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14. ABSTRACT
The overall objective of proposed study is to develop an adaptive socket system that detects in-socket residual limb motion and dynamically adjusts internal socket negative pressure to optimize fit and performance. Specific Aim 1: Test relationship of pressure variance waveforms with residual limb movement in-socket. Hypothesis 1: Pressure data will detect distal displacement and lateral shift of the residual limb inside the socket. Specific Aim 2: Characterize the effects of residual limb movement in-socket on residual limb health. Hypothesis 2: Increased socket motion decreases residual limb perfusion and disrupts skin barrier function. Specific Aim 3: Compare the long-term effects of adaptive Elevated Vacuum Suspension (EVS) to pin-locking and suction suspension systems. Hypothesis 3.1: Adaptive EVS improves amputee performance and residual limb health as compared to pin-locking/lanyard, suction, and static EVS. Hypothesis 3.2: Adaptive EVS improves functional performance, pistoning control, and user comfort as compared to pin-locking/lanyard, and static EVS.

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TABLE OF CONTENTS

	Page
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	17
5. Changes/Problems	18
6. Products	19
7. Participants & Other Collaborating Organizations	21
8. Special Reporting Requirements	22

1. **INTRODUCTION:**

The **overall objective** of proposed study is to develop an adaptive socket system that detects in-socket residual limb motion and adjusts internal socket negative pressure to optimize fit. Novel technologies to be tested include a "smart" vacuum suspension system capable of monitoring socket movement relative to the residual limb. Probe-based and imaging modalities to assess residual limb circulation and skin health will be employed to assess the functional significance of the adaptive socket system in comparison to existing standard of care suspension systems.

2. **KEYWORDS:**

prosthesis, transtibial, transfemoral, residual limb, vacuum, suspension

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Major tasks/goals

1. **Task1. IRB/HRPO approval –** completion -100%

2. **Task-2: perform Aim 1:** Test relationship of pressure variance waveforms with residual limb movement in socket. completion -100%

3. **Task-3 Perform Aim 2:** Characterize the effects of residual limb movement in-socket on residual limb health. Completion 100%, interim analysis completed data provided to WillowWood for vacuum level setting for the main study.

4. **Task-4 Perform Aim 3:** Compare the long-term effects of adaptive Elevated Vacuum Suspension (EVS) to pin-locking and suction suspension systems. Completion 100%. The final data presented in this technical report, publication/s have been planned and will be submitted in next 12 months.

Note: *The PI moved to a new Institution in Aug 2018 – reported in Year 2 report. The Award transfer occurred for Indiana University on 04/15/2019. The award end date was 09/29/2019. Because of delays on the transfer process and global pandemic NCE were obtained. The new award end date was 03/29/2021.*

What was accomplished under these goals?

1. **Major Activities.**

Aim 1 - Aim 1 visits were completed during year one. The details of the progress have been reported in Year 1 & 2 reports. The studies resulted in a multiple conference presentations. Data was collected on 20 subjects (10 transtibial and 10 transfemoral prosthesis users). The experiment proved the hypothesis that pressure waveform magnitude is directly proportional to movement of the limb within the socket. This was previously shown in a benchtop experiment, but this was the first evidence presented from human subject data. Further, the experiment showed that the quality of socket fit can be detected through movement data.

Aim 2 - Aim 2 studies began in year two and we completed (n=15) prior to moving to IU from OSU. An interim analysis of the data was performed to determine if n=15 subjects provided sufficient data to complete Aim 2. Major objective was to develop a standardized approach to quantitatively measure residual limb skin health using noninvasive imaging (hyperspectral imaging and laser speckle flowmetry) and probe-based approaches (laser doppler flowmetry, transcutaneous oxygen, transepidermal water loss, surface electrical capacitance), these data resulted in a publication. Using measurements from Aim 1 subjects a standardized approach to quantitatively measure residual limb health in individuals with lower limb loss was developed and reported. The study reported that compared to intact limb controls, resting residual limb physiology in people that have had transfemoral or transtibial amputation is characterized by lower transcutaneous oxygen tension and poorer skin barrier function.

The data from this study was presented at the MHSRS 2019 as invited oral presentation. The presentation concluded that socket movement is a critical factor that should be controlled in order to preserve limb health while using a prosthesis. By maximizing vacuum pressure setting and expect to achieve the best limb environment.

Aim 3. For this aim, work initiated at all three sites i.e., Indiana University (IU), Walter Reed (WRNMMC) and WillowWood (WW). There were two sub aims:

Sub Aim 3a: **Compare the effect of prosthetic suspension on residual limb health as compared to standard of care**.

- An overall improvement of limb health following 16 weeks of use of elevated vacuum suspension (EVS) was noted. The improvement was manifested as better skin barrier function (low TEWL) and improved skin hydration.
- At one region of interest affected by high superficial pressure exerted by EVS, superficial perfusion was compromised as measured after 10-15 mins doffing of the EVS. These ischemic responses of the superficial vasculature but not that of the deeper vessels can be explained by possible deformities of the superficial microvasculature in response to the mechanical pressure caused by EVS. As reported, such limitations in focal superficial blood flow is expected to be followed by reactive hyperemia (PMID: 21178967).
- Skin elastography as measured by HUSD displayed no significant changes indicating that the biomechanical property of skin (*eg.* stiffness) was not adversely affected by the use of EVS for 16 weeks.

Sub Aim 3b: **Compare the effect of prosthetic suspension on functional performance and patient reported outcomes as compared to standard of care**.

- Self-reported outcomes were generally better with the EVS vs. SoC socket; in particular, greater socket comfort scores exceed the standard error of measurement and approach the minimal detectable change. Socket comfort scores were 2.1± 3.1 points higher (p=0.048) in the EVS vs. SoC socket, improving for all but one participant (though reporting 10/10 in SoC and 9/10 in EVS).
- The study of biomechanical parameters indicated clear benefit of EVS *vs* SoC socket. Although, the mobility/functional outcomes (i.e., CHAMP) remain comparable between the two groups, the biomechanical measurement such as self-selection of a faster walking speed (strong indicator for enhanced community ambulation and functional independence, particularly among older individuals) and steps with the intact limb (suggesting an elevated level of confidence in the prosthetic limb to facilitate taking longer steps with the limb contralateral to amputation) clearly established the benefit of EVS.

STUDY DESIGN AND ELIGIBILITY CRITERIA

Subaim 3a: A total of n=59 subjects were enrolled at IU (n=29) and WW (n=30) sites. The studies initiated mid-2019, however, due to global pandemic research activities were greatly affected at both sites. The studies were on hold and got reinitiated fall of 2020 at IU and WW sites. All human studies were approved by IU Institutional Review Board (IU site) or Advarra (WW) and HRPO. Declaration of Helsinki protocols was followed, and patients gave their written informed consent.

The eligibility criteria for the study is listed below:
Inclusion Criteria: 1) Ages of 18 and above; 2) unilateral trans-tibial or trans-femoral amputee; 3) ambulate at a K2 level or higher; 4) at least 3 months post-amputation per physician discretion; 4) residual limb length greater than 6.5 inches in length; 5) able to follow directions and give informed consent on their own or through Legally Authorized Representative; 6) must be able to ambulate without assistance. An external assistance device such as cane or walker will be permitted; 7) adequate arterial blood flow of the index stump as evidenced by TcOM > 30 mmHG, measured within the past 12 months.

