Effects of Vibration on Vertical and Joint Stiffness in Ankle Instability and Healthy Subjects

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ABSTRACT

Effects of Vibration on Vertical and Joint Stiffness in Ankle Instability and Healthy Subjects

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Doctor of Philosophy

Some have suggested acute increases in musculotendinous stiffness ($k$) following whole body vibration (WBV). Others propose that chronic ankle instability (CAI) may alter $k$ of the lower extremity. Changes in proprioceptive activity and/or gamma motoneuron activation post-WBV and/or due to CAI could lead to alterations in $k$. However, little is known about acute effects of WBV on $k$ and less is known about changes in $k$ with CAI. **PURPOSE:** Assess differences in vertical and joint $k$ between healthy and CAI subjects during single-limb landings and detect alterations in $k$ measures post-vibration. **METHODS:** Subjects were identified as CAI via the FAAM, MAII and special testing. Thirty-five CAI subjects (17 males, 18 females; age = 22 ± 7 yr; height = 1.73 ± 0.23 m; mass = 70 ± 30 kg) and 35 matched healthy subjects (17 males, 18 females; age = 23 ± 5 yr; height = 1.73 ± 0.21 m; mass = 70 ± 35 kg) qualified for this study. Kinetic (2000 Hz) and kinematic (250 Hz) data were recorded during several jump landings pre- and post-WBV. Five repetitions of WBV, at 26 Hz and 4 mm amplitude, were introduced between pre- and post-WBV jump trials. The jump task included a double-limb jump followed by a single-limb landing and a subsequent contralateral hop. Vertical $k$ ($\Delta$vertical GRF/center of mass vertical displacement), hip, knee and ankle joint $k$ ($\Delta$joint moment/$\Delta$joint angle) were calculated, averaged across five successful pre-WBV and across six post-WBV trials. An ANOVA was used to detect between-group differences, while an ANCOVA was used to analyze within-group differences post-WBV using pre-measures as covariates. A pseudo-Bonferroni adjustment was performed prior to statistical analysis ($p < 0.01$). **RESULTS:** No between-group differences were observed for any of the variables ($F_{1,68} = 0.020$ to 1.400, $p = 0.240$ to 0.890). A significant increase in vertical $k$ was observed post-WBV for the healthy group ($t_{67} = 2.760$, $p = 0.008$), but not for the CAI group ($t_{67} = 0.370$, $p = 0.720$). The CAI group did demonstrate a decrease in ankle ($t_{67} = -3.130$, $p = 0.003$) and knee ($t_{67} = -3.490$, $p = 0.001$) joint $k$ post-vibration. No other within-group differences were observed post-WBV ($p > 0.01$). **CONCLUSIONS:** It appears that WBV does acutely increase vertical $k$ in healthy subjects. However, this treatment effect was not observed in CAI. Further research is needed to assess how $k$ is regulated in CAI subjects and why CAI subjects responded differently to WBV.

Keywords: ankle, instability, stiffness, vibration
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Introduction

Ankle sprains are the most common injury among the physically active. The average cost of treating ankle sprains in the USA is over $2 billion dollars each year. More athletic games and practices are missed due to ankle sprains than from any other injury. Up to 73% of people who sustain an ankle sprain will develop chronic symptoms after the first sprain. Chronic ankle symptoms often include self-reported disability and impaired physical activity. One of the most common self-reported symptoms is a feeling of “giving way” of the involved ankle during activities of daily living. This repetitive “giving way” is frequently used to define chronic ankle instability (CAI).

There are two subtypes of CAI discussed in the literature. Mechanical instability occurs when extensive damage to the static stabilizers (e.g. ligaments) causes pathological laxity. Functional instability (FI) is defined as chronic instability with intact joint integrity. Functional instability appears to respond to conservative treatment. Despite comprehensive rehabilitation, however, even some FI patients continue to experience chronic symptoms. Several variables are thought to contribute to FI. Decreased force sense, kinesthesia, joint position sense, strength, balance and postural control have been observed in FI patients. Increased peroneus longus electromechanical delay and latency have also been observed in FI subjects. Functional instability is usually attributed to the sensorimotor deficits that accompany joint and ligamentous injury. However, recent research suggests that mechanical and functional instability are not mutually exclusive. Current thinking suggests CAI patients have some degree of sensorimotor deficits and mechanical instability. Even mild mechanical instability accompanied by sensorimotor deficits may alter the stiffness of the leg and lower extremity joints, potentially predisposing CAI patients to further injury.
To model vertical stiffness of the leg, the human body is usually represented as a simple mass-spring system (mass block sitting on springs). Vertical stiffness is calculated by dividing the peak vertical ground reaction force by the vertical displacement of the center of mass during a dynamic activity. Hip, knee and ankle joint stiffness are components of vertical stiffness. Joint stiffness is related to the joint’s ability to resist rotational and translatory movements. Stiffer springs may result in increased joint stability. If ankle joint stiffness is altered due to injury, joint stability may also be affected. Some have reported a decrease in muscle stiffness of the leg following ankle injury. Others have observed an increase in ankle joint stiffness in CAI subjects. Due to the limited research, alterations in vertical and joint stiffness that occur with CAI remain unclear.

One intervention that may acutely alter muscle stiffness of the lower extremities is whole body vibration (WBV). Vibration training employs low frequency and high amplitude sensory input to purportedly enhance strength, power, and athletic performance. Several investigators have reported acute changes in strength, power, and jump height after a single session of vibration training. Some propose the immediate effects of WBV are due to increases in muscle stiffness. Previous jump-landing data collected in our biomechanics lab revealed altered activation patterns of both proximal and distal muscles of the involved lower extremity in a CAI population. These alterations in CAI muscle activity may play a role in changing the dynamic stiffness of the lower kinetic chain. Even though Cronin et al lacked the statistical power to show a significant difference in muscle stiffness after one session of WBV, they still asserted that there was an observable increase in passive stiffness of the plantarflexors post-WBV in healthy subjects. Some theorize that an acute increase in muscle stiffness post-vibration may be due to heightened gamma activation. Others contend that WBV does not
increase muscle spindle sensitivity in healthy college students. They speculate other mechanoreceptors may enhance proprioceptive feedback, which can have an immediate effect on performance. In light of the conflicting results and theories, it is obvious that further research is required to understand the acute effects of WBV on stiffness.

