking on different inclines. Empty Box: without vestibular stimulation, Diagonal Stripes Box: with vestibular stimulation. X: mean values, *: $p < 0.05$, **: $p < 0.01$.

Table 1. Pre-Hoc Test. This table demonstrated the effect of bilateral vestibular stimulation on each gait characteristic while walking on 0%, 3%, 6%, and 9% grades of inclines. This Pre-Hoc tests were used to identify the priorities from multiple dependent variables. A paired t-test with Bonferroni correction was used to compare the effect of bilateral VS on each gait parameter while walking on 0%, 3%, 6%, and 9% grades of inclines. The gait characteristics were as follows: stance time (StanceTime, %), stance time variability (StanceTimeV, %), double support time (DoubleSupport, %), double support time variability (DoubleSupportV, %), step length (StepLength, mm), step length variability (StepLengthV, mm), step time (StepTime, ms), step time variability (StepTimeV, ms), step width (Stepwidth, mm), step width variability (StepwidthV, mm), foot clearance (Footclearance, mm), foot clearance variability (FootclearanceV, mm). No: Walking on the treadmill without bilateral vestibular stimulation, VS: walking on the treadmill with bilateral vestibular stimulation. NS: the p-value is larger than 0.05, indicating not significant.

StanceTime(%)	**0% of Grade**	**3% of Grade**	**6% of Grade**	**9% of Grade**	**StanceTimeV(%)**	**0% of Grade**	**3% of Grade**	**6% of Grade**	**9% of Grade**
No	63.56 (1.27)	63.49 (1.13)	63.62 (1.15)	63.62 (1.03)	**No**	1.62 (0.39)	1.65 (0.33)	1.75 (0.44)	1.78 (0.65)
VS	63.57 (1.20)	63.61 (1.32)	63.37 (1.39)	63.01 (1.95)	**VS**	1.75 (0.39)	1.79 (0.41)	1.87 (0.57)	1.86 (0.69)
Pre hoc (p value)	NS	NS	NS	NS	**Pre Hoc (p value)**	NS	NS	NS	NS
Doublesupport (%)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	**DoubleSupportV(%)**	0% of Grade	3% of Grade	6% of Grade	9% of Grade
No	25.12 (2.54)	24.99 (2.27)	25.23 (2.31)	25.25 (2.08)	**No**	1.44 (0.39)	1.41 (0.35)	1.49 (0.37)	1.49 (0.49)
Vs	25.13 (2.41)	25.23 (2.65)	24.75 (2.77)	24.48 (2.62)	**VS**	1.44 (0.38)	1.47 (0.38)	1.55 (0.59)	1.52 (0.57)
Pre hoc (p value)	NS	NS	NS	NS	**Pre Hoc (p value)**	NS	NS	NS	NS
Steplength (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	**StepLengthV (mm)**	0% of Grade	3% of Grade	6% of Grade	9% of Grade
No	537.27 (57.27)	536.82 (54.56)	546.55 (57.88)	547.88 (61.44)	**No**	134.73 (30.83)	129.95 (34.73)	141.94 (40.07)	141.77 (45.11)
Vs	551.02 (65.72)	554.57 (65.94)	552.42 (65.84)	559.15 (68.91)	**VS**	147.78 (26.66)	146.87 (31.52)	152.22 (35.69)	153.18 (46.22)
Pre hoc (p value)	0.004	0.013	NS	0.002	**Pre Hoc (p value)**	NS	NS	NS	NS
Steptime (ms)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	**StepTimeV (ms)**	0% of Grade	3% of Grade	6% of Grade	9% of Grade