Exclusion Criteria: 1) conditions that prevent wearing a prosthetic socket; such as existing scab, ulcer, or keloid scar on amputation stump; 2) cognitive deficits or mental health problems that would limit ability to consent and participate fully in the study protocol; 3) women who are pregnant or who plan to become pregnant in the near future.
Following consent, the arterial blood flow was measured on the index stump if not already completed per standard of care within the previous 12 months prior to enrollment. Those with an TcOM < 30 mm Hg were excluded and no further study activities were completed. These subjects were recorded as a screen fail and were not enrolled. Once enrolled, the subjects went through the following study visits and activities:

a) **Baseline visit**. During this visit, subject demographics and medical history was collected. Subjects were asked to walk on a treadmill for 10 mins. The residual and sound (intact) limb health measurements were recorded before and after the activity period with the exception of conducting post-activity ultrasound and digital imaging. The purpose of acquiring ultrasound and digital imaging is to determine changes in skin structure on a long-term basis; thus, these measurements were only collected during pre-activity procedures to observe changes in a resting state. The following limb health measurements were collected: Surface Electrical Capacitance (SEC); TEWL measurement; Hyperspectral Imaging; Ultrasound (pre-activity only); Digital Image (pre-activity only).

b) **Socket fitting visits**: The visit included the following activities: 1) limb shape capture, measurements or tracing; 2) diagnostic static fitting and diagnostic dynamic fitting; 3) delivery of definitive "research" socket. The subject was allowed to return for no greater than 4 adjustments (within 0-2 weeks from baseline) to obtain a comfortable fit in order to complete this study. If subject required more than 4 adjustment visits, the participation from the study ended.
Once the subject was comfortable with the fitting, the date was noted by the study team as "*research socket use start date".* From this date, the subject began wearing the test socket for next 16 weeks.

c) Final study visit (16 weeks post *research socket use start date*). The activities described in baseline visit were repeated and data was recorded.

Subject demographics and medical history

Characteristics	Total		IU		WW	
	N	%	N	%	N	%
Total	59	100	29	49.15	30	50.85
Gender						
Male	51	86.44	23	79.31	28	93.33
Female	8	13.56	6	20.69	2	6.67
Age (median, IQR)	57	(39-63)	56	(39-61)	57.5	(41-64)
BMI	29.42	(26.52-35.28)	31.22	(26.49-36.01)	27.82	(26.93-34.51)
Race						
Black or African American	10	16.95	9	31.03	1	3.33
Whites	47	79.66	19	65.52	28	93.33
Other	2	3.39	1	3.45	1	3.33
Ethnicity						
Hispanic	1	1.69	0	0	1	3.33
Non-Hispanic	58	98.31	29	100	29	96.67
Insurance						
Medicaid/HIP	6	20.69	6	20.69	NA	
Medicare/Medicare Advantage	18	62.07	18	62.07	NA	
Other	2	6.9	2	6.9	NA	
Self-pay/No Insurance	3	10.34	3	10.34	NA	
Smoking						
Never	11	39.29	11	39.29	NA	
Former	9	32.14	9	32.14	NA	
Current	8	28.57	8	28.57	NA	
Medical History						
DM	19	32.2	16	55.17	3	10
Insulin Dependent	10	17.24	9	56.25	1	32.14
Heart Disease	10	16.95	8	27.59	2	6.67
High blood Pressure	16	55.17	16	55.17	NA	
Stroke/TIA	1	1.69	1	3.45	0	0
Previous revascularization	4	6.78	4	13.79	0	0
PVD	2	3.39	1	3.45	1	3.33
Bleeding tendencies	5	8.47	3	10.34	2	6.67
Clotting disorders	4	13.79	4	13.79	NA	
Asthma	3	5.08	3	10.34	0	0
Other chronic lung disease	3	5.08	3	10.34	0	0
Drug/Alcohol Abuse	6	20.69	6	20.69	NA	
Depression	4	13.79	4	13.79	NA	
Other mental illness	4	13.79	4	13.79	NA	
Migraine/Headaches	4	6.78	4	13.79	0	0
Obesity	11	18.64	8	27.59	3	10
Thyroid disease	3	5.17	3	10.71	0	0

High Cholesterol	11	37.93	11	37.93	NA	
Kidney disease	7	11.86	7	24.14	0	0
Dialysis	4	57.14	4	57.14	0	0
Other Medical History	7	24.14	7	24.14	NA	
Initial TCOM (median, IQR)	55.24	(41.7, 67.24)	55.24	(41.7, 67.24)	NA	

NA=Not available

METHODS

Transepidermal Water Loss (TEWL): TEWL is characterized by the constitutive evaporation of water that initiates from the deeper, more hydrated layers of the epidermis and dermis, which moves towards the more superficial stratum corneum (SC) layer, in the absence of sweat gland activity. Depending on climate conditions, a portion of this water evaporates through the SC. If the functional integrity of skin is compromised, more water can escape, and subsequently TEWL increases. TEWL was measured with DermaLab® (Cortex Technology ApS, Hadsund, Denmark) which is based on open chamber mechanism that follows the basic theory of Fick's law of diffusion. The set-up consists of a chamber made up of two pairs of temperature and humidity sensors, placed inside a hollow cylinder. When using these, it is essential to maintain a controlled environment as many factors such as probe size, airflows from atmosphere and the body, room temperature, air convection, and ambient humidity can easily alter results. In most cases it is desirable that TEWL value is measured under steady state conditions. It takes about 10 minutes for the sensor to warm up and equilibrate with the ambient temperature and humidity Consequently, after the probe is placed on the skin, the water loss rate (TEWL value) progressively increases over time and then plateaus when equilibrium is restored. The measurement at plateau is reported by the equipment as the TEWL value and is recorded by the study coordinator. It is only under such steady conditions that meaningful measurement of the functional integrity of the skin can be measured.

Skin hydration or skin Electrical Conductance (SEC). Hydration of the skin surface is directly proportional to electrical conductance. SEC was measured with DermaLab® (Cortex Technology ApS, Hadsund, Denmark) with a hydration probe.

Hyperspectral Imaging or Spatial Frequency Domain Imaging

Biological tissues are composed of a variety of chromophores--molecules that absorb light of characteristic wavelengths. This is useful in many clinical applications because some of the more dominant chromophores in tissue are markers of important clinical parameters, such as oxygenation. However, it is difficult to quantify the absorption of tissue because it is turbid. The attenuation of light in tissue is a function of both its absorption and scattering properties. The hyperspectral Imaging was performed using Clarifi™ Imaging SFDI system (Modulim, Irvine CA).

Ultrasound. B-mode and Elastography mode image acquisition and processing was performed using Hitachi-Aloka ultrasound system (Hitachi Aloka Medical Corporation, Chiyoda-ku, Japan) to non-invasively determine anatomical changes in skin and underlying structures.

