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THE EFFECT OF DIFFERENT HOPPING CONDITIONS ON LEG STIFFNESS OF MEN AND WOMEN

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ABBREVIATIONS

NMS	Neuro-musculo-skeletal system
Fr	Frequency
F	Force
F peak	Peak force
dL	Vertical displacement of center of mass
∆L	Changes in length of leg
k leg	Leg stiffness
gct	Ground contact time
ct	Cycle time
N	Newton
cm	Centimeter
COM	Center of mass
SSC	Stretch- shortening cycle
BW	Body weight
H	Height
TMH	Trochanter major height

ABSTRACT

"The Effect of Different Hopping Conditions on Leg Stiffness of Men and Women"

İlkşan DEMİRBÜKEN,PT

Purpose: Stiffness is considered as a regulated property of neuromuscular system. In this study, stiffness is used as a parameter to quantify neuromuscular system's behavior to avoid the complexities by looking at the overall behavior of NMS. The purpose of our study was to assess the effect of increase in hopping frequency and added weight on leg stiffness of men and women.

Materials and Methods: The study group consisted of 22 healthy subjects (11M-11F) ranged in age from 20 to 43 years. Subjects performed two-legged hopping on a force platform. The subjects performed three hopping tasks; preferred, fast as possible and with added mass of 10% bodyweight. F (ground reaction force), fr (frequency) and ct (ground contact time) was measured in three different hopping conditions. Then ΔL (changes in length of leg) and k leg (leg stiffness) was calculated.

Results: Leg stiffness increased with an increase in hopping frequency (p=0.000), while the added mass did not change it. The leg stiffness of male subjects was greater than female subjects in three hopping conditions (p=0.067, power=0.454).

Conclusion: Leg stiffness increased by increasing frequency and added mass have only significant effect on increase in ground contact time. The most important reason for increasing leg stiffness is a significant decrease in vertical displacement of COM. It will provide basic knowledge to select different clinical assessment and treatment programs.

Key Words: leg stiffness, gender, frequency, added mass, hopping test

PREFACE AND PURPOSE

The basic systems of human movement that react together within the body system are nervous, muscular and skeletal systems as supported by the cardio respiratory system. Functionally combined they are termed neuro-musculo-skeletal (NMS) system which have millions of nerves, hundreds of muscles and bones are integrated within the human body to react together to effectuate locomotion (1, 2). It is too hard to understand how the numerous parts of the NMS system interact to produce locomotion. Understanding the interrelationship between locomotion and NMS needs more research on lower extremity mechanics (3).

To further understand these complexities, currently studies focus on the STIFFNESS component of the lower extremity mechanics. In particular, sports and clinical biomechanists are typically interested in the role of stiffness in lower extremity mechanics. Greater understanding of the role of the stiffness will provide stronger knowledge for understanding locomotion more clearly (4). Because stiffness has often been mentioned as a regulated property of the neuromuscular system and thought to be an important factor for performance, risk of injury and functional ability (4,5,6,7).

Stiffness of lower extremity is adaptable to conditions and these adjustments of the leg are made by adjusting the components of stiffness. In this current study we looked for the change in components of leg stiffness by using hopping test by means of change in hopping conditions. We chose the hopping at preferred (normal) frequency, hopping at highest frequency and hopping at normal frequency with added weight as different hopping conditions. This study will be the first one that has the combination of these three hopping conditions in this field. Earlier studies have used similar methods and trials but in different combinations. Based on previous studies it was proposed that leg stiffness would increase with increasing hopping frequency and with added mass on subjects' body weight. The first goal of this study was to examine if leg stiffness is actually changed, as we expected. In addition, the second goal of this study was to see how people change their leg stiffness in different hopping conditions such as, normal hopping frequency, highest hopping frequency and the normal hopping frequency with added weight. Based on biomechanical differences that exist between male and female we also hypothesized that men and women would show different changes on leg stiffness. Therefore, the third goal of this study was to see if men had greater leg stiffness than women at different hopping conditions. And the fourth goal of this study was to see if men and women responded differently to changes in these hopping conditions.

The results of this study will be useful for further studies in lower extremity biomechanics of unhealthy subjects and provide a scientific basis for physical therapy and sport medicine fields in development of optimal treatment programs of lower extremity problems.

INTRODUCTION

Biomechanics is a science that helps to understand the mechanical function of locomotion. It studies on the relationships and interactions that the various part of body, segments and systems have with each other to contribute the function. Biomechanics can be defined as " the science concerned with the internal and external forces acting on a human body and the systematical effects produced by these forces"(8). Locomotion is one of the subject in biomechanics that is hard to understand its interaction with NMS .

To understand more clearly this complexity studying on STIFFNESS component of lower extremity is popular nowadays.

Stiffness is defined as "stiffness or young's modulus of elasticity of a material, is determined by slope of the load deformation curve during the elastic response range and is representative of the material's resistance to load as structure deforms. In other words, stiffness is the resistance of a material to deformation; stress per unit strain. And the young modulus that is referred to as a gradient of the stress-strain curve in Hookean region. Young modulus provides a standard measure of stiffness for comparing different materials. The larger the young's modulus, the stiffer the material. This is a response in most materials including bones, tendons and ligaments" (9). The concept of stiffness has its origin from physics, as part of Hooke's Law. Objects that obey this law are deformable bodies (10).They can store and return elastic energy. Hooke's law is defined as;

$$\boxed{F=k.x}$$

Where F is the force, which is required to deform a material, x is the dimensions of material which is deformed, k is constant and is referred to as the spring constant which is the description of an ideal spring and mass system stiffness. It can be considered as a proportionally constant (4). For a linear elastic material, the strain is a linear function of the stress applied. Providing that its shape is temporarily changed (10). An ideal spring is mass less, moves in only one direction and has a stiffness that is independent of time, length and velocity. Furthermore, the mass of the system is supposed to be concentrated at a point at one end of spring (4,7). This spring mass system is applicable to some kind of locomotion of animals as an ideal model

The animals which have legs use a variety of gaits to move from one place to another. Although there are remarkable differences in body shapes and sizes among animals, some features of their gaits are quite similar. Running, hopping and trotting animals all move much like a bouncing ball (11, 12, 13, 14, 15, 16). For example, observing the motion of the ball on the ground it can be seen that ball first totally compressed and suddenly bounced by the reaction forces. Running is often referred to as a bouncing gait because of the movements of center of mass during running are similar to a bouncing ball (3). And the hopping is an unipedal or bipedal bouncing motion that is developmentally similar to skipping and typically appears between 4 and 7 years old (17). Recent studies have shown that during running and hopping, the action of the body's many musculoskeletal elements including muscles, tendons, and ligaments are integrated together and humans maintain similar center of motion so that the overall musculoskeletal system behaves like a single spring (12,13,18,19). As a result, these gaits can be modeled by using a simple spring mass system consisting of a single linear 'leg spring' and a point mass that is equivalent to body mass (11,20,21,22,23,24,25,26,27,28,29).