Increasing joint stiffness could be an effective intervention for patients post-sprain and might aid in preventing chronic instability. Whole body vibration may be a practical way to acutely enhance joint stability by increasing stiffness of the involved ankle. Therefore our purpose was threefold. The primary purpose of this study was to reveal any differences in vertical and lower extremity joint stiffness between CAI and healthy subjects. We hypothesized that CAI subjects would demonstrate altered vertical and lower extremity joint stiffness compared to matched, healthy subjects. The secondary purpose was to observe the acute effects of WBV on vertical and joint stiffness in CAI and healthy subjects. We hypothesized that WBV would acutely alter vertical and joint stiffness in both groups. Lastly, we wanted to determine if a correlation existed between a passive measure of joint stiffness and a dynamic one. We hypothesized that a relationship would not exist between the different stiffness measures. A better understanding of potential alterations in vertical and lower extremity joint stiffness in CAI subjects during a dynamic task may aid in discovering contributing factors of chronic instability. Understanding the effects of WBV on stiffness in the CAI population might lead to more effective interventions in the treatment and prevention of CAI.
Methods

Study Design

This study implemented a controlled laboratory design. A pre-test, post-test repeated measures statistical design was used to assess differences in the following dependent variables: vertical stiffness, hip, knee and ankle joint stiffness. These variables were measured during a jump-landing task before and after one session of WBV in CAI and healthy subjects.

Subjects

Seventy physically active, college-age subjects were recruited for this study. A power analysis was performed with previously collected data. The power analysis predicted at least 35 subjects in each group would be needed to observe pre-test, post-test differences in vertical stiffness. Therefore, 35 healthy (17 males, 18 females; age = 23 ± 5 years; mass = 70 ± 35 kg; height = 173 ± 21 cm) and 35 CAI (17 males, 18 females; age 22 ± 7 years; mass 70 ± 30 kg; height 173 ± 23 cm) subjects were recruited to participate. Subjects who reported a history of two or more ankle sprains, chronic “giving way”, scored ≤ 90% on the Functional Ankle Ability Measure (FAAM) ADL section and/or scored ≤ 80% on FAAM Sports section (Appendix A), reported at least 2 “yes” answer on questions 4-8 of the Modified Ankle Instability Instrument (Appendix B) (Tab. 1), and demonstrated negative anterior drawer and talar tilt special tests (Appendix C) (Tab. 2) qualified as CAI subjects. Ankle arthrometry was performed to quantify special testing (Tab. 2). Ankle sprains were defined as an inversion injury causing pain, swelling and loss of function (e.g. antalgic gait). Healthy subjects reported normal scores on both questionnaires and reported one or fewer ankle sprains in lifetime. Healthy subjects were matched to CAI subjects according to age (± 5 years), height (±2 inches), weight (±10 pounds),
and gender. Healthy subjects’ involved ankle was determined by matching leg dominance to involved ankle of corresponding CAI subjects. Leg dominance was defined as the leg you would use to kick a ball. Subjects were excluded if they had a history of cardiovascular disease, neurological disorder, pregnancy within the last year, lower extremity surgery within the last two years, ankle surgery within lifetime, or ankle sprain within the last 90 days. All subjects who qualified to participate in this study read and signed the university’s approved informed consent form (Appendix D) prior to participation.

Instrumentation

Ankle Arthrometry

An ankle arthrometer (Blue Bay Research, Navarre, FL) was used to measure passive ankle joint stiffness in the frontal and sagittal planes. For frontal plane stiffness, 4,000 N·mm torque was applied to assess inversion-eversion rotation (measured in degrees). A one hundred Newton force was applied to assess anterior-posterior displacement (measured in millimeters) in the sagittal plane.

Ground Reaction Forces

An embedded force plate (AMTI OR6-5, Newton, MA) was used to measure 3-dimensional ground reaction forces (GRF) during landings (2000 Hz). Peak vertical GRF was used to calculate vertical stiffness. Ground reaction force data were also used to calculate hip, knee and ankle joint moments.
Kinematics

To estimate center of mass excursion and to measure lower extremity joint angles, kinematic data was collected at 250 Hz using ten Vicon cameras (6 MX13+, 2 MXF20, and 2 MXT20) running on Vicon Nexus 1.6.1 software (Vicon, Centennial, CO). Kinetic and kinematic data were synchronized using the Vicon system. For data collection, subjects dressed in spandex clothing. Subjects also wore a standard shoe (Nike T-Lite V RX) during study participation.

Procedures

All subjects participated in one session. The first part of the session consisted of completing the questionnaires, recording anthropometric data, reviewing data collection procedures, reading and signing the informed consent form. Once the consent form was signed and dated, ankle arthrometry was collected bilaterally with the foot bare. With the ankle positioned at 90°, three repetitions in the anterior-posterior direction and three repetitions of inversion-eversion rotation were performed. After arthrometry was recorded, shoes were donned and reflective markers were placed. Fifty-nine reflective markers were applied to each subject to estimate their center of mass position and to measure three-dimensional hip, knee and ankle angles. Single markers were placed on the following landmarks: sternum, both acromion processes, C7 spinous process, inferior angles of the scapulae, T7 spinous process, both lateral humeral epicondyles, both dorsal wrists (mid-styloid processes), both posterior-superior iliac spines (PSIS), both anterior-superior iliac spines (ASIS), both greater trochanters, both medial femoral condyles, both lateral femoral condyles, both medial malleoli, both lateral malleoli, bilateral dorsal foot, bilateral dorsal 2nd metatarsal heads, bilateral 5th metatarsal bases, and
bilateral insteps (Fig. 1). Rigid, three marker clusters were placed on the heels of both shoes, rigid four marker clusters were placed on both thighs and bilateral shanks, and a 4-marker cluster headband was donned.

After marker placement, subjects performed a 5 minute treadmill walk with speed standardized to leg length\textsuperscript{33} and practiced the jump-landing task. Prior to jump landing practice, a quiet standing motion trial was recorded for 3 seconds. At this time, the subject was also recorded while flexing-extending and abducting-adducting each hip three times ($\geq 20^\circ$ for each movement). This was done in order to enhance the estimation of the hip joint center by using a moving or “functional” hip\textsuperscript{34} in our kinematic model. Subjects practiced the jump-landing task 5 times. During these 5 practice trials, subjects were asked to jump as high as possible and still perform the task correctly. The involved PSIS marker was tracked during each attempt to measure vertical height. Maximum vertical height was determined for each subject from an average of 3 practice jumps (the high and low practice trials were excluded).