Statistical Analysis

Patient demographics and clinical characteristics at baseline are described using frequencies and percentages for categorical data and median and the interquartile range (IQR) for continuous data. We used Shapiro-Wilk test to determine the skewness of the continuous variables and the appropriateness of the use of non-parametric measures of describing continuous variables. We used median (Inter-quartile range) to describe the continuous measures of limb health such as TEWL, Hydration, and Oxygenation levels at each visit and each location of the measurements in the sound (SL) and residual limbs (RL). To normalize the measures, we created the fold change (FC) in residual limbs compared to its contralateral part in sound limb at post-activity (FC to SL for post-activity) and the fold change in residual limb at post-activity compared to the value in the same limb at pre-activity (FC to Pre-activity for RL). These computed measures were then compared between visit 2 and visit 3 at each location of the limb. In a bivariate analysis, we used two-sided equality of the median test for the matched pairs of observation. For the multivariable analysis, controlling for the effect of age, sex, race, BMI, and diabetes status, we used random effect models by using generalized least square estimations assuming an exchangeable correlation structure and cluster variance, clustered at patient and centers. Floating bars with median and interquartile ranges for bivariate comparison between visit 2 and visit 3 for each measurement location and coefficient plot (95% CI) from the multivariable analyses were created for visual presentation of the results. All analyses and the visualizations were done using Stata MP 14.1, Stata Corporation, College Station, Texas.

2. Specific Objectives.

IRB/HRPO approval – gained approval to conduct Aim 1 and Aim 2 studies. HRPO approval obtained to continue studies at IU once the IRB protocol was approved.

Aim 1 – Test the relationship of pressure variance waveforms with residual limb movement in socket.

Aim 2 – Characterize the effects of residual limb movement in socket on residual limb health.

Aim 3 - Compare the long-term effects of adaptive Elevated Vacuum Suspension (EVS) to the standard of care (SoC) suspension (pin, suction or standard vacuum) systems.

3. Significant Results.

Aim 1 - Pistoning and horizontal movement was dependent on socket fit. Horizontal motion generally attributed 20-50% of the total motion depending on socket fit condition. Vacuum pressure data had a positive correlation to the inductive sensor data, indicating it can detect global and local changes in fit.

The loose socket generally resulted in the most horizontal movement and the tight socket resulted in the most distal movement. Interestingly, horizontal movement still occurred in the tight socket. Transfemoral subjects tended to have more horizontal motion than transtibial subjects, likely due to anatomy of the residual limb. Vacuum pressure profiles correlated positively with the data.

Aim 2 – One of the major objectives, was to develop a standardized approach to quantitatively measure residual limb skin health using noninvasive imaging (hyperspectral imaging and laser speckle flowmetry) and probe-based approaches (laser doppler flowmetry, transcutaneous oxygen, transepidermal water loss, surface electrical capacitance). Using measurements from Aim 1 subjects a standardized approach to quantitatively measure residual limb health in individuals with lower limb loss was developed and reported. The study reported that compared to intact limb controls, resting residual limb physiology in people that have had transfemoral or transtibial amputation is characterized by lower transcutaneous oxygen tension and poorer skin barrier function.

We performed analysis for Aim 2 on n=15 with unilateral limb loss (8 transfemoral; 7 transtibial), who wore an elevated vacuum suspension prosthesis. These subjects were recruited as part of the randomized crossover study proposed in Aim 2. Subjects completed 4 data collection visits; one baseline visit and three experimental visits. Each visit was scheduled one month apart. Post-activity TEWL and TCOM correlated with socket movement (near significant p=0.07 and significant p=0.03 respectively) so that when socket movement decreased, health outcome improved. These outcomes did not correlate with the average atmospheric pressure within the socket (p=0.3 for both).

When socket fit was accounted for by normalizing the average atmospheric pressure by socket movement, TEWL and TCOM indicate a stronger correlation with movement (p=0.02 and p=0.01, respectively). Pre-activity SEC and TEWL, and post-activity SEC, did not correlate (all p>0.3) with either socket movement or average atmospheric pressure.

AIM-3a. Limb Health analysis.

Transepidermal water loss (TEWL). High TEWL is a measure of poor skin barrier function. TEWL of the residual limb was compared between baseline (**visit 2**) and post 16 weeks of Elevated Vacuum Suspension (EVS) use as compared to the standard of care (SoC) suspension (pin, suction or standard vacuum) use (**visit 3**). The long-term use of EVS does not adversely affect skin barrier function at first glance. However, when the TEWL of the residual limb was normalized with the sound limb and compared between baseline and post 16 weeks of EVS use, a significant improvement of the skin barrier function was observed at the superior and posterior side exposed to EVS when compared to baseline. These data validated our pilot study (n=10) where after 16 wks of use, elevated vacuum suspension (EVS) significantly improved skin barrier function as compared to SoC sockets when all areas were considered together. Specifically, the areas of high stress (superior) on residual limb displayed the lowered TEWL values (**Figure 1**).

Skin hydration or skin electrical conductance (SEC). The skin hydration is widely regarded as an important indicator of skin health. The amount of glycosaminoglycans (GAGs) in the epidermis and dermis decline with age, leading to a decrease in the capacity to retain moisture within the skin and an increase in skin dryness (PMID: 33521019, 27378089). The electrical conductance of the skin, which measures hydration level of the stratum corneum was measured using a hygrometer. The use of EVS for 16 weeks significantly improved skin hydration of the residual limb compared to the baseline. Such improvement of skin property remained significant even after normalization of the data with that of the sound limb (**Figure 2**). This improvement in skin hydration is well aligned and as expected inversely related to the TEWL values.

Hyperspectral imaging (SFDI). Spatial frequency domain imaging (SFDI) is a technique to separate the effects of scattering and absorption, and consequently, to approximately quantify a set of chromophores. The technique works by shining different patterns of light on the tissue, recording a video of the remitted light, and processing the movie acquired (**Figure 3**). Because of the differences in absorption between oxyhemoglobin (HbO_2, **Table 2**) and deoxyhemoglobin (HbR, **Table 3**), it is an excellent measure of tissue oxygenation. These values can then be used to calculate the arterial oxygenation (StO_2, **Table 1**). Other measurements performed using this system include total hemoglobin levels in **superficial (0-1mm HbT1**, **Table 5**) or *papillary dermis microvasculature* and **subsurface (1.5-3 mm)** (HbT2, **Table 4**), *reticular dermis macrovasculature*. Data indicated no significant changes in StO2 or HbR was noted either between visit 2 and visit 3 or when the same data was analyzed following normalization with the sound limb. Compared to HbT2, HbT1 values showed a marginal but significant decrease post 16 weeks of EVS as compared to the baseline values at visit 2. When normalized with the sound limb, these changes were noted in the areas of high stress. Pressure induced structural deformities may account for compromised superficial perfusion. Because the deep intact perfusion HbT2 does not indicate any change, it is likely that the vacuum is causing the compression of the superficial capillaries thereby compromising superficial blood flow. The data suggest a slight reduction in perfusion within *papillary dermis in the superior site* of the residual limb following 16 weeks of EVS use as compared to their baseline.