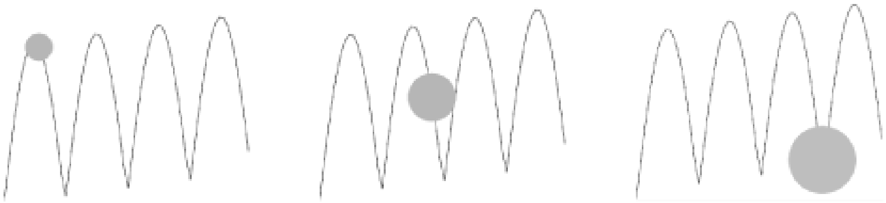

Figure 1. A bouncing ball, the natural motion animation example

There are several definitions of leg stiffness used by biomechanists including vertical stiffness, leg stiffness and joint stiffness. Vertical stiffness usually describes linear movements that occur in the vertical direction such as hopping and it serves as the mechanism by which the direction of the downward velocity of the body is reversed during extremity contact (4,30). If the research focuses at the joint level, the joint stiffness can be calculated by using joints moments and joint angle data (4,11,31). In the simplest application of a spring-mass model, the spring mass system only moves vertically, in activities of hopping. In this purely vertical model, the stiffness of the leg spring is the most important determinant of the amount of time that the feet are on the ground and leg stiffness represents average combined stiffness of all the limbs in contact with the ground (11,16,18,19,32). Mc Mahon and Cheng developed a method for calculating leg stiffness for such cases. This method takes into account the runners' horizontal velocity, time of contact, resting length of leg and peak ground reaction force. If the mass moves purely in the vertical direction, as in hopping, leg stiffness becomes exactly like vertical stiffness (4,27). In the present study, we used only force platform to measure peak vertical forces, vertical velocity and the position of center of mass.

The overall stiffness of the leg influences the mechanics and kinematics of the interaction with the ground (11,24,33,34).The transfer of kinetic and gravitational potential energies with each step of walking is similar to an inverted pendulum. During walking, the center of mass reaches its highest point and potential energy reaches its maximum value at the middle of stance phase. When potential energy is maximum, kinetic energy reaches its minimum value. Thus, this mechanism conserves substantial mechanical energy by the inverted pendulum mechanism during walking. The motion of center of mass is different in bouncing gaits. In bouncing gaits, the leg spring is compressed during the first half of the ground-contact phase by the flexion of the leg. This compliance causes the vertical displacement and gravitational potential energy of center of mass to reach their minimum values at mid-stance phase. Also kinetic energy of center of mass reaches its minimum value at the same time with potential energy. As a result, the exchange of kinetic and potential energy cannot conserve the mechanical energy. Substantial mechanical energy is conserved through the storage and return of elastic energy in elastic tissues. The peak ground reaction force is maximum when the maximum leg compression occurs. During the second half of the ground-contact phase leg spring goes to extension and leg lengthens (3,11,28,35,36,37,38)

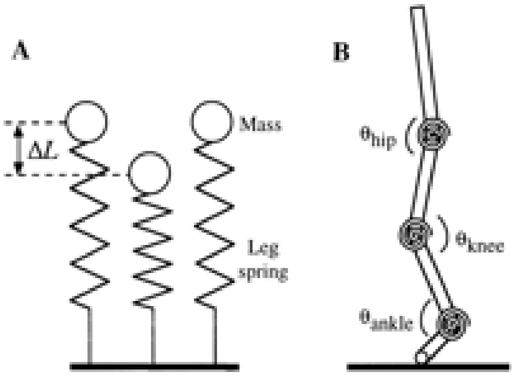

Figure 2. Single linear "leg spring" represents mechanical behavior of lower extremity during ground-contact phase. Mass is equivalent to body mass. Model is shown at beginning of ground-contact phase (*left*), middle of ground-contact phase (*middle*), and at end of ground-contact phase (*right*). During first half of ground-contact phase, leg spring is compressed by a distance ΔL, represents the change in leg length (vertical displacement of COM. θ, represents joint angle at instant of touchdown).

The leg stiffness depends on geometry and touchdown geometry of musculoskeletal system, stretch reflex activation, muscle activation, alignment and moment arm of ground reaction force vector, muscle stiffness, tendon stiffness and muscle –tendon unit stiffness (3,11,18,19,20,39,40,41,42). The stiffness of muscle-tendon unit depends on the both muscle and tendon stiffness (19). Muscle stiffness describes the stiffness properties specifically exhibited by tenomuscular tissues. Muscle stiffness also affects rotational stiffness of the joints and it depends on its activation level (number of actin-myosin cross brigdes) (43,44,45,46). In addition, the joint stiffness is up to contributions from all structures within and around the joint. Muscle, tendon, skin, subcutaneous tissue, facia, ligaments, joint capsule and cartilage are examples of these structures. From these terms, it can be concluded that stiffness has an active and passive components. Active components include muscle activation, stretch reflexes, co activation of muscles around joint, ground moment arm and energy conservations. Passive stiffness can be defined as the length –tension relationship of contractile materials of musculoskeletal system that is correlated to muscle thickness, body mass (31). All these factors combined and have an effect on overall leg stiffness.