The jump-landing task (Fig. 2) consisted of a bilateral jump followed by a single-leg landing onto the involved leg and a subsequent hop onto the non-involved leg, in a direction contralateral to the landing leg. Subjects were asked to look at a taped “X” on the lab wall directly in front of them while performing the jump landings. Subjects started the jump 1 m from the center of the force plate for each trial.

Prior to the WBV training session, subjects successfully performed five jump-landing trials with a 60 second rest interval between jumps. Jump height was monitored during data collection to ensure height was within $\pm 5\%$ of the calculated maximum vertical height. If jump height was not in this range or subject failed to perform the proper landing and subsequent hop,
the trial was considered unsuccessful. After 5 successful jump landings were recorded, subjects performed five 60-second repetitions of WBV (Galileo 2000) at 26 Hz and 4 mm amplitude. Subjects maintained a static squat position with knees flexed at 40° (per goniometer) and their feet set on the number two position during each WBV repetition. Surgical tubing, placed at the proper buttock height, was used to visually assess proper positioning during the entire repetition. A 60-second rest interval was observed between each WBV repetition. Successful post-training landing trials were collected immediately following the last WBV repetition and at 1-minute intervals for the first five minutes post-WBV (total of six post-WBV trials). Subjects were instructed to perform the jump-landing task exactly as they did prior to WBV. Trials were excluded if subject did not perform the task correctly, did not land completely on the force plate, did not reach ± 5% of their previously calculated maximum height and/or post-WBV trials were not within 5 minutes post-vibration.

Data Reduction

Three-dimensional coordinates for the reflective markers and the ground reaction forces were recorded using VICON Nexus software. The raw marker coordinates and kinetic data were then imported into Visual 3D software (C Motion, Germantown, MD, USA) and smoothed using a 4th order low-pass Butterworth filter. A 10 Hz cutoff frequency was determined to be appropriate using a residual analysis technique. The smoothed motion data was then used to calculate three-dimensional ankle, knee, and hip joint kinematics in Visual 3D. A custom, static model was developed for each individual incorporating the subject’s height and mass. The coordinate system convention used was: + medial-lateral (X) was towards the subject’s right, + anterior-posterior (Y) was forward and + proximal-distal (Z) was up. A 6-degree of freedom pose estimation was used. Midway between the two ASIS markers defined the origin of the
pelvic coordinate system. Unit vectors created from the ASIS and PSIS markers were used to generate the orthogonal axes. Right hand rule was used to determine the order of vector multiplication to identify the orthogonal axes. All 4 pelvic markers were used to track the pelvic segment. Estimation of the functional hip joint center has been previously described. To develop the thigh segment coordinate system, a superior vector was created along a line from the femoral intracondylar mid-point to the hip joint center. Next, a vector was created along a line between the two femoral condyles. Again, the right hand rule determined the order of vector multiplication to identify orthogonal axes. Thigh tracking markers included the greater trochanter and a rigid 4-marker cluster. Estimation of the knee joint center was mid-point between femoral condyles. To develop the shank segment coordinate system a superior vector was created connecting the mid-point of the malleoli to the knee joint center. Next, a vector was created along a line connecting the malleoli. Orthogonal axes were determined using the same vector multiplication procedures used for the thigh. Shank tracking markers consisted of a rigid 4-marker cluster. Estimation of the ankle joint center was the mid-point between the malleoli. To develop the foot segment coordinate system a vector was created connecting the 2nd metatarsal marker to the superior heel marker. Next, a vector was created connecting the heel marker and the malleolar mid-point. Orthogonal axes were determined using the same vector multiplication procedures described earlier. Foot tracking markers included: rigid 3-marker clusters on the heel, instep, dorsal foot, 2nd metatarsal head and 5th metatarsal base.

Hip, knee and ankle joint angles were computed using a Cardan rotation sequence of flexion-extension, abduction-adduction, and internal-external rotation. Three-dimensional internal joint moments (normalized to body mass) were calculated from the synchronized kinematic and force data using a standard inverse dynamics approach. Head, trunk, arm, and
forearm segments were created based on reflective markers from the static trial. All segments were used to estimate whole body center of mass position (see Hanavan for inertial properties\textsuperscript{39} and Dempster for mass\textsuperscript{40}).

*Kinematic and Kinetic*

Vertical position of the center of mass was calculated in Visual3D and then exported to Microsoft Excel (Microsoft Inc., Redmond, WA). Since peak vertical GRF was of primary interest, vertical stiffness was calculated using unfiltered peak vertical GRF’s. From peak vertical GRF and center of mass vertical displacement data, during the time interval of impact to peak vertical GRF, vertical stiffness ($\Delta$vertical GRF/$\Delta$vertical displacement of center of mass) was calculated for each trial and then averaged for pre-WBV trials and post-WBV in Microsoft Excel (Microsoft Inc., Redmond, WA). Internal joint moments were calculated in Visual 3D using previously filtered kinematic and kinetic data. Joint (or torsional) stiffness during landing for the hip, knee and ankle joints in the sagittal plane was calculated and averaged in Microsoft Excel (Microsoft Inc., Redmond, WA). This was accomplished by dividing the absolute change in joint moment by the absolute change in joint angle from impact to peak angle (Fig. 3-6).

*Statistical Analysis*

SAS version 9.2 (SAS Institute Inc., Cary, NC) was used to analyze the data. To account for multiple comparisons, a pseudo-Bonferroni adjustment was performed prior to statistical analysis ($p < 0.01$). An ANOVA using group as the independent variable was performed to evaluate pre-WBV differences between the treatment groups for the following dependent variables: vertical stiffness, hip, knee and ankle joint stiffness. An ANCOVA, using pre-WBV
measures as covariates, was used to identify specific treatment effects (pre-test to post-test) for the same dependent variables within each group. Finally, a Pearson correlation was performed to detect any relationship between ankle joint stiffness during a dynamic task and the sagittal ankle arthrometry measure.