Ultrasound imaging of the skin. In the ultrasound image of the healthy skin, basic layers that may be distinguished are skin and subcutaneous tissue, and bone may be used as a reference anatomical position (**Figure 4**). The higher the resolution of the structures needed, the lower the depth of ultrasound beam penetration into the skin. Modern ultrasound systems have numerous and diverse applications including vascular imaging, visualizing 3D structures in motion and measuring the stiffness of tissues (elastography mode). The ultrasound transducer generates pulses that pass-through tissue and reflect back producing echoes. The echoes of reflected and scattered ultrasound waves from tissue boundaries and within tissues respectively result in a B-mode image (Figure 4). The amplitude of the echo relates to brightness of the image. Ultrasound in elastography mode is a dynamic technique that uses ultrasound to non-invasively assess the mechanical stiffness of tissue by measuring tissue distortion in response to external stretch. The elastogram is represented as a color map with a range of colors from red (soft/high strain) to green (intermediate/equal strain) to blue (hard/no strain). This data can also be semi-quantitated using a visual scoring system based on the colors or using strain-ratio measurements usually provided in the elastography software. No major changes were noted in skin stiffness after 16 weeks of EVS use as compared to the baseline, suggesting the use of the device does not result in any significant anatomical changes in residual limb skin (**Figure 5**).

AIM-3b. Patient reported outcomes and Functional analysis

Sub aim 3b studies were planned to be performed and completed at WRNMMC, however, due to COVID-19 pandemic, a low volume of patients (overall, and especially within the O&P clinic) made it difficult to enroll subjects at that site as recruitment activities were effectively on hold across their entire portfolio of projects. The WRNMMC team approached the IU team and it was agreed that IU would perform functional studies on n=10 subjects for Subaim 3b in the Neuroscience Gait lab. The subjects were enrolled and data was sent to WRNMMC for analysis. The report is provided herein:

Ten participants with unilateral lower limb loss (mean[standard deviation] age = 53.3 [11.2] yr, stature = 180.4[6.1] cm, body mass = 94.9[17.1] kg) completed a pre/post (A-B) study design in which all outcomes were replicated twice, first in their standard of care (SoC) socket (i.e., the prosthesis worn at time of study enrollment) and second in the Elevated vacuum suspension (EVS) socket (i.e., the study prosthesis); outcomes collections were separated by ~16 weeks to allow sufficient acclimation to the EVS socket (mean time between visits = 104 days). At both visits, participants completed three categories of outcomes**:** **(1)** demographic, medical, and subjective questionnaires (Socket Comfort Score, Veterans RAND 36-Item Health Survey [VR36], and National Institute of Health [NIH] Patient-Reported Outcomes Measurement Information System [PROMIS]), **(2)** a functional/mobility assessment (Comprehensive High-Activity Mobility Predictor; CHAMP), and **(3)** a biomechanical gait evaluation (at a self-selected walking speed). Two participants were lost to follow-up; thus, the summary below reflects the eight participants (n=8) who completed both study visits, replicating outcomes in the standard of care (SoC) and test (EVS) sockets. All values are reported as means (standard deviations).

Socket comfort scores were 2.1± 3.1 points higher (p=0.048) in the EVS vs. SoC socket, improving for all but one participant (though reporting 10/10 in SoC and 9/10 in AV). **VR36** scores improved with the EVS socket, in the *Role Limitations due to Physical Health* (p=0.049), *Social Functioning* (p=0.048), and *General Health* (p=0.018) domains, but were similar (p>0.31) between sockets for all other domains (i.e., *Physical Functioning, Energy/Fatigue, Emotional Well-being,* and *Pain*). **PROMIS** scores were similar (p=0.77) between sockets.

Aggregate **CHAMP scores** were similar (p=0.46) between the EVS (17.1±7.8]) and SoC (17.9 ±7.6) sockets. Participants walked faster (p=0.039) with the EVS vs. SoC socket (1.18±0.14 m/s vs. 1.11±0.12 m/s, respectively), with similar cadence (p=0.13) and step width (p=0.21). With the EVS vs. SoC socket, participants took longer (p=0.037) steps with the non-amputated limb.

Self-reported outcomes were generally better with the EVS vs. SoC socket; in particular, greater socket comfort scores exceed the standard error of measurement (SEM=1.2) and approach the minimal detectable change (MDC=2.7). From the VR36, reducing limitations due to physical health while improving overall social functioning and general health further support possible benefits of the EVS vs. SoC sockets. Despite similarities in mobility/functional outcomes (i.e., CHAMP), a few key biomechanical parameters further identify potential functional benefits of the EVS vs. SoC sockets. Specifically, self-selecting a faster walking speed (strong indicator for enhanced community ambulation and functional independence, particularly among older individuals), as well as taking longer steps with the intact limb (suggesting an elevated level of confidence in the prosthetic limb to facilitate taking longer steps with the limb contralateral to amputation).

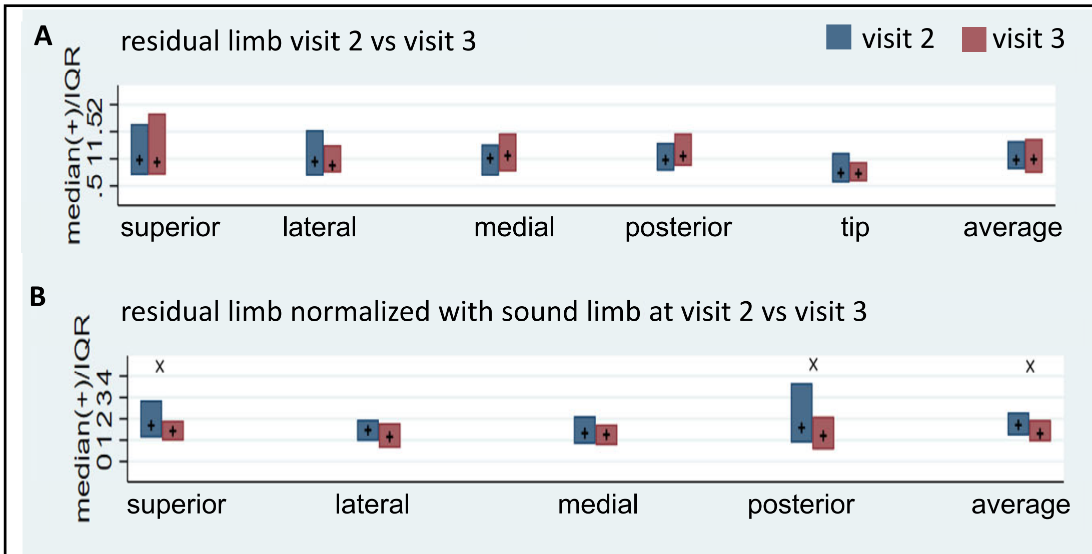

Figure 1: Transepidermal water loss (TEWL) was measured as a function of skin barrier function. (A) The TEWL of the residual limb was compared between visit 2 and visit 3. No significant change in barrier function was observed. (B) The TEWL of the residual limb was normalized with the sound limb and compared between visit 2 and visit 3. Significant decrease in TEWL was observed at the superior and posterior side compared to baseline. (n=59) The bar indicates the inter-quartile range (25th percentile to 75th percentile of the measurement) by each visit and site. The "+" inside the bar indicates the median value.x, $p < 0.05$; xx, $p < 0.01$; xxx, $p < 0.001$.