To understand more about the determinants that affect the leg stiffness and the interaction between ground, several studies have been done by using different types of locomotion on humans and animals. For example, in previous studies it has been revealed that animals which explout bouncing movements, like running and hopping, can keep their leg stiffness constant at all speeds and they can change the angle swept by the leg to adjust for different speeds (16). Also, vertical stiffness changes with speed. Although leg stiffness can remain almost the same at all running speeds, humans are capable of changing their leg stiffness during bouncing gaits (24,29,32). There are several studies that have shown that leg stiffness is adjustable. When humans hop in place, the stiffness of the leg can be increased by more than double its size to accommodate increases in hopping frequency or increases in hopping height at a given frequency (19,24,32). Furthermore, recent evidence reveals that the stiffness of the leg spring can be increased by more than double during forward running at a given speed to allow a range of stride frequencies (23,24). A stiffer leg spring allows humans to run with a higher stride frequency at the same forward speed. Similarly, when humans bounce vertically on a compliant board, the stiffness of the leg springs can change by twofold in response to changes in knee angle (36,47,48,49). Also, all previous studies of hopping and running on elastic surfaces of different stiffness have shown that humans maintain spring-like leg behavior and adjust leg stiffness to conserve the center of mass dynamics (25,47,50). As the stiffness of surface was increased, the leg stiffness was decreased. If the surface was compliant the muscle level activation must be increased to achieve the same stiffness level. Consequently, leg plus surface stiffness remained constant by this modification of nervous system. Also, it has been revealed that this adaptation occurs within first step on a new surface (29). Therapists who are aiding individuals in gait or observing individuals walking over various surfaces must be aware of this capability of nervous system. These studies clearly show that it is possible to change the leg stiffness during bouncing movements.

Some of these studies explain how the leg stiffness is adjusted in different hopping conditions like hopping on different surfaces and hopping with different frequencies. Recent studies showed that it is possible to change leg stiffness for different surfaces by a combination of changes primarily in ankle stiffness and knee angle excursion (19,24). By increasing the angle swept by the leg spring, the vertical displacement of the center of mass and ground contact time can be reduced and at the same time, the leg stiffness can be maintained (23). And, when humans run with increased knee flexion, the stiffness of the leg spring appears to decrease indicating greater vertical displacement of center of mass (42).

Previous investigations have assessed the effects of added mass on leg stiffness. One of these studies reported that contact period increased 2.6% while carrying an additional 19% of body mass. Especially, it was mentioned that adding mass during unipedal hopping decreased preferred hopping frequency and hip flexion, on the other hand vertical stiffness, ankle dorsiflexion or knee flexion stayed the same (51). Another study looked at the effect of three different frequencies and added weight of %10 and %20 of subjects' body weight. They assessed if the combination of hopping frequency and added mass affected vertical stiffness, contact period and lower extremity kinematics during hopping. It was reported that vertical stiffness increased with increased hopping frequency but did not change with added mass. But added weight had an affect on increasing the contact duration time and ankle dorsiflexion (52).

Also in this study, the gender effect on leg stiffness was examined. Because it is known that women and men have different physical, biological and biomechanical properties which influence the biomechanical behaviors of females and males through functional activities. Smith at al. have shown that during walking women exhibited significantly more pelvic obliquity range and less vertical COM displacement comparing with men (53). When women and men were examined at frontal plane it was seen that women demonstrated more abduction of hip and knee at the stance phase of running comparing with men (54). Also men tend to have larger muscles (greater cross sectional areas) and greater muscle strength than women (31,55,56,57,58). Additionally, a relationship have been shown to exist between cross sectional areas, strength and stiffness (28,51,59). Sepic at al.(60) demonstrated that women strength ranged from 62 to 70 percent of the men for ankle muscle strength and there was a significant difference in ankle range of motion between male and female. Female demonstrated greater range than men. Also comparing the flexibility properties of men and women, it is well known that tendon structures of men are more resistant to stretching than women and young modulus in women are significantly lower than men indicating they have more compliant tendon structures compared to men (56). Besides these differences, previous studies revealed that women had lower stiffness of tendon structures and lower active muscle stiffness(61).

MATERIALS AND METHODS

Subjects

Eleven male and eleven female volunteers with no reported knee abnormalities or recent musculoskeletal injuries participated in this study. Also the subjects had no history of surgery in their lower extremities or of neurological disorders. Subjects were asked to participate in this study voluntarily. They were recruited from students and teachers of Maastricht University, Faculty of Health Science. Subjects ranged in age from 20 to 43 years.

Experimental Design

Before collecting data; the heights, the weights, the trochanter major heights from ground, the added weights according to subjects' weights and ages were recorded. The subjects were informed about the trials and their questions were answered. Measurements of leg stiffness were determined by requiring subjects to perform two-legged hopping on a force platform (Kistler 9281 A, natural frequency 1 KHz) sampled at 1000 Hz via an analogue to digital converter (12 bit, National Instruments). The protocol for hopping test was approved by Maastricht University, Movement Science department.

Protocol for Hopping Test

To assess the effect of different frequency on leg stiffness subjects were asked to hop in place with training shoes and with their hands on their hips at two separate hopping frequencies. Hopping was performed first at their preferred rate (normal frequency; subjects chosen their own frequency which was the preferred frequency for current study), then at the highest frequency that they were able to do (here, highest frequency was the voluntary peak force of subjects). Subjects were instructed that each hop must be a continuous motion and were allowed as much practice as needed until they felt comfortable at the asked frequency. The following instructions were given to the subjects:

1. leave the ground between hops and keep it as a continuous motion;
2. put hands on your hips;
3. stop hopping when you get the stop sign.

To assess the effect of added weight on leg stiffness on bipedal hopping, prior to data collection participants wore a custom-made weight vest containing 10% of their body mass. The frequency of hopping was the preferred frequency for hopping with added weights. The subjects hopped following the instructions above.

Collecting Data

We used a force platform to measure vertical forces. Each subject performed 5 hopping tests for each conditions. Subjects put their feet on the middle area of the force platform that was signed before. Then subjects performed hopping as the instructions above. The amplifier was reseted for each of measurement. The subjects were not on the platform when the computer was reset for each hopping. During the measurements force platform did not make any contact with the surroundings. When the subjects felt comfortable at the asked frequency, we started collecting data for 4 seconds for each hopping trial, which is consistent with 8 hops. At the end of each hopping test, the subjects were allowed to take rest for 1 minutes in order to maintain the beginning conditions without any fatigue symptoms. Thus, first trial contained 5 times hopping at normal frequency and 1 min. rest, and second 5 times hopping at highest frequency and 1 min. rest and last 5 times hopping at normal frequency with added weights. After each measurement, data was stored for further analysis. Each trial was coded. We gave the subjects codes from A to V, and the codes for the hopping conditions as A for normal hopping frequency and B for highest hopping frequency and C for hopping in normal frequency with added weights. The numbers of the trials were given from 1 to 5 for each hopping in each hopping condition. For example, CA2 means that third subject who was coded as C performed second hopping trial at normal hopping frequency.