No	619.25 (55.81)	617.46 (49.98)	626.74 (59.73)	628.25 (58.61)	**No**	15.89 (4.40)	15.29 (4.88)	17.02 (5.44)	17.23 (6.31)
Vs	631.39 (57.41)	635.24 (59.53)	632.09 (59.36)	639.64 (58.15)	**VS**	17.41 (4.35)	17.49 (4.75)	18.07 (4.89)	18.37 (6.27)
Pre hoc (p value)	0.027	0.028	NS	0.028	**Pre Hoc (p value)**	NS	0.05	NS	NS
Stepwidth (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	**StepWidthV (mm)**	0% of Grade	3% of Grade	6% of Grade	9% of Grade
No	537.27 (57.27)	536.82 (54.56)	546.55 (57.88)	547.88 (61.44)	**No**	14.88 (2.78)	13.89 (3.71)	16.15 (2.79)	15.51 (3.38)
Vs	551.02 (65.72)	554.57 (65.94)	552.42 (65.84)	559.15 (68.91)	**VS**	15.05 (2.83)	15.21 (2.94)	15.86 (3.27)	15.60 (3.32)
Pre hoc (p value)	NS	NS	NS	NS	**Pre Hoc (p value)**	NS	NS	NS	NS
Footclearance (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	**FootClearanceV (mm)**	0% of Grade	3% of Grade	6% of Grade	9% of Grade
No	231.98 (30.79)	243.32 (33.02)	253.18 (32.94)	260.40 (34.52)	**No**	35.96 (20.12)	39.32 (20.05)	44.75 (23.52)	51.98 (28.11)
Vs	235.01 (32.22)	246.48 (30.59)	256.58 (32.66)	264.25 (34.94)	**VS**	38.92 (18.97)	43.96 (21.28)	49.89 (25.43)	61.22 (27.55)
Pre hoc (p value)	NS	NS	NS	0.008	**Pre Hoc (p value)**	0.022	0.003	NS	0.001

Table 2. This table demonstrated the effect of bilateral vestibular stimulation on selected gait characteristic from Pre-Hoc while walking on 0%, 3%, 6%, and 9% grades of inclines. Step length (StepLength, mm), step time (StepTime, ms), step time variability (StepTimeV, ms), foot clearance (Footclearance, mm), foot clearance variability (FootclearanceV, mm). No: Walking on the treadmill without bilateral vestibular stimulation, VS: walking on the treadmill with bilateral vestibular stimulation. NS: the p-value is larger than 0.05, indicating not significant. NA: the interaction was didn't reach the significance level. The Post-Hoc can't be performed. Therefore, NA represented not available.

StepLength (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	The effect of inclines	The effect of MV	Interaction
No	537.27 (57.27)	536.82 (54.56)	546.55 (57.88)	547.88 (61.44)	p = 0.001	p < 0.001	p = 0.005
VS	551.02 (65.72)	554.57 (65.94)	552.42 (65.84)	559.15 (68.91)			
PostHoc (p value)	p = 0.004	p = 0.014	NS	p = 0.034			
StepTime (ms)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	The effect of inclines	The effect of MV	Interaction
No	619.25 (55.81)	617.46 (49.98)	626.74 (59.73)	628.25 (58.61)	p = 0.008	p = 0.001	p = 0.028
VS	631.39 (57.41)	635.24 (59.53)	632.09 (59.36)	639.64 (58.15)			
PostHoc (p value)	p = 0.027	p = 0.028	NS	p = 0.028			
StepTimeV (ms)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	The effect of inclines	The effect of MV	Interaction
No	15.89 (4.40)	15.29 (4.88)	17.02 (5.44)	17.23 (6.31)	NS	p = 0.024	NS
VS	17.41 (4.35)	17.49 (4.75)	18.07 (4.89)	18.37 (6.27)			
PostHoc (p value)	NA	NA	NA	NA			
FootClearance (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	The effect of inclines	The effect of MV	Interaction
No	231.98 (30.79)	243.32 (33.02)	253.18 (32.94)	260.40 (34.52)	p < 0.001	p < 0.001	NS
VS	235.01 (32.22)	246.48 (30.59)	256.58 (32.66)	264.25 (34.94)			
PostHoc (p value)	NA	NA	NA	NA			
FootClearanceV (mm)	0% of Grade	3% of Grade	6% of Grade	9% of Grade	The effect of inclines	The effect of MV	Interaction
No	35.96 (20.12)	39.32 (20.05)	44.75 (23.52)	51.98 (28.11)	p < 0.001	p < 0.001	p = 0.003

VS	38.92 (18.97)	43.96 (21.28)	49.89 (25.43)	61.22 (27.55)			
PostHoc (p value)	NS	$p = 0.003$	NS	$p = 0.001$			