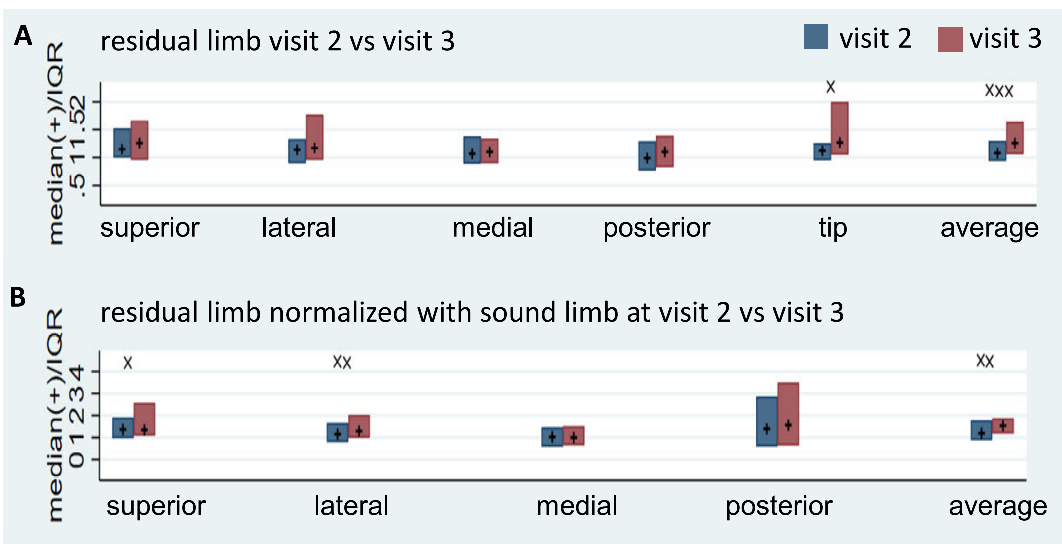

Figure 2: Skin hydration or skin electrical conductance (SEC). (A) The SEC of the residual limb was compared between visit 2 and visit 3. (B) The SEC of the residual limb was normalized with the sound limb and compared between visit 2 and visit 3. (n=59) The bar indicates the inter-quartile range (25th percentile to 75th percentile of the measurement) by each visit and site. The "+" inside the bar indicates the median value. x, $p < 0.05$; xx, $p < 0.01$; xxx, $p < 0.001$.

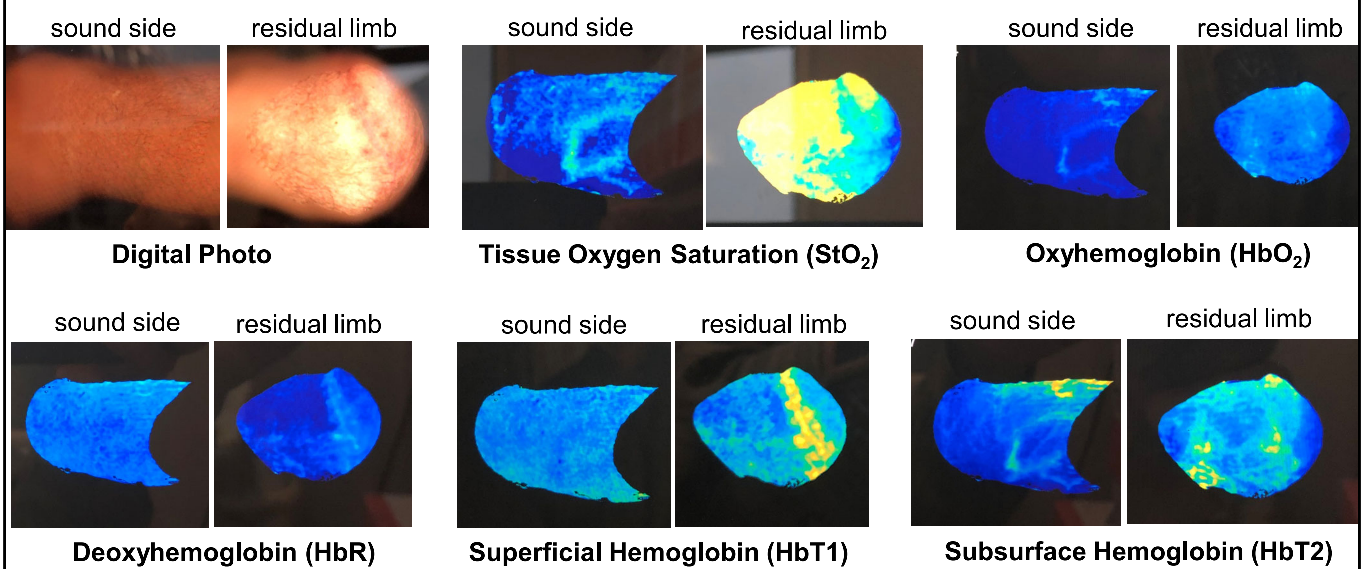

Figure 3: Hyperspectra imaging or SFDI. Representative images of residual limb and sound limb showing tissue oxygenation status and perfusion taken non-invasively using spatial frequency domain imaging (SFDI).

residual limb was compared between visit 2 and visit 3

	Site 1=Superior		Site 2=Lateral		Site 3=Medial		Site 4=Posterior		Average (Limb-All Sites)	
Visit 2 (median, IQR)	0.96	(0.32, 1.77)	1.11	(0.7, 2.33)	1.15	(0.59, 2.77)	1.06	(0.72, 2)	1.15	(0.76, 1.91)
Visit 3 (median, IQR)	0.94	(0.27, 1.18)	0.96	(0.5, 1.25)	1	(0.17, 1.38)	1.01	(0.66, 1.36)	0.97	(0.76, 1.25)
p-value	0.6076		0.1221		0.1102		0.8601		0.1996	

residual limb was normalized with the sound limb and compared between visit 2 and visit 3

	Site 1=Superior		Site 2=Lateral		Site 3=Medial		Site 4=Posterior		Average (Limb-All Sites)	
Visit 2 (median, IQR)	0.89	(0.39, 2)	0.87	(0.37, 1.75)	1.2	(0.55, 2.94)			1.13	(0.61, 1.6)
Visit 3 (median, IQR)	1.04	(0.36, 2.79)	0.81	(0.2, 1.22)	0.98	(0.07, 1.77)			1.05	(0.38, 1.76)
p-value	0.7111		0.4421		0.0201				0.7428	

Table 1: Tissue Oxygen Saturation (StO_2) of the residual limb was compared between visit 2 and visit 3 (top) and normalized with the sound limb and compared between visit 2 and visit 3 (bottom panel). (n=59)

residual limb was compared between visit 2 and visit 3

	Site 1=Superior		**Site 2=Lateral**		**Site 3=Medial**		**Site 4=Posterior**		**Average (Limb-All Sites)**	
Visit 2 (median, IQR)	1	(0.22, 1.95)	1.5	(0.6, 3.25)	1.36	(0.44, 2.5)	1.2	(0.65, 2)	1.18	(0.59, 2.22)
Visit 3 (median, IQR)	0.45	(0.06, 0.92)	0.84	(0.42, 1.43)	0.63	(0.13, 1.48)	1	(0.38, 1.32)	0.83	(0.32, 1.43)
p-value	0.0009		0.1686		0.0522		0.8506		0.0807	

residual limb was normalized with the sound limb and compared between visit 2 and visit 3