Data Analysis

Data was recorded and analyzed by custom-made software developed with MATLAB 6.5.1 version that is a high-level technical computing language and interactive environment for algorithm development, data visualization, data analyses, and numerical computation. It has mathematical functions for linear algebra, statistics, filtering, optimization, and numerical integration. In MATLAB program the forces, position of center of mass, velocity and acceleration curves were determined. The average for five consecutive hops was used for analysis. MATLAB was programmed to our commands to figure out the graphs of all subjects' data as peak forces, acceleration of COM, velocity of COM and position of COM (fig.3,4,5). Beside these graphs, the program shows the subject's average peak forces, vertical displacement of COM, frequency, ground contact time and leg stiffness when we entered the subject's body weight for each individual.

Leg stiffness (kleg) is the ratio of the peak ground reaction force to the maximum displacement of the leg spring (ΔL). The peak vertical ground reaction force (F_{peak}) occurs at the same time as maximum leg compression.

$$k_{\text{leg}} = \frac{F_{\text{peak}}}{\Delta L}$$

The force exerted by the feet on the platform in vertical direction is

$$\mathbf{F=m.\,(g + a)}$$

where m is the mass of body, g is the gravitational force and a is the acceleration of center of mass.

The acceleration of the center of the body was calculated from ratio between *Fnet* and *m*.

$$a = \frac{F_{\text{net}}}{m}$$

In the equation above, *Fnet* is the difference between total force and the multiplication of mass and gravitational force.

$$\mathbf{Fnet = Ftot - m.g}$$

Since velocity is the integral of acceleration it can be obtained directly by numerically integrating the signal measured with the force plate (5). The integration constant must be known in order to calculate the absolute velocity. The value of the integration constant was used as zero when the center of mass reached its maximum compression, thus at peak force.

Vertical displacement of the COM (*dL*) was calculated from numeric double integration of the acceleration data. Integration constants for velocity were based upon steady-state performance criteria wherein the mean vertical COM velocity is zero. Since the goal was to determine COM displacement, the integration constant for position was set arbitrarily to zero at touchdown. Displacement of the center of mass was measured during each contact phase (62).

Contact duration is the time on the force platform from initial contact to take off. The values for each subjects were calculated from the differences between the time at take off phase and the time at initial contact.

$$\mathbf{gct = t2 - t1}$$

In the equation above *gct* represents ground contact time, *t2* is the time at take off and *t1* is the initial contact time.

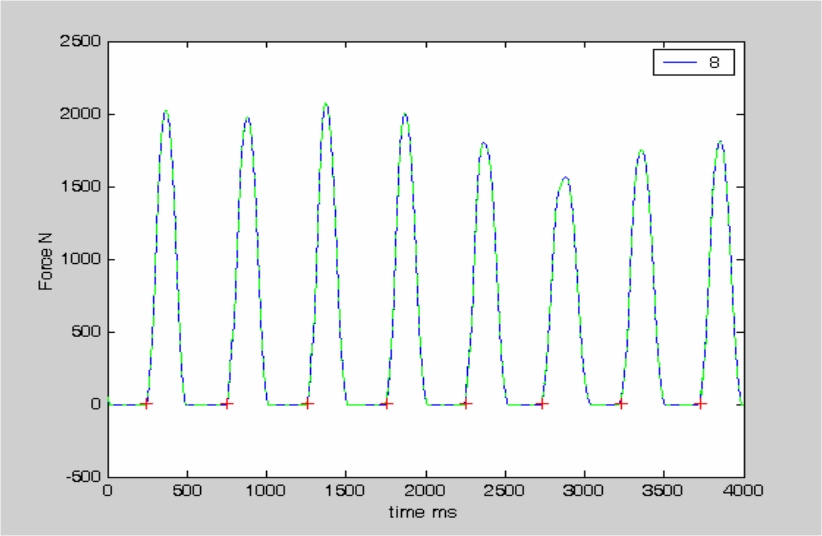

Fig. 3. **Forces (N) during 4000 ms of hopping trial**

The curves represent the average forces of five consecutive hops of subject during 4000 msn. The subject performed 8 consecutive hops during 4 sec. at preferred hopping frequency.

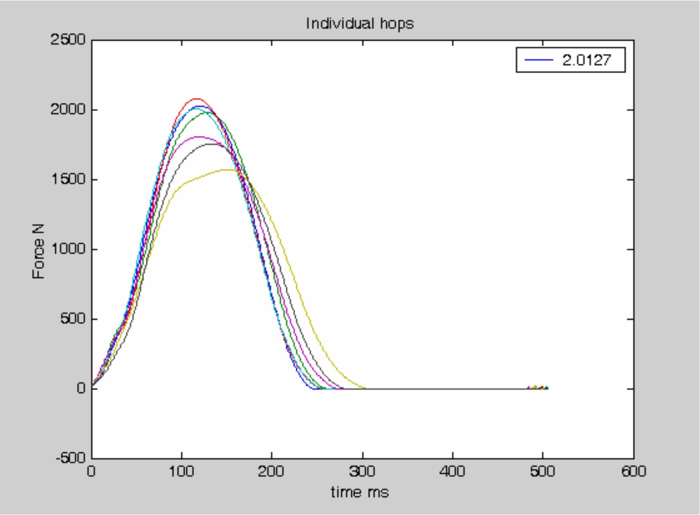

Fig. 4. Average of Forces (N)

In this figure, the forces of 8 consecutive hop combined together to obtain average peak force of one hopping trial. As it is seen in the figure the ground contact time of one hop takes time between 200 ms and 300 ms. The vertical ground reaction forces begin to rise as the subjects hit the ground and reach their maximum value (peak force) at the middle of the ground contact time. In the second half of ground contact phase ground reaction forces decrease till the beginning value zero.