Results

Passive vs. Dynamic Stiffness Measures

No correlation ($r = 0.08$, $p = 0.50$) was observed between the two different ankle joint stiffness measures. It appears there is no relationship between ankle joint stiffness during a dynamic task and our passive measure of ankle joint stiffness (ankle arthrometry).

Vertical Stiffness

There was no significant difference in pre-WBV vertical stiffness measures between the two groups ($F_{1,68} = 1.400$, $p = 0.240$). No significant post-intervention change in vertical stiffness was observed for the CAI group ($t_{67} = 0.370$, $p = 0.720$). However, a significant increase in vertical stiffness post-vibration was observed for the healthy group ($t_{67} = 2.760$, $p = 0.008$).

Ankle Stiffness

No between-group pre-WBV differences existed for ankle stiffness ($F_{1,68} = 0.190$, $p = 0.660$). Ankle stiffness decreased post-WBV for the CAI group ($t_{67} = -3.130$, $p = 0.003$), but not for the normal group ($t_{67} = .0450$, $p = 0.960$).
Knee Stiffness

No between-group pre-WBV differences existed for knee stiffness (F_{1,68} = 0.050, p = 0.830). Knee stiffness decreased post-WBV for the CAI group (t_{67} = -3.490, p = 0.0008), but not for the normal group (t_{67} = -2.140, p = 0.040).

Hip Stiffness

No between-group pre-WBV difference existed for hip stiffness (F_{1,68} = 0.100, p = 0.750). Hip stiffness did not change post-WBV for the CAI group (t_{67} = 0.720, p = 0.480) or for the normal group (t_{67} = 1.840, p = 0.071).

Summary of Results

No relationship was observed between dynamic ankle joint and passive ankle joint stiffness measures. No between-group differences were observed for any of the pre-WBV variables. No treatment effect was observed for hip joint stiffness for either group (Tab. 6). However, the CAI group did exhibit less ankle (Tab. 4) and knee (Tab. 5) joint stiffness post-WBV compared to their pre-WBV measures. No other differences were noted at the ankle or knee for either group. The healthy group did demonstrate increased vertical stiffness post-WBV (Tab. 3), but this was not observed in the CAI group (Tab. 3).

Discussion

Between-Group Differences

We anticipated between-group differences in vertical stiffness, hip, knee and ankle joint stiffness between CAI and healthy subjects. However, our results reveal that there were no between-group differences for these variables. The current data do not support the idea that a lack of vertical, hip, knee or ankle joint stiffness contributes to CAI.
These findings contradict previous assertions. Mora et al proposed that CAI subjects generally exhibit a decrease in ankle stiffness. They suggested that this decrease in ankle stiffness could predispose the CAI population to chronic re-injury. However, Mora et al observed peroneal electromechanical delay as an indirect measure of ankle stiffness by comparing the delay during stance between a healthy and a CAI group. Since the current study used a direct measure of ankle stiffness during a dynamic task, it is not surprising that our results do not concur with those of Mora. In addition, Mora et al specifically investigated changes in muscle stiffness, while our data did not discriminate between the various components (bone, structural alignment, inert tissues and contractile tissues) that comprise joint stiffness. Others have concluded that CAI subjects demonstrate increased sagittal plane ankle stiffness as a possible compensation for lack of stability. Wikstrom et al used an ankle arthrometer to measure passive anterior-posterior ankle stiffness in CAI and healthy patients. Although they found CAI subjects exhibited increased passive ankle joint stiffness relative to the normal group, others have consistently reported decreased sagittal and frontal plane passive ankle stiffness in this population using an ankle arthrometer. However, no significant correlation (sagittal plane: \( r = 0.08, p = 0.50 \)) between dynamic joint stiffness and passive ankle stiffness (ankle arthrometry) was observed in the current study. Our data suggest that a passive measure of joint stiffness cannot be generalized to predict joint stiffness during a dynamic task.

Our findings, using a different methodology and measure of stiffness, do not agree with the cited literature. However, our normal group did exhibit a mean vertical stiffness (non-normalized) of 26.5 kN·m\(^{-1}\) compared to the CAI group’s 21.4 kN·m\(^{-1}\). Due to the inherent variability in single-leg landing strategies between genders, it is possible that larger sample sizes might reveal a significant difference in vertical stiffness between CAI and healthy subjects.
Otherwise, perhaps our CAI subjects were able to compensate for any passive deficiencies by altering neuromuscular control strategies during landing.

Several investigators have noted alterations in neuromuscular control strategies in CAI subjects during a variety of tasks: agility, gait, postural control, and even during drop and single-limb landings. Notably, Caulfield and associates observed increased dorsiflexion and knee flexion positions prior to and during early landing in CAI. Other drop landing data partially support these results. Fu et al observed increased tibialis anterior activity prior to landing during unanticipated 30 cm drop-landings. An increase in tibialis anterior activity, ankle dorsiflexion position and knee flexion position could be a possible CAI compensatory pattern used to increase stability during landing. Others have observed that CAI subjects rely more on proximal segments to maintain postural control during demanding tasks. The literature corroborates that CAI subjects consistently demonstrate changes in ankle and knee joint positions and an increased reliance on proximal segments to compensate for any lingering ankle deficiencies during dynamic tasks. These compensatory control strategies may help CAI subjects regulate stiffness during landings and could explain why no significant between-group differences were observed for vertical stiffness.

Our vertical stiffness measures are similar to published data. Chang et al reported average leg stiffness values ranged from 19.2 kN·m⁻¹ to 32.0 kN·m⁻¹ during single-limb hopping at different frequencies (2.0 to 2.4 Hz). Our average vertical stiffness measures ranged from 21.4 kN·m⁻¹ to 26.5 kN·m⁻¹, which are within Chang’s reported range. The present vertical stiffness results are also supported by unpublished data. Cameron compared lower-limb stiffness during a similar jump-landing task between a unilateral chronic ankle sprain group and a group with no history of ankle sprains. Using similar methods and measurement techniques as the current
study, Cameron found that there was not a significant difference in leg stiffness between the two groups. According to Cameron and our findings, it appears there is no significant difference in vertical stiffness between CAI and healthy subjects during a single-limb landing task.