	Site 1=Superior		**Site 2=Lateral**		**Site 3=Medial**		**Site 4=Posterior**		**Average (Limb-All Sites)**	
Visit 2 (median, IQR)	0.72	(0.15, 1.88)	0.93	(0.29, 2.33)	1.52	(0.35, 3.69)			1.04	(0.52, 1.92)
Visit 3 (median, IQR)	0.96	(0.17, 2)	0.56	(0.11, 1.73)	0.5	(0, 2.3)			1.06	(0.17, 1.85)
p-value	1		0.2295		0.0052				0.243	

Table 2: Oxyhemoglobin (HbO_2). (top), HbO_2 content of the residual limb was compared between visit 2 and visit 3. (bottom) HbO_2 content of the residual limb was normalized with the sound limb and compared between visit 2 and visit 3. (n=59)

residual limb was compared between visit 2 and visit 3										
	Site 1=Superior		Site 2=Lateral		Site 3=Medial		Site 4=Posterior		Average (Limb-All Sites)	
Visit 2 (median, IQR)	0.83	(0.55, 1.78)	0.8	(0.39, 1.2)	0.9	(0.4, 1.38)	0.85	(0.5, 1.17)	0.87	(0.61, 1.07)
Visit 3 (median, IQR)	0.71	(0.39, 1.12)	0.9	(0.6, 1.28)	0.73	(0.48, 1.23)	0.84	(0.6, 1)	0.87	(0.6, 1.08)
p-value	0.644		0.2559		0.8746		1		0.6516	
residual limb was normalized with the sound limb and compared between visit 2 and visit 3										
	Site 1=Superior		Site 2=Lateral		Site 3=Medial		Site 4=Posterior		Average (Limb-All Sites)	
Visit 2 (median, IQR)	0.96	(0.35, 1.63)	1.05	(0.5, 2.08)	1.02	(0.44, 1.87)			0.93	(0.55, 1.75)
Visit 3 (median, IQR)	0.69	(0.25, 1.08)	1.03	(0.48, 2.33)	0.91	(0.44, 1.35)			1.01	(0.44, 1.54)
p-value	0.5224		0.7552		0.6358				0.766	

Table 3: Deoxyhemoglobin (HbR). (top) HbR content of the residual limb was compared between visit 2 and visit 3. (bottom) Deoxyhemoglobin (HbR) content of the residual limb was normalized with the sound limb and compared between visit 2 and visit 3. (n=59)

residual limb was compared between visit 2 and visit 3										
	Site 1=Superior		Site 2=Lateral		Site 3=Medial		Site 4=Posterior		Average (Limb-All Sites)	
Visit 2 (median, IQR)	0.73	(0.21, 1.54)	0.75	(0.4, 1.37)	0.58	(0.26, 1.46)	0.74	(0.32, 1)	0.83	(0.37, 1.42)
Visit 3 (median, IQR)	0.75	(0.35, 1.33)	1.14	(0.4, 1.86)	0.77	(0.12, 1.36)	0.94	(0.41, 1.33)	0.8	(0.4, 1.23)
p-value	1		0.8601		0.8601		0.7011		0.7608	
residual limb was normalized with the sound limb and compared between visit 2 and visit 3										
	Site 1=Superior		Site 2=Lateral		Site 3=Medial		Site 4=Posterior		Average (Limb-All Sites)	
Visit 2 (median, IQR)	0.5	(0.1, 0.96)	0.62	(0.18, 1.33)	0.48	(0.08, 1.4)			0.6	(0.27, 0.86)
Visit 3 (median, IQR)	0.7	(0.27, 1.18)	0.71	(0.13, 1.47)	0.48	(0.17, 1.11)			0.6	(0.25, 1.18)
p-value	0.8746		0.5114		0.7359				0.883	

Table 5: Subsurface Hemoglobin (HbT2). (top) HbT2 of the residual limb was compared between visit 2 and visit 3. (bottom) HbT2 of the residual limb was normalized with the sound limb and compared between visit 2 and visit 3. (n=59)

residual limb was compared between visit 2 and visit 3										
	Site 1=Superior		Site 2=Lateral		Site 3=Medial		Site 4=Posterior		Average (Limb-All Sites)	
Visit 2 (median, IQR)	0.91	(0.63, 1.29)	1.01	(0.61, 1.5)	0.94	(0.65, 1.4)	1	(0.73, 1.21)	0.96	(0.75, 1.19)
Visit 3 (median, IQR)	0.7	(0.5, 1)	0.92	(0.67, 1.16)	0.93	(0.63, 1.24)	0.94	(0.64, 1.23)	0.94	(0.68, 1.09)
p-value	0.0226		1		0.7608		0.7552		0.302	
residual limb was normalized with the sound limb and compared between visit 2 and visit 3										
	Site 1=Superior		Site 2=Lateral		Site 3=Medial		Site 4=Posterior		Average (Limb-All Sites)	
Visit 2 (median, IQR)	1.26	(0.67, 2.19)	1.43	(0.83, 3.8)	1.31	(0.78, 2.81)			1.37	(0.89, 2.34)
Visit 3 (median, IQR)	0.89	(0.47, 1.69)	1.29	(0.72, 2)	1.31	(0.95, 1.97)			1.14	(0.69, 1.78)
p-value	0.0009		0.0137		0.066				0.0725	

Table 4: Superficial Hemoglobin (HbT1). (top) HbT1 of the residual limb was compared between visit 2 and visit 3. (bottom) HbT1 of the residual limb was normalized with the sound limb and compared between visit 2 and visit 3. (n=59)