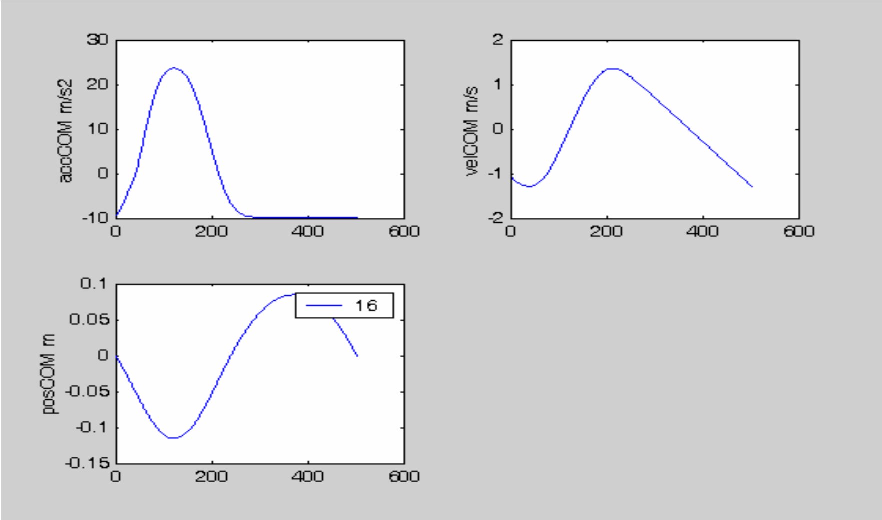

Fig. 5. Kinematics of Center of Mass at ground contact phase

a. acceleration of COM (m/s2)

b. position of COM (m)

c. velocity of COM (m/s)

a. To be able to hop, inertia must be overcome. COM has negative value of acceleration at the beginning of hopping to help to overcome inertia. Related to Fig.3 , increase in acceleration is proportional to increase in ground reaction force. And both acceleration and ground reaction force reach their maximum point b. The position of COM is at its minimum point where the ground reaction force and acceleration reach their maximum. At that point, maximum vertical displacement of COM occurs.

Statistical Analyses

Statistical analyses were performed using the SPSS.11 for windows package. In this study Mann-Whitney U test was performed to evaluate differences in body weight, height, trochanter major height from ground and age between gender. In addition Friedmann variance analyses were used to determine if there is a significant difference between adjusting leg stiffness for normal hopping frequency, highest hopping frequency and normal hopping frequency with added weight. When the results were significant Wilcoxon Signed Ranks with Bonferroni was performed as a post-hoc test to see if there was a significant difference between variables among conditions. Statistical significance was set at $\alpha < 0.05$ for all analyses. We also performed power test to see the power of sample size for each condition and variable.

RESULTS

In the data-analyzing phase, it was discovered that some data, which belonged to one female subject and one male subject were incomplete Thus, two subjects' data were removed and two subjects were excluded from study. The data was analyzed for 10 female and 10 male subjects ranged in age from 20 to 43. The subject characteristics are shown in Table 1.

Table 1 . Characteristics of the Subjects

GENDER		BW	H	TMH	AGE
FEMALE	*Mean*	59.8	1.67	83.9	23.7
	range	50-75	1.61-1.80	76-90	20-32
	N	10	10	10	10
	S.D	7.96	0.077	6.11	3.62
MALE	*Mean*	77.8	1.83	82.6	27.6
	range	73-84	1.75-1.98	80-1.04	22-43
	N	10	10	10	10
	S.D	3.85	0.061	29	6.83
TOTAL	*Mean*	68.8	1.75	83.25	25.65
	range	50-84	1.61-1.98	76-1.04	20-43
	N	20	20	20	20
	S.D	11.06	0.107	20.41	5.68

a. BW is bodyweight
b. H is height
c. THM is trochanter major height
d. Mean is mean values.
e. SD is standard deviation
f. N is number of subjects

The characteristics of subjects were looked for to compare if there were significant differences between female and male subjects by Mann Whitney U test. The body weight, height and trochanter major height differences were significant (p=0.000). On the other hand there was no significant difference between ages of the subjects (p=0.128)

i. Overall Results

Results from statistical analyses revealed that the hopping conditions had a significant effect on parameters that we calculated (frequency=fr, peak force=F, vertical displacement of center of mass=dl, leg stiffness=k (p=0.000) and ground contact time= ct (p=0.002)). Additionally, gender had a significant overall effect (p=0.005). If we looked at Mann Whitney U test results to see the gender effect on variables separately, it was seen that gender had a significant effect on only peak forces in three hopping conditions (p=0.011, p=0.041, p=0.010) and p value was significant for leg stiffness at normal hopping frequency and normal hopping frequency with added weight (p=0.006, p=0.002).

ii. Parameters of Leg stiffness Of All Subjects at Different Hopping Conditions

a. Frequency For all subjects, added weight had an affect on normal frequency to increase. But the results from Wilcoxon Signed Rank test with bonferroni showed that this difference was not significant. Therefore, the added mass had no significant effect on changing frequency. The results from Wilcoxon Signed Rank test revealed that there was a significant differences between the preferred hopping frequency and highest frequency with a high power (p=0.000, power=1.000) (Table 2)

The highest frequency that the subjects could reach was 4.16 Hz (Table 2)

b. F peak The peak reaction force was different from each other in three hopping conditions. The peak forces of all subjects increased by 3 % with added weight and decreased 20 % with increasing frequency. The Wilcoxon Signed Rank test with bonferroni indicated significant differences between peak forces at normal hopping frequency and highest hopping frequency statistically (p=0.000, power=0.997). There were no significant differences in peak forces when 10 % of body mass was added to subjects' body mass.

c. Ground contact time The ground contact phase was shorter during highest frequency (189.4) than normal hopping frequency (286.6). Ground contact time at normal hopping frequency was significantly different from the ground contact time at highest frequency. Also, it was found that added mass had a significant effect on increasing ground contact time comparing with normal hopping frequency. It can be pointed out that added mass only had a significant effect on increasing ground contact time (p=0.015).

d. Vertical displacement of center of mass dL Comparing the amount of vertical displacement of center of the mass in three condition, it can be said that the vertical displacement of center of mass was lowest at highest frequency hopping (dL=0.0285). The amount of vertical displacement of center of mass was greater when subjects performed hopping with added weight (dl=0.1115) than hopping at normal frequency (dl=0.1065). During the ground contact phase, the center of mass moved downward by only 4 % more on hopping at preferred frequency with added mass than hopping at preferred frequency.

The effect of different conditions on changing vertical displacement of center of mass was statistically significant (p=0.000). The vertical displacement of COM was significantly different between hopping condition at preferred frequencies and hopping condition at highest frequency with high observed power (p=0.002, power=1.000). By increasing mass, vertical displacement did not change significantly at normal frequencies. Wilcoxon Signed Rank test showed that this difference was because of the hopping condition at highest frequency.

e. Leg stiffness - K leg As we mentioned before, experimental condition had a significant effect on changing leg stiffness (p=0.000). The results from Wilcoxon Signed Rank test showed that the leg stiffness at normal frequency and at normal hopping frequency with added weight was significantly different from the leg stiffness at highest frequency (p=0.000). Leg stiffness was significantly affected by changing frequency, while the added mass did not change leg stiffness significantly.