In addition, our data suggest that CAI does not cause any significant alterations in hip, knee or ankle joint stiffness. Even though no significant group differences were observed for these measures, joint stiffness is a composite variable derived from two estimated measures (joint angle and joint moment) which introduces error into the calculation. Small errors in both or either measurement might have been compounded during the calculation of joint stiffness. This compounding error effect may help explain the lack of significant difference observed between groups. Further research may find a connection between altered joint stiffness and CAI. However, our hip, knee and ankle joint stiffness measures are supported by other data. Schultz et al reported 0.47 hip, .14 knee, and -.23 ankle stiffness (N·m·BW⁻¹·height(cm)⁻¹/degree) during single-limb drop landings in subjects with knee laxity. Our corresponding stiffness measures were: 0.47 hip, 0.36 knee and 0.27 ankle (N·m·BW⁻¹·height(cm)⁻¹/degree). The discrepancy in knee stiffness values may be due to differences in subject populations (knee laxity vs. CAI and healthy subjects) and tasks. According to our results, however, it appears that altered hip, knee and ankle joint stiffness do not contribute to CAI.

**WBV Treatment Effect**

The current results do support our hypothesis that WBV would alter stiffness in the healthy group. A significant increase in vertical stiffness post-WBV was observed for the healthy group. These findings are partly substantiated by previous data. Cronin et al used a damped oscillation technique to measure plantarflexor stiffness before and after WBV in a healthy group. Their vibration intervention was identical to ours with the exception of
amplitude. Cronin et al found no significant difference in plantarflexor stiffness post-WBV, but
asserted that there was an observable increase. They proposed that lack of adequate power to
show significance was due to a small sample size (n = 11 per group). While they reported a
non-significant 8.1% increase in passive plantarflexor stiffness post-WBV, we found a
significant 17% increase in vertical stiffness post-WBV for the healthy group. Increased vertical
stiffness during landing post-WBV may help explain acute increases in jump performance others
have observed.

Many opinions still exist about the possible effects of WBV on stiffness. Several
investigators have reported increases in strength resulting from WBV training. Some have even
observed acute increases in strength, power, and jump height after only one session.
Perhaps the most plausible explanation for this acute phenomenon is that WBV increases the
external forces acting on the body up to 15 g. These additional loads would increase
proprioceptive input from the treated joints and muscles. Increases in sensory feedback have
been shown to increase motor unit recruitment and may alter muscle stiffness. If WBV did
increase motor unit recruitment in the lower extremity muscles of the healthy group, an increase
in the stiffness of those musculotendinous units would be expected. This could explain the
increase in vertical stiffness that was observed in our healthy subjects.

Even if sensory feedback is responsible for an increase in motor unit recruitment and
therefore stiffness, we cannot conclude which proprioceptor(s) is/are responsible for the increase.
Even though the muscle spindle has been implicated as a probable source, recent studies cast
doubt on this theory. Conceivably, the most logical explanation is the tonic vibration reflex
(TVR). The TVR has been found to stimulate muscle contraction by altering the firing rate of
the Ia muscle spindle afferents. However, TVR is typically observed in relaxed muscle and
with direct vibration to the muscle’s tendon. Neither of these conditions was met in our study and therefore cannot explain our results. Furthermore, other investigators have reported that TVR actually attenuates the stretch reflex\textsuperscript{59,60} and therefore could potentially decrease musculotendinous stiffness. In addition, it appears that WBV does not alter peroneus longus electromechanical delay, reaction time, peak electromyography or average electromyography.\textsuperscript{31} Alterations in these variables would be expected if WBV did facilitate the gamma motoneuron system of the peroneus longus. Nor does WBV seem to potentiate the knee stretch reflex in healthy subjects.\textsuperscript{57} It is obvious that further research is necessary to confirm our results and to clarify the exact mechanism(s) that caused increased vertical stiffness in our healthy subjects post-WBV.

It is interesting to note that CAI subjects did not demonstrate increased vertical stiffness post-WBV. Martin et al suggested that “an increase in discharge rate and recruitment of additional motor units can be hindered by vibration”.\textsuperscript{58} They also suggested that since high threshold motor units are stimulated by vibration and tend to be more fatigable, it is reasonable that muscle fatigue could develop at a faster rate with vibration.\textsuperscript{58} Fatigue has been found to decrease the sensitivity of muscle spindles by increasing threshold\textsuperscript{61}. Fatigue has also been reported to decrease lower extremity musculotendinous stiffness.\textsuperscript{62} Perhaps the excitatory response of WBV was countered by some level of fatigue in the CAI group.

Alternating isometric contractions have been used to induce ankle fatigue.\textsuperscript{63} Maintaining a 40° squat during WBV requires significant isometric contraction and may have induced fatigue in our CAI group. Furthermore, although CAI subjects may compensate for passive deficiencies by altered landing strategies, these compensatory patterns may be less efficient. Utilizing inefficient control strategies to compensate for instability may predispose CAI to fatigue faster.
Unfortunately, no study could be found comparing fatigue rates between CAI and healthy subjects. In addition, even if our CAI subjects tended to fatigue faster than healthy subjects, we do not know whether peripheral and/or central fatigue occurred during our intervention. However, we believe fatigue is still a plausible explanation for the CAI group’s altered response to WBV.

On the other hand, some have reported that fatigue does not alter stiffness in this population. Kuenze et al measured ankle stiffness before and after an ankle fatiguing protocol. They concluded that fatigue did not affect ankle stiffness in CAI subjects. However, they measured ankle stiffness using a partial weight-bearing inversion-eversion cradle method. Although this device has been deemed valid and reliable, it is difficult to compare their static stiffness measure with our measure of stiffness during a dynamic task. Again, our data suggest that other measures of stiffness may not accurately predict joint stiffness during dynamic activities.