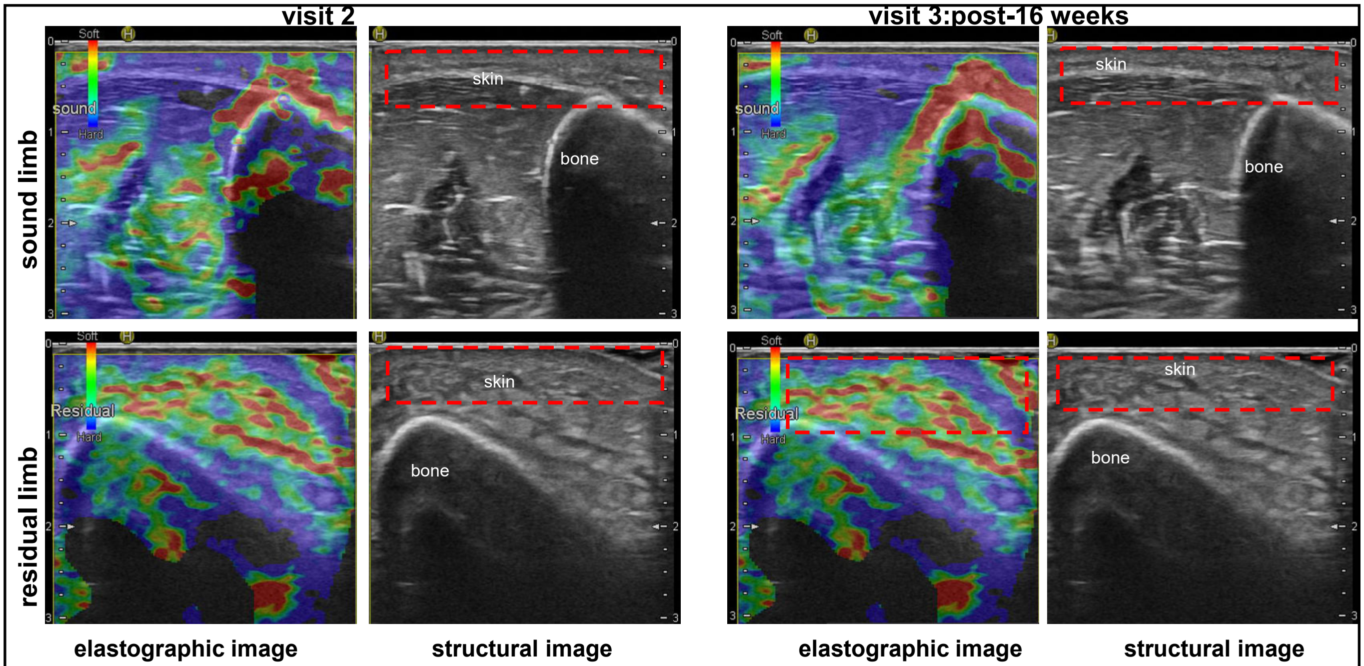

Figure 4: Representative ultrasound images taken in elastomode and B-mode at baseline (visit 2) and after 16 weeks post EVS use (visit 3)

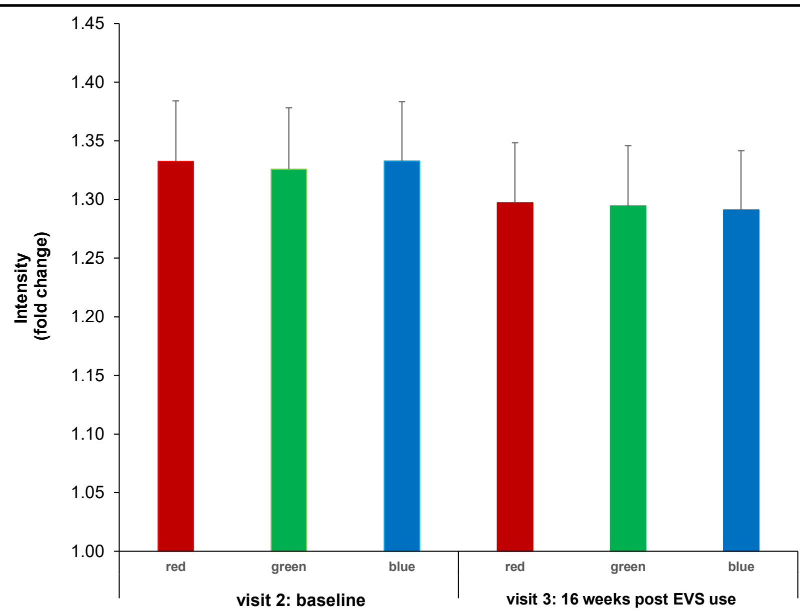

Figure 5: Quantification of signals in elastomode. No major significant changes were noted in any of the RGB signals indicating no changes in the stiffness or elasticity changes in skin following 16 weeks of EVS use as compared to baseline.

Other Achievements.
The following publications as part of the progress of this project:

Wernke MM, Schroeder RM, Haynes ML, Nolt LL, Albury AW, Colvin JM. Progress Toward Optimizing Prosthetic Socket Fit and Suspension Using Elevated Vacuum to Promote Residual Limb Health. Rehabil Res Dev. 2016;53(6):1121-1132.

Rink CL, Wernke MM, Powell HM, Tornero M, Gnyawali SC, Schroeder RM, Kim JY, Denune JA, Albury AW, Gordillo GM, Colvin JM, Sen CK. Standardized Approach to Quantitatively Measure Residual Limb Skin Health in Individuals with Lower Limb Amputation. Adv Wound Care (New Rochelle). 2017 Jul 1;6(7):225-232.

Wernke, M.M., et al. Progress Toward Optimizing Prosthetic Socket Fit and Suspension Using Elevated Vacuum to Promote Residual Limb Health. Adv Wound Care (New Rochelle) 6, 233-239 (2017).

Roy, S., Mathew-Steiner, S. & Sen, C.K. Limb Health and Prosthetics. in IntechOpen, DOI: 10.5772/intechopen.83819. (Available from: https://www.intechopen.com/online-first/residual-limb-health-and-prosthetics, 2019).

Roy, S., Mathew-Steiner, S. & Sen, C.K. Prosthetics and limb health in extreme sports. in Extreme and Rare Sports: Performance Demands, Drivers, Functional Foods, and Nutrition (eds. Datta, S. & Bagchi, D.) (CRC Press, Boca Raton, FL, 2019).

What opportunities for training and professional development has the project provided?

Training Activities:

1. Dr. Matt Wernke presented Multiaxial In-Socket Movement and Its Relationship to Fit in the 44th Academy Annual Meeting and Scientific Symposium. February 16, 2018. Podium Presentation. Training Activities contd:

2. Presented (podium) in 2018 Military Health System Research Symposium. Kissimmee, FL. August 21, 2018.

3. Presented on How Does Socket Motion Impact Limb Health? A Preliminary Report in the American Orthotic and Prosthetic Association Annual Meeting. Vancouver, BC Canada. September 28 2018. Poster Presentation.

4. Presented (podium) in 2019 Military Health System Research Symposium. Kissimmee, FL. August 2019.

How were the results disseminated to communities of interest?

In addition to the aforementioned training and professional development activities, results were disseminated to the military community at the 2018, 2019 Military Health System Research Symposium. Both oral and poster presentations.

1. Presented (podium) in 2018 Military Health System Research Symposium. Kissimmee, FL. August 21, 2018.

2. Presented on How Does Socket Motion Impact Limb Health? A Preliminary Report in the American Orthotic and Prosthetic Association Annual Meeting. Vancouver, BC Canada. September 28 2018. Poster Presentation.

3. Presented (podium) in 2019 Military Health System Research Symposium. Kissimmee, FL. August 2019.

What do you plan to do during the next reporting period to accomplish the goals?

Not applicable. This is final report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

This is the first work to correlate in socket motion with vacuum pressure waveform. This exciting discovery means that vacuum pressure waveform data that is recorded in an elevated vacuum suspension socket system can be used as measure of socket fit. Data obtained from limb health measurements, functional and patient reported outcomes (PRO) demonstrate that the use of EVS has beneficial effect on overall limb health, PRO and functional assessments, including self-selecting a faster walking speed (strong indicator for enhanced community ambulation and functional independence, particularly among older individuals), as well as taking longer steps with the intact limb (suggesting an elevated level of confidence in the prosthetic limb to facilitate taking longer steps with the limb contralateral to amputation).

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Results will support industry partner WillowWood's further efforts with the design and development of the "adaptive" socket system. This work led to the development of a new algorithm for establishing suspension level based on movement. This dynamic EVS was used in aim 3. The prototype algorithm worked without any reported issues during the study and was able to dynamically change the vacuum pressure setting in response to the amount of movement of the limb within the socket.