Table 2. Effect of different hopping conditions on parameters of leg stiffness of subject

N=number of subjects analyzed * significant between conditions (comparing with normal frequency)

	Frequency (Hz) N=20	**F peak (N)** N=20	**Ground contact time (ms)** N=20	**Vertical displacement of COM (m)** N=20	**Leg stiffness (kN/m)** N=20
Preferred	2.14 ± 0.26	2313.72 ± 603.06	286.60 ±41.78	0.1065 ± 0.03	24.1 ± 9.94
Highest	4.16 ± 0.56*	1844.47 ± 382.25*	183.43±22.81*	0.0285 ± 0.02	70.12 ± 22.0 *
Added	2.20 ± 0.40	2320.01 ± 556.05	300.39±40.02*	0.1115 ± 0.04	24.2± 10.7

* $p < 0.05$ Friedman Variance analysis

.

iii. Gender Effect on Parameters of Leg Stiffness at Different Hopping Conditions

The male and female subjects hopped at different preferred frequencies, but not significantly different from each other (Table 3). For high frequency, condition females performed a hopping at a frequency (4.19 Hz) which is greater than male's highest frequency (4.13 Hz). When we added % 10 kg of subjects body weight as an added weight then it was seen that female subjects decreased their normal frequency and male subjects increased it. The gender affect on overall frequencies was not significant statistically (Table 3).

Table 3. Frequencies in different conditions

N=number of subjects analyzed

Frequency (Hz)	Female ((N=10)	Male (N=10)	P value
Preferred	2.07 ± 0.18	2.21 ± 0.31	0.162
Highest	4.19 ± 0.65	4.13 ± 0.49	0.307
Added	2.04 ± 0.23	2.36 ± 0.48	0.173

* $p < 0.05$ Mann- Whitney U test

The peak reaction force was significantly greater in males than females in three conditions (p=0.011, p=0.041, p=0.010) but the difference was related to increased body mass in the male population. The female subjects weighed 76 % of male subjects in the population. The differences between bodyweights of the male and female subjects was also significant as mentioned before (p=0.000). So, the peak forces of women were 75 % of men at the preferred hopping frequency parallel to ratio between body masses. When the forces normalized to bodyweight, the differences between peak forces of men and women disappeared (table 5). The female and male subjects reacted differently to added weight. The peak force of female subjects increased with added weight approximately 3 %. But the male subjects decreased their peak forces 1.5 % with added weight and decreased by increasing of frequency. So, there is no direct relationship between added weight of 10 % of body weight and peak forces.

As seen in Table 4, the differences between peak forces of males and females are different from each other. For the conditions at preferred hopping frequency and with added mass at normal hopping frequency, the differences between peak forces of male and female subjects were remarkable. The peak forces values of males were pretty greater than females. On the other hand, the differences between peak forces of male and female subjects at highest frequency were not substantially different (Table 4).

Table 4. F peak in different hopping conditions in female and male subjects

N=number of subjects analyzed
^ = male subjects significantly greater than female subjects

Force (N)	Female (N=10)	Male (N=10)	P value
Preferred	1985.62 ± 543.31	2641.83 ± 483.06^	0.011
Highest	1655.61 ± 263.35	2033.32 ± 399.35^	0.041
Added	2037.66 ± 490.03	2602.63 ± 485.24^	0.010

^ $p < 0.05$ Mann- Whitney U test

Table 5. F peak normalized to BW in different hopping conditions in female and male subjects

N=number of subjects analyzed

Force (N)	Female (N=10)	Male (N=10)	P value
Preferred	33.99 ± 14.5	33.94±13.7	0.912
Highest	27.67 ± 10.5	25.51± 9.2	0.315
Added	34.06± 14.9	33.44± 12.2	0.971

* $p < 0.05$ Mann- Whitney U test

Ground contact time of female and male subjects at three different hopping conditions was not significant statistically. Female subjects performed longer ground contact time while hopping at all hopping conditions comparing with the male subjects (Table 6).

Table 6. Ground contact time in different hopping conditions in female and male subjects

N=number of subjects analyzed

Ground contact time (msn)	Female (N=10)	Male (N=10)	P value
Preferred	299.8 ± 46.53	273.36 ± 33.45	0.140
Highest	191.19± 27.12	187.68 ± 18.87	0.940
Added	313.88 ± 41.70	286.90 ± 35.18	0.212

* $p< 0.05$ Mann- Whitney U test

The amount of vertical displacement in female subjects are greater than male subjects in hopping conditions at preferred frequency and with added weight, on contrast male subjects had greater displacement of COM in condition at highest frequency. Additionally, the differences between vertical displacement of center of the mass among gender were not significant statistically (Table 7).

Table 7. Vertical displacement of center of the mass in different conditions in male and female subjects

N=number of subjects analyzed

Vertical displacement of COM (cm)	Female (N=10)	Male (N=10)	P value
Preferred	0.1150± 0.02	0.0980 ± 0.03	0.186
Highest	0.0270 ± 0.01	0.0290 ±0.02	0.290
Added	0.1270 ± 0.04	0.0960 ± 0.04	0.082

* $p < 0.05$ Mann- Whitney U test

The leg stiffness of male subjects was greater than female subjects in hopping conditions at normal frequency and with added weight (p=0.006, p=0.002). At highest frequency the differences between leg stiffness of men and women was not significant statistically. The leg stiffness of female subjects at the preferred hopping frequency was 61 % of the leg stiffness of male subjects. The leg stiffness of female subjects became 85 % of male subjects' leg stiffness when subjects wanted to hop at their highest frequency. With added weight the differences of leg stiffness between male and female subjects again became remarkable and the leg stiffness of female subjects was 55 % of male subjects in this current study (Table 8).

Table 8. Leg stiffness in different conditions between female and male subjects

N=number of subjects analyzed

^ = male subjects significantly greater than female subjects

Leg stiffness (kN/m)	Female (N=10)	Male (N=10)	P value
Preferred	18.3 ± 6.05	29.9 ± 9.86 ^	0.006
Highest	65.3 ± 21.15	74.9 ± 22.48	0.880
Added	17.2 ± 6.17	31.3 ± 9.88 ^	0.002

^ $p < 0.05$ Mann- Whitney U test

DISCUSSION

Stiffness is considered as a regulated property of the neuro-musculo-skeletal (NMS) system. Stiffness and spring mass model was studied to avoid the complexities by looking at the overall behaviour of NMS. In this study, the adaptation property of leg stiffness is tested by hopping test to asses the effect of different conditions on leg stiffness of men and women. Better understanding the adjustment mechanism of leg stiffness that is used by men and women would provide stronger knowledge about locomotion and NMS. To this aim we tested three hypothesis, 1) leg stiffness would increase by increasing frequency 2) leg stiffness would increase with added weight 3) men would have greater value of leg stiffness than women.

The results from this study demonstrates that leg stiffness increases by increasing frequency which is consistent with our first hypothesis but it does not change with added weight in contrast to our expectation. With added weight, increase in leg stiffness was hypothesized because by increasing mass of the spring system it is expected that the vertical force applied to ground would increase and would have a significant effect on increase in leg stiffness. As expected men have greater value of leg stiffness then women at all conditions and also it is seen that men and women react different from each other to different hopping conditions. Women are more compliant and they reacted different from men to different hopping conditions by parameters of leg stiffness.

It is possible that, the differences between men and women in biomechanical and viscoelastic properties of musculoskeletal system lead to differences in stiffness parameter of NMS. In previous studies it was found that stiffness parameter of men at the preferred hopping frequency was 24.18 kN/m and 19.85 kN/m for women (61). These results are almost the same with our study. Leg stiffness in female subjects during the hopping task was approximately 61 % of the leg stiffness in male subjects, which resembles mass differences between them. Several studies have observed that leg stiffness in females is lower than the leg stiffness observed in males (61,63). Leg stiffness represents the overall stiffness of musculoskeletal elements of lower extremities in contact with ground (11,16,18,19,32). It has passive and active components (63). One of the passive components of stiffness is the mass of the muscle group in leg (64). The women in our study had shorter leg lengths and lower body weights; therefore, they have smaller leg mass. It has been shown that large muscle mass increases leg stiffness (16,61). The ratio between body mass and leg stiffness indicated the leg stiffness differences between men and women is related with differences in body mass in our experiment. The activation and preactivation time of muscle influences the stiffness as an active component (5). For men, this earlier preactivation time may lead to greater tension and therefore increased stiffness (43). They could be one of the possible explanations of the differences between leg stiffness of men and women.

In the previous study, Granata et al.(64) performed preferred hopping frequency and hopping at 3.0 Hz so that they achieved different hopping frequencies. Stiffness of leg increased in both female and male subjects by increasing frequency. But the significant differences between leg stiffness of male and female subjects maintained. In our study, subjects performed their highest frequency as 4.16 Hz, which is the average of both gender. And the significant differences in leg stiffness values of men and women disappeared (kleg for men=74.9 and kleg of women = 65.32). It can be concluded that by increasing frequency women demonstrated different strategies and increase their leg stiffness significantly. Women chose smaller displacement of COM than men and decrease the differences of peak forces between them.

When subjects were asked to hop at their preferred hopping frequency women and men performed different hopping frequencies. The women subjects demonstrated lower hopping frequency than men did. Earlier studies reported that the women and men demonstrated similar preferred hopping frequency, which is unlike our finding (61). One possible explanation why women performed lower frequency than men as a preferred frequency could be the active and passive components of stiffness differences between men and women. The mean value of preferred hopping frequency of all subjects was 2.14 Hz in our study. It was reported in the previous studies that preferred hopping frequency was 2.2 Hz (24). Granata et al. (64) described the preferred hopping frequency as 2.34 Hz on two legged hopping and Jones et al.(65) reported as 2.06 Hz on one legged hopping. Considering the differences, the results of this study are similar to previous studies and reflect a fundamental property of the human NMS. Cavagna demonstrated that leg stiffness during two legged hopping was greater than one-legged hopping (62). Because of leg stiffness is greater in two legged hopping, the preferred frequency of leg spring could be greater than preferred frequency of one legged hopping.

If we look at the frequency change across different conditions we realize that when subjects asked to hop at their highest frequency, women performed higher frequency than men but this difference was not significant. The maximum value that was achieved by women was 4.19 Hz. The value for highest hopping frequency and the differences for highest hopping frequency between men and women have not been reported yet in scientific literature. And other hopping condition, hopping with added weight increased frequency comparing with preferred frequency. This finding is not consistent with previous studies. It was reported that the preferred hopping frequency decreased with an additional 19 % of body mass during unipedal hopping (51). In addition, if we looked at gender women decreased their preferred hopping frequency with additional weight while men increased their frequency.

At the highest frequency, the leg spring stiffness is adjusted to greater stiffness in both female and male subjects. The subjects experience smaller vertical forces, which result in smaller vertical displacement. Although both these parameters decreased, comparing with the decrease in vertical displacement of COM decrease in peak force was not substantially. Peak force decreased slightly while the vertical displacement of COM decreased significantly. Thus the leg stiffness increased at highest frequency significantly. This finding of our study is identical to findings of earlier studies (23,24,32). But some other studies have found that peak vertical ground reaction forces increase by increasing the frequency of hopping (18,66).

In all hopping conditions, males demonstrated greater peak vertical ground reaction forces than females related to increased body mass in male population. The peak force in the leg spring is nearly proportional to body weight (16). The peak forces normalized to bodyweight demonstrated this relation clearly. The peak reaction forces of males were remarkable greater than females at normal hopping frequency and normal hopping frequency with added weight. But when both female and male subjects performed highest frequency hopping, the differences between peak forces of female and male subjects significantly decreased in our study. One possible explanation of this result could be the differences in vertical displacement of COM between men and women across conditions. As it was seen in results the differences between vertical displacement of COM of men and women became quite smaller at highest frequency, furthermore at the highest frequency women demonstrated smaller value of vertical displacement than men. As a result the differences of leg stiffness between men and women at highest frequency became smaller unlike other two conditions

In earlier studies, it has been reported that with increasing hopping frequency hip flexion, knee flexion and ankle dorsiflexion decreases (51). Thus, the vertical displacement of COM decreases by increasing hopping frequency. Also it has been reported that with additional mass ankle dorsiflexion increased significantly while hip flexion and knee flexion were not effected (51,52). Thus, vertical displacement was not significantly changed with added mass. Vertical displacement of COM was influenced by body mass like peak force. Both of them increased by increasing mass (16). This finding is consistent with our study. In our study peak forces increased by 3% with added weight while the vertical displacement of COM also increased by 9 % with comparing normal frequency. This could be one possible explanation why leg stiffness did not effect significantly by adding mass. And on other possible explanation could be immediately adjustment property of leg spring system. As it was mentioned before, previous studies showed that people adjust their leg stiffness within first step on a new surface (29). And this indicates that stiffness is adjusted immediately. Maybe with added mass, they have already adjusted their stiffness before measurement. Maybe, adding mass can change leg stiffness if it is applied in the middle of measurement.

Furthermore about COM displacement, researches suggest that COM motion is modulated with hopping frequency or running cadence (23,61,64). By modifying COM displacement it is possible to maintain a constant value of peak acceleration and ground reaction force independent of hopping frequency. The touchdown geometry and flexion of the joint cause vertical displacement of COM to increase (11,42). Thus, increased flexibility and larger range of motion in females may indicate greater COM displacement at normal hopping frequencies. In this current experiment, we did not record video analyses and flexion degrees of joints of subjects. For further studies, it can be taken into account. Here it is needed to point that the aim of this study was to identify overall effects of gender on leg stiffness. And these interesting results of our study points at interesting future studies.

One of the parameters of the leg stiffness that was observed in current study was ground contact time. Ground contact time was significantly different from each other at all hopping conditions. Here, the important point is added mass condition had only significant effect on increasing ground contact time. This result agrees with earlier study of Austin et al.(51). They reported this increase in ground contact time as a significant main effect of added mass (51,66). Also it is seen that aerial phase of subjects decreased with added weight to maintain almost the same cycle time with normal frequency conditions. This reduction leads to lower hopping heights at normal hopping frequency with added weight.

Here hopping height was calculated from the equation below;

$$\boxed{X=V*t+0.5*G*t^2}$$

Where X is hopping height, t is half the duration of the aerial phase, G is the gravity (10 m/s^2), and V is impact velocity (calculated from G and half the duration of the aerial phase).

If we focus on the lower hopping heights of men and women at hopping with added weight separately , it is seen that male subjects decreased their height almost half value of height at normal hopping frequency condition. Here, cycle time decreased while contact time increased comparing with normal frequency conditions. Thus, smaller aerial time makes these differences in hopping height. For female subjects, the cycle time increased slightly since ground contact time increased. Although cycle time increased slightly, greater increase in ground contact time with added weight caused the shorter aerial time that leads to shorter heights.

In contrast to this increase in ground contact time with added weight, by increasing frequency ground contact times decreased in our study. On the other hand it is known that shorter ground contact times are related with increasing leg and ankle stiffness (18,66). The primary mechanism for reducing the ground contact time was that the subjects increased their leg stiffness nearly three times from 24.18 kN/m during hopping at preferred frequency to

64.78 kN/m during hopping at highest frequency. These results are similar to the study by Arampatsiz et al (66). And the decline in contact time across frequency was significant. One interesting point here is since the frequency is same between men and women, the results suggest that the aerial phase is longer in men as their ground contact time is shorter than women. It means men had higher hopping height at hopping highest frequency condition to achieve higher aerial phase with shorter ground contact. When hopping heights is calculated it supports that idea (hopping height of men= 4056 mm, hopping height of women= 2996 mm)

The female and male subjects reacted differently at three hopping conditions by parameters of leg stiffness. When we looked the results of the parameters of leg stiffness between men and women we thought that the differences between hopping heights of men and women could be one of the reasons for these different reactions between hopping conditions. When women and men performed hopping at their preferred frequency, they chose different frequencies. Therefore, different duty cycles occurred between genders. So, women chose higher hopping height comparing with men at normal hopping frequency and hopping with added weight. The compliance of women musculo skeletal system has a favourable effect on storage of strain energy in these structures (63). Storage and use of elastic energy lead to higher performance (26). Performing highest hopping is one of the variables that is effected by performance. The higher hopping height of women could be explained by this property of women musculo skeletal system.

On the other hand, at highest frequencies the differences between frequencies of men and women disappeared. Since the frequency is same between gender, men performed higher aerial time than women did. So that, higher hopping height than women. The higher performance of men could be considered at this situation.

Hopping height, frequency and running velocity are related with performance (6,26,66). In terms of performance some level of stiffness is required for optimal utilization of the stretch- shortening cycle (SSC) promotes storage and use of elastic strain energy during concentric and eccentric contractions (5,9,36,67,68,69). Activation of stretch reflex is one of the potential contributor to the SSC (9,70,71). It is known that men have higher reflex response than women (64,72). The differences of hopping height during hopping activity at the highest frequency (which is almost same between gender) between men and women could be explained by differences in reflex response which is related with SSC and therefore performance. Also greater leg stiffness causes higher performance which is needed to perform higher hopping height (19,66,67).

Thus, some amount of stiffness is needed to optimize the performance and decrease the risk of injury. These parameters are important for training and rehabilitation programs. Most often flexibility is taken into clinical assessments. Flexibility is best defined as the behavior of muscle-tendon units in response to stretch especially at muscle lengths used during daily activities. In contrast, stiffness is the amount of deformation proportional to load applied (72). From a clinical perspective, assessment of stiffness parameter may provide additional insight into training and rehabilitation programs. So that, further researches are needed to include stiffness assessment and the acute and chronic effect of different kind of exercises on leg stiffness in different clinical conditions of NMS.

Leg stiffness is an adjustable parameter. It may change with an existing pathology. Further studies can be proposed to examine adjustment of stiffness to different pathologies. The results of our study will provide a scientific basis for researches in this field. Also, the result of our study on the differences between women and men in adjustment mechanism to different conditions can considered in further studies.

To further understand the differences between men and women and the mechanism they use to adjust leg stiffness, further studies should be performed including EMG and video analyses . Because it is known that posture during hopping and muscle activities influence the leg stiffness (64). In this study, the gender effect on leg stiffness was measured with a quite low observed power (p=0.454). To see the differences between leg stiffness of male and female subjects clearly further studies should be performed with larger sample.

CONCLUSION

Leg stiffness is adjustable to different conditions of locomotion. Leg stiffness is affected by changing frequency proportionally while added mass has no effect on changing leg stiffness. Added weight has only significant effect on increase in ground contact time. The most effective reason for increasing leg stiffness at highest hopping frequency is the decrease in vertical displacement of COM. Men has greater leg stiffness than women at all hopping conditions. Women achieve almost the same leg stiffness with men at highest frequency. Women and men have significant differences in peak vertical ground forces, which is proportional to body weight. Women and men react different from each other to different hopping conditions. Women are more compliant and react differently from men to different hopping conditions by parameters of leg stiffness. To see the main reason what causes this different behavior to adjustment of leg stiffness further studies should be done with larger samples including EMG and video analyses.

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APPENDIX