Padua et al, using a similar measure during a hopping task, found that fatigue did not alter vertical stiffness in healthy subjects either. However, they did observe alterations in joint movement strategies post-fatigue. Specifically, they reported that fatigued subjects used an ankle-dominant strategy, relying more on the ankle musculature to regulate stiffness. An ankle-dominant strategy is prevalent even in non-fatigued human hopping and appears to be magnified with fatigue. It is reasonable that a fatigued CAI group, utilizing an ankle-dominant strategy to regulate stiffness during a single-limb landing, may compensate by using altered neuromuscular control strategies. Some have reported that modified control strategies alter lower extremity stiffness. If the CAI group’s altered strategies were coupled with fatigue, then a discrepancy in the treatment effect between groups would be expected.
The lack of pre-post difference in vertical stiffness for the CAI group might also be explained by the significant decreases in knee and ankle joint stiffness post-WBV. If the CAI group relied on an altered strategy during landing post-WBV, this could explain the decrease in ankle and knee joint stiffness we observed. Others have reported altered neuromuscular control of the knee and ankle in the sagittal plane during closed-chain tasks in CAI subjects.\textsuperscript{50, 80} These investigators concluded that fatigue tended to amplify the alterations in CAI control strategies.\textsuperscript{80} As mentioned above, lower extremity stiffness can be altered when control strategies are modified.\textsuperscript{66} Perhaps the decrease in knee and ankle stiffness observed in the CAI group could be explained by altered control strategies that were exaggerated by WBV-induced fatigue. If WBV created acute neuromuscular fatigue and magnified altered control strategies, then changes in CAI knee and ankle joint stiffness would also be expected.

Even though the deafferentation theory has somewhat been refuted,\textsuperscript{17} another potential explanation for the CAI group’s altered response to WBV may be the inability to properly sensitize the neuromuscular system using sensory input. This inability might be explained by some type of concurrent nerve and/or sensory receptor injury during the ankle sprain(s). It has been documented that up to 27\% of patients with grade II and 86\% of patients with grade III sprains sustain injury to the peroneal and/or tibial nerves.\textsuperscript{81} These investigators proposed that the likely cause of nerve injury during an ankle sprain was nerve traction or a hematoma in the epineural sheath.\textsuperscript{81} They also stated that rehabilitation with these types of patients is “markedly prolonged”.\textsuperscript{81} Perhaps, this inability to properly sensitize the neuromuscular system is a contributing factor to re-occurring sprains and the chronic sensation of “giving way” that define CAI. Re-occurring sprains and/or episodes of “giving way” may further traction the injured neural tissue and perpetuate the problem. Varying degrees of neural involvement during the
initial and subsequent injuries may also help explain why some CAI patients demonstrate increased peroneus longus latency and electromechanical delay, increased strength, and altered proprioception while others do not.

Our CAI subjects may have demonstrated an altered response to WBV because of sensory integration deficits. Deficits in CAI sensory integration could include some type of sensory neglect. Neglect of sensory input may be a compensation to errant information coming from disrupted afferent neurons and/or receptors. Due to our definition of ankle sprain it is probable that some of our subjects sustained grade II (and possibly grade III) sprains. Unfortunately, the present data cannot confirm this. It is possible, however, that some of our CAI subjects sustained some type of nerve and/or sensory receptor injury during their ankle injuries. If nerve and/or sensory receptor injury led to sensory neglect in some of our subjects, it could have altered their ability to properly integrate sensory information. Due to altered sensory integration, the facilitatory effects of WBV might have been ignored or overridden by an inhibitory response from higher centers of control. This may explain why our CAI subjects regulated stiffness differently than the healthy subjects post-WBV. However, additional research is needed to identify if fatigue, altered neuromuscular control, sensory neglect, a combination of these variables, and/or other factors are responsible for the change in stiffness regulation that our CAI group demonstrated post-WBV.

**Conclusion**

There appears to be no difference in vertical stiffness, hip, knee or ankle joint stiffness between CAI and healthy subjects. No relationship was observed between dynamic joint and ankle arthrometry stiffness measures either. Caution should be used when generalizing passive
stiffness measures to predict ankle joint stiffness during a landing task. Whole body vibration created a 17% increase in vertical stiffness in the healthy group. However, further research is needed to reveal the exact mechanism for increased stiffness post-WBV in healthy subjects. This treatment effect was not observed in the CAI group. The CAI group did demonstrate a decrease in knee and ankle joint stiffness post-WBV. It is unclear why the CAI group responded this way. It appears that WBV may have altered the CAI group’s ability to regulate stiffness in a normal fashion. Further investigation is needed to understand why these two groups responded differently and if CAI subjects have difficulty regulating stiffness during other types of perturbation. It is clear, however, that WBV is not an effective way to increase lower extremity joint stiffness in CAI patients. In addition, the present data do not support the idea that a lack of vertical, hip, knee or ankle joint stiffness contributes to CAI.
References


15. McKeon PO, Hertel J. Spatiotemporal postural control deficits are present in those with chronic ankle instability. *BMC Musculoskelet Disord* 2008;9:76.


Table 1. Group means and standard deviations of Functional Ankle Ability Measure (FAAM) for activities of daily living (ADL) and sports sub-scores and for the Modified Ankle Instability Instrument (MAII).

<table>
<thead>
<tr>
<th>Group</th>
<th>FAAM-ADL</th>
<th>FAAM-Sports</th>
<th>MAII (#4-8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAI</td>
<td>84% ± 8.1</td>
<td>67% ± 14</td>
<td>3 &quot;yes&quot; ± 1.4</td>
</tr>
<tr>
<td>Healthy</td>
<td>100% ± 0.0</td>
<td>100% ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>
Table 2. Group means and standard deviations for ankle arthrometry and summary of special testing. No significant between-group differences when comparing involved ankles or within-subject differences for either group when comparing involved to non-involved ankles ($p \leq 0.01$).

<table>
<thead>
<tr>
<th>Measure</th>
<th>CAI Involved</th>
<th>CAI Non-Involved</th>
<th>Healthy Involved</th>
<th>Healthy Non-Involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior-Posterior Displacement (mm)</td>
<td>12 ± 2.9</td>
<td>13 ± 3.4</td>
<td>12 ± 3.5</td>
<td>13 ± 3.4</td>
</tr>
<tr>
<td>Anterior Drawer (±)</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Inversion-Eversion Rotation (°)</td>
<td>44 ± 13</td>
<td>44 ± 11</td>
<td>38 ± 9.8</td>
<td>39 ± 11</td>
</tr>
<tr>
<td>Talar Tilt (±)</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
</tbody>
</table>
Table 3. Means and standard deviations for vertical stiffness normalized to body weight (BW/m) for: pre-vibration, post-vibration and treatment effect (post – pre). *Significant treatment effect for healthy group ($p < 0.01$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-WBV $K_{\text{vertical}}$</th>
<th>Post-WBV $K_{\text{vertical}}$</th>
<th>Post-Pre $K_{\text{vertical}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAI</td>
<td>31.17 ± 21</td>
<td>32.04 ± 19</td>
<td>0.87 ± 10.9</td>
</tr>
<tr>
<td>Healthy</td>
<td>38.57 ± 30</td>
<td>45.10 ± 43</td>
<td>6.53* ± 27.0</td>
</tr>
</tbody>
</table>
Table 4. Group means and standard deviations of average ankle joint stiffness (N·m/degree) normalized by body mass for: pre-WBV (Pre), post-WBV (Post) and treatment effect (post-pre). *Significant treatment effect for CAI group ($p < 0.01$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-$K_{\text{ankle}}$</th>
<th>Post-$K_{\text{ankle}}$</th>
<th>$K_{\text{ankle}}$ Post-Pre</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAI</td>
<td>4.69 ± 2.44</td>
<td>3.92 ± 1.82</td>
<td>-0.77* ± 0.99</td>
</tr>
<tr>
<td>Healthy</td>
<td>4.62 ± 1.83</td>
<td>4.71 ± 2.11</td>
<td>0.09 ± 0.90</td>
</tr>
</tbody>
</table>
Table 5. Group means and standard deviations of average knee joint stiffness (N·m/degree) normalized by body mass for: pre-WBV (Pre), post-WBV (Post) and treatment effect (post-pre). *Significant treatment effect for CAI group ($p < 0.01$).

<table>
<thead>
<tr>
<th>Group</th>
<th>$\text{Pre-}K_{\text{knee}}$</th>
<th>$\text{Post-}K_{\text{knee}}$</th>
<th>$K_{\text{knee Post-Pre}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAI</td>
<td>$6.05 \pm 2.54$</td>
<td>$5.45 \pm 2.46$</td>
<td>$-0.60^* \pm 0.96$</td>
</tr>
<tr>
<td>Healthy</td>
<td>$6.18 \pm 2.45$</td>
<td>$5.81 \pm 2.44$</td>
<td>$-0.37 \pm 1.11$</td>
</tr>
</tbody>
</table>
Table 6. Group means and standard deviations of average hip joint stiffness (N·m/degree) normalized by body mass for: pre-WBV (Pre), post-WBV (Post) and treatment effect (post-pre). No significant treatment effect observed ($p \leq 0.01$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-$K_{\text{hip}}$</th>
<th>Post-$K_{\text{hip}}$</th>
<th>$K_{\text{hip}}$ Post-Pre</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAI</td>
<td>8.47 ± 5.20</td>
<td>8.90 ± 6.01</td>
<td>0.43 ± 3.39</td>
</tr>
<tr>
<td>Healthy</td>
<td>7.99 ± 7.20</td>
<td>9.10 ± 11.1</td>
<td>1.11 ± 4.58</td>
</tr>
</tbody>
</table>
Figure 1: Complete marker set for motion analysis. Top panel: Anterior view of marker set with subject holding arms out to side. Bottom panel: Posterior view of marker set with subject in same position.
Figure 2: Jump-landing task. Top panel: Bilateral jump. Middle panel: Single-limb landing. Bottom panel: Contralateral hop.
Figure 3: Plot of vertical stiffness (N/m) during one representative landing trial. Landing defined as impact to peak vertical ground reaction force. Vertical stiffness equals peak vertical ground reaction force divided by the change in vertical displacement of the center of mass.
Figure 4: Plot of average ankle joint stiffness ($K_{\text{ankle}}$) during landing (impact to peak angle). Slope of the line equals average ankle stiffness during landing (or change in joint moment divided by the change in joint angle). Plot is representative of one trial.
Figure 5: Plot of average knee joint stiffness ($K_{\text{knee}}$) during landing (impact to peak angle). Slope of the line equals average knee joint stiffness during landing (or change in joint moment divided by the change in joint angle). Plot is representative of one trial.

$$K_{\text{knee}} = \frac{\Delta \text{Moment}}{\Delta \text{Angle}}$$
Figure 6: Plot of average hip joint stiffness ($K_{\text{hip}}$) during landing (impact to peak angle). Slope of the line equals average hip joint stiffness during landing (or change in joint moment divided by the change in joint angle). Plot is representative of one trial.
Appendix A: Functional Ankle Ability Measure Questionnaire

Foot and Ankle Ability Measure (FAAM)

Activities of Daily Living subscale

Please answer every question with one response that most closely describes to your condition within the past week. If the activity in question is limited by something other than your foot or ankle mark not applicable (N/A).

No difficulty = 4, Slight difficulty = 3, Moderate difficulty = 2, Extreme difficulty = 1

Unable to do = 0, or N/A

Standing:

Walking on even ground:

Walking on even ground without shoes:

Walking up hills:

Walking down hills:

Going up stairs:

Going down stairs:

Walking on uneven ground:

Stepping up and down curbs:

Squatting:

Coming up on your toes:

Walking initially:

Walking 5 minutes or less:

Walking approximately 10 minutes:

Walking 15 minutes or greater:

Because of your foot and ankle how much difficulty do you have with:

Home Responsibilities:

Activities of daily living:
Personal care:
Light to moderate work (standing, walking):
Heavy work (push/pulling, climbing, carrying):
Recreational activities:
How would you rate your current level of function during your usual activities of daily living from 0 to 100 with 100 being your level of function prior to your foot or ankle problem and 0 being the inability to perform any of your usual daily activities?
________ %

Sports subscale
Because of your foot and ankle how much difficulty do you have with:
Running:
Jumping:
Landing:
Starting and stopping quickly:
Cutting/lateral movements:
Low impact activities:
Ability to perform activity with your normal technique:
Ability to participate in your desired sport as long as you would like:

How would you rate your current level of function during your sports related activities from 0 to 100 with 100 being your level of function prior to your foot or ankle problem and 0 being the inability to perform any of your usual daily activities?
________ %
Overall, how would you rate your current level of function?
Normal, Nearly normal, Abnormal, or Severely abnormal
Appendix B: Modified Ankle Instability Instrument Questionnaire

Instructions

This form will be used to categorize your ankle instability. A separate form should be used for the right and left ankles. Please fill out the form completely. If you have any questions, please ask the administrator of the survey. Thank you for your participation.

1. Have you ever sprained an ankle?
   a. Right ankle?
   b. Left ankle?
2. Have you ever seen a doctor for an ankle sprain?
3. Did you ever use a device (such as crutches) because you could not bear weight due to an ankle sprain?
4. Does your ankle ever feel unstable while walking on a flat surface?
5. Does your ankle ever feel unstable while walking on uneven ground?
6. Does your ankle ever feel unstable during recreational or sport activity?
7. Does your ankle ever feel unstable while going up stairs?
8. Does your ankle ever feel unstable while going down stairs?
9. Have you ever had rehabilitation on your ankle due to a sprain?
10. Have you ever had an injury to your knee?
    If yes, please explain:
        Side (right or left) | Injury | Date

        ___________________ | ___________________ | _________  
        ___________________ | ___________________ | _________  
11. Have you ever had an injury to your leg below the knee?
    If yes, please explain:
        Side (right or left) | Injury | Date

        ___________________ | ___________________ | _________  
        ___________________ | ___________________ | _________  

Number of previous ankle sprains:

   Left______ Right______

How long since your last ankle sprain?

   Left______ Right______
Appendix C: Physical Exam and Special Testing

Is there **swelling** present? + -

Is there **ecchymosis** present? + -

**Anterior Drawer Test**
- Right Ankle + -
- Left Ankle + -

**Talar Tilt Test**
- Right Ankle + -
- Left Ankle + -
Appendix D: Informed Consent Form

Consent to be a Research Subject

Introduction
This research study is being conducted by Mark Coglianese, doctoral candidate in the Department of Exercise Sciences, under the sponsorship of Professor Ty Hopkins at Brigham Young University. The purpose of this study is to observe the acute effects of whole body vibration on vertical and lower extremity joint stiffness of subjects with functional ankle instability (FAI) and healthy controls. You were invited to participate in this study because you have: (1) a history of ankle instability and have remained physical activity (or no history of ankle instability and are active for control group), (2) no known neurological disorders, (3) no ankle sprains with the past 3 months, (4) no other lower extremity injuries (not including ankle sprains) within the past 6 months, and (5) no previous lower extremity surgeries within the last two years.

Procedures
Your participation in this study will include one visit to the biomechanics lab (124 RB) that will last about 2 hours. During your visit, you will were identified as: a) ankle instability or b) healthy control via questionnaires (FAAM and MAII) and an ankle exam by a licensed Physical Therapist. During the rest of your visit: 1) the procedures will be explained to you 2) you will read and then sign this form after all questions have been answered 3) you will be provided a pair of spandex shorts, a spandex shirt and a standard cross-training shoe to wear during the study 4) you will walk on a treadmill for 5 minutes at a standardized pace (by leg length), 5) you will practice the jump landing task, 4) an investigator will abrade your skin with sandpaper and clean the site with alcohol prior to placing eight skin electrodes over muscles of your involved leg with double-sided tape, and 4) several reflective markers will also be placed on your body with double-sided tape to allow for motion analysis (images will not reveal personal identity). Once all electrodes and markers are in place, you will be asked to stand in a semi-squat position (with knees flexed to 40 degrees) in order to record the EMG activity from all 8 muscles while you are in this position. You will also be asked to perform a forward jump onto an embedded force plate, landing on the involved leg, followed by a contralateral hop (hop to the opposite side of the landing leg).

Data collection: The task will consist of jumping onto a force plate with your involved foot and then performing a subsequent lateral hop (hop to the other side onto your other foot). Five successful trials will be recorded. You will then be asked to stand on a vibration platform in a semi-squat position 5 times. You will be allowed to gently rest your finger-tips on a handrail for balance (but not for support). Your feet and legs will experience an intense vibration which some describe as a “tingling” sensation. Each repetition will last 60 seconds with a one-minute rest interval between repetitions. After the vibration repetitions are completed, 6 more jump landings will be recorded (immediately after vibration and at one-minute intervals up to 5 minutes post-vibration). During data collection ground reaction forces will be recorded by the force plates, 10 infra-red cameras will be used to record motion from the reflective markers (images will not reveal personal identity), and muscle activity will be recorded by the eight wireless, surface electrodes.
**Risks/Discomforts**
You will be subjected to few risks. You’ll be asked to perform a jump landing task several times. Since you have a history of ankle instability you could be at risk of rolling your ankle, however the possibility of this is very low because the tasks we are asking you to perform are no more demanding than your normal daily activities. The acute effects of vibration create no known risks in this population. Certified health care professionals (athletic trainer and/or physical therapist) will be onsite for all sessions to assess any potential problems. Also, skin irritation may be caused by double-sided tape used for reflective markers and electrodes. All sites will be properly cleaned at the end of your participation to minimize this risk. All investigators will adhere to OSHA guidelines when appropriate.

**Benefits**
There are no direct benefits from participation in this study; however, the results from this study may benefit society by improving rehabilitation of functional ankle instability. No information will be available at this time to improve ankle instability.

**Confidentiality**
All information provided will remain confidential and will only be reported as group data with no identifying information. All data will be kept in a locked storage cabinet and only those directly involved with the research will have access to them. After the research is completed, all original data will be destroyed.

**Compensation**
You may receive extra credit points for your participation in this study. The availability of extra credit points and the number of extra credit points you receive is up to your instructor. If you choose not to participate in this study, an equal number of extra credit points can be earned by completing an assignment of equal time commitment. You will receive monetary compensation at the end of the study for your participation in the form of $25 cash. No partial compensation will be given if the study is not completed. No compensation will be given if you are excluded from the study for any reason.

**Participation**
Participation in this research study is voluntary. You have the right to withdraw at anytime or refuse to participate entirely without jeopardy to your class status, grade or standing with the university. Your participation in this study can be terminated by the investigator if you are unable to comply with the research procedures.

**Questions about the Research**
If you have questions regarding this study, you may contact Mark Coglianese, MPT (801)422-9156, markcoglianese@byu.edu or Dr. Ty Hopkins at (801)422-1573, ty_hopkins@byu.edu.
Questions about your Rights as Research Participants
If you have questions you do not feel comfortable asking the researcher, you may contact the IRB Administrator, Brigham Young University, A-285 ASB Campus Drive; Provo, UT 84602; (801) 422-1461; irb@byu.edu.

___________________________________     ____________
Signature         Date