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

No changes in any of the major objectives of proposed study. We eliminated the crossover design for Aim 3, thus, significantly shortening of the study duration to 16 from 32 weeks. This decision was implemented following discussion with other participating investigators.

Actual or anticipated problems or delays and actions or plans to resolve them

There were significant delays in getting the Aim 3 started that included:

a. Award transfer process.
b. Reassembling the research team including hiring of a prosthetist and clinical research nurse coordinator.
c. Redesigning existing space at new PI Institution for completing the study visit and socket fitting activities.
d. Ongoing COVID-19 pandemic delayed all aspects of this study.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable

6. PRODUCTS:

- ### Publications, conference papers, and presentations

Journal publications.

1. Wernke MM, Schroeder RM, Haynes ML, Nolt LL, Albury AW, Colvin JM. Progress Toward Optimizing Prosthetic Socket Fit and Suspension Using Elevated Vacuum to Promote Residual Limb Health. Rehabil Res Dev. 2016;53(6):1121-1132.

2. Rink CL, Wernke MM, Powell HM, Tornero M, Gnyawali SC, Schroeder RM, Kim JY, Denune JA, Albury AW, Gordillo GM, Colvin JM, Sen CK. Standardized Approach to Quantitatively Measure Residual Limb Skin Health in Individuals with Lower Limb Amputation. Adv Wound Care (New Rochelle). 2017 Jul 1;6(7):225-232.

3. Wernke, M.M., et al. Progress Toward Optimizing Prosthetic Socket Fit and Suspension Using Elevated Vacuum to Promote Residual Limb Health. Adv Wound Care (New Rochelle) 6, 233-239 (2017).

4. Roy, S., Mathew-Steiner, S. & Sen, C.K. Limb Health and Prosthetics. in IntechOpen, DOI: 10.5772/intechopen.83819. (Available from: https://www.intechopen.com/online-first/residual-limb-health-and-prosthetics, 2019).

5. Roy, S., Mathew-Steiner, S. & Sen, C.K. Prosthetics and limb health in extreme sports. in Extreme and Rare Sports: Performance Demands, Drivers, Functional Foods, and Nutrition (eds. Datta, S. & Bagchi, D.) (CRC Press, Boca Raton, FL, 2019).

Books or other non-periodical, one-time publications.

1. Roy, S., Mathew-Steiner, S. & Sen, C.K. Limb Health and Prosthetics. in IntechOpen, DOI: 10.5772/intechopen.83819. (Available from: https://www.intechopen.com/online-first/residual-limb-health-and-prosthetics, 2019).

2. Roy, S., Mathew-Steiner, S. & Sen, C.K. Prosthetics and limb health in extreme sports. in Extreme and Rare Sports: Performance Demands, Drivers, Functional Foods, and Nutrition (eds. Datta, S. & Bagchi, D.) (CRC Press, Boca Raton, FL, 2019).

Other publications, conference papers and presentations.

1. Dr. Matt Wernke presented Multiaxial In-Socket Movement and Its Relationship to Fit in the 44th Academy Annual Meeting and Scientific Symposium. February 16, 2018. Podium Presentation.

2. Presented (podium) in 2018 Military Health System Research Symposium. Kissimmee, FL. August 21, 2018.

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4. Presented (podium) in 2019 Military Health System Research Symposium. Kissimmee, FL. August, 2019.

- **Website(s) or other Internet site(s)**

Significant changes in use or care of human subjects
None

- **Technologies or techniques**

None

- **Other Products**

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

PERSONNEL

Name:	Dr. Chandan K. Sen, PhD
Project Role:	PI
Nearest person month worked:	2
Contribution to Project:	Dr. Sen is responsible for the overall coordination and leadership of the project; including experimental design, analysis and interpretation of study outcomes.

Name:	Dr. Sashwati Roy, PhD
Project Role:	Co-I
Researcher Identifier (ORCID ID):	NA
Nearest person month worked:	1
Contribution to Project:	Dr. Roy serves as the Director of Clinical Research at IU Health Comprehensive Wound Center. She is closely working with the clinical research team and ensure compliance, recruitment and retention.

Name:	Dr. Subhadip Ghatak, PhD
Project Role:	Co-I
Nearest person month worked:	2
Contribution to Project:	Dr. Dr. Ghatak will acquire residual limb health measurements while closely working with Dr. Roy and optimizing the measurements.

Name:	Dr. Gayle Gordillo, MD
Project Role:	Co-I
Nearest person month worked:	1
Contribution to Project:	Her role is to support analysis and interpretation of residual limb skin health outcomes.

Name:	Ms Amy Miller, CCRP
Project Role:	Clinical Research Manager
Nearest person month worked:	1
Contribution to Project:	Study staff management, help in reporting, IRB, HRPO approvals, DSMB.

Name:	Bryce Hockman
Project Role:	Clinical Research coordinator
Nearest person month worked:	1
Contribution to Project:	Clinical Research coordinator on the project and supports study visits, consenting data entry.

Name:	Mr. Jeff Denune, CPO
Project Role:	Prosthetist
Nearest person month worked:	1
Contribution to Project:	His primary role in socket shape capture, fabrication and fittings related to the project.

Name:	Mr. Jim Colvin, MS
Project Role:	WillowWood Lead
Nearest person month worked:	1

Contribution to Project:	His primary role was leading the WillowWood team during the course of the project.
Name:	Dr. Matthew Wernke, PhD
Project Role:	Research Engineer
Nearest person month worked:	3
Contribution to Project:	His primary role was to lead technology development, coordinate IRB, oversee research, and analyze outcomes.
Name:	Mr. Alex Albury, CPO
Project Role:	Prosthetist
Nearest person month worked:	2
Contribution to Project:	His primary role was socket shape capture, fabrication, fittings, and data collection related to the project.
Name:	Dr. Christopher Dearth, PhD
Project Role:	Site PI; WRNMMC
Nearest person month worked:	1
Contribution to Project:	Overall project management and coordination of local study activities
Name:	Dr. Bradford Hendershot, PhD
Project Role:	Co-I; WRNMMC
Nearest person month worked:	1
Contribution to Project:	Assistance with local project management and oversight of Aim 3 data collection/analyses
Name:	Dr. Ashley Knight, PhD
Project Role:	Co-I; WRNMMC
Nearest person month worked:	1
Contribution to Project:	Assistance with local project management and oversight of Aim 3 data collection/analyses
Name:	Mr. Julian Acasio, MS
Project Role:	Co-I; WRNMMC
Nearest person month worked:	3
Contribution to Project:	Analyses of functional data obtained as part of Aim 3
Name:	Ms. Heidi Mahatan, MA
Project Role:	Program Manager, Research Coordinator; WRNMMC
Nearest person month worked:	1
Contribution to Project:	Regulatory and programmatic support

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

1. WillowWood Global LLC, Ohio, US
Participated in designing, subject recruitment as well as study visits and collection of data for Aim1, 2 and Aim 3a.

1. Henry M. Jackson Foundation for the Advancement of Military Medicine (HjF) and Walter Reed National Military Medical Center (WRNMMC), Bethesda, MD, USA.
Participated in designing and data analysis for the studies performed for Aim 3b.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS: