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# Assessing non-invasive imaging devices to detect temperature differentiation and lymphatic/venous flow to the head and neck during head down tilt, supine, and sitting positions The NIID Study (non-invasive imaging device)

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**Article**

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# Abstract

Under the influence of standard developmental physiology on the Earth's surface (1 gravity equivalent known as "1G"), 70% of body fluids reside below the level of the heart.Fluid shifts towards the cephalic region during microgravity have also been speculated to contribute towards spaceflight associated neuro-ocular syndrome (SANS).Although a significant barrier to spaceflight, the underlying mechanism of SANS is not well understood, partially due to the spaceflight environment and limited medical capabilities including invasive diagnostic testing. Thus, non-invasive approaches to studying real-time fluid shifts in weightlessness could serve as critical areas of research to further SANS study and effective countermeasure protocol development. For continuous fluid shift monitoring and management, the goal is to establish baseline assessments utilizing real time point of care noninvasive imaging devices (NIID). NIID will first need to be quantified and validated through ground-based analogues, with subsequent acquisition of near continuous imaging from arrival in the weightlessness of LEO and during the time of adaptation, which may be variable among crew members. The purpose of this study was to examine temperature differential alterations, superficial venous flow patterns (head, neck, upper torso), and venous flow patterns along the lymphatic ventromedial bundles of the medial calves and thighs. Imaging was obtained in three different positions using three standards of care medical NIIDs: SnapShotTM by Kent Imaging, Scout by WoundVisionTM and LymphScannerTM by Delfin. 30 volunteers were assessed for temperature and lymphatic/venous flow using three different non-invasive devices that can measure temperature, perfusion, and image the lymphatic and venous structures. The volunteers were assessed in the sitting, supine, and 6-degree head down tilt (HDT) positions, with pre/post assessments for the HDT position. Venous and lymphatic flow patterns may vary upon position changes (sitting vs. supine vs. 6-degree head down tilt), and in response to MLD performance in the treatment group. Fifteen subjects per group (N = 30) completed all visits and were used in the full analysis. The sitting and supine position data did not show statistical significance at any of the time points and with the devices used. The HDT data from SnapShot (looking at perfusion, deoxy and oxyhemoglobin) and Wound Vision Scout (thermal imaging) did not show statistical significance between the control and treatment groups at any time points. No significant difference for any of the within-group comparisons were found with the Lymphscanner. A significant reduction in left temple TDC for the treatment group was found at post-30-minutes (5.77, 95% CI: 1.24, 10.30) and post-180-minutes. A significant reduction in right subclavicular TDC for the treatment group was found at post-180-Minutes (5.09, 95% CI: 0.16, 10.03). A significant increase in the left ventromedial bundle for the treatment group was found at post-90-Minutes (5.72, 95% CI: 0.34, 11.10) and post-120-Minutes (5.85, 95% CI: 0.47, 11.23). A significant increase in the right ventromedial bundle for the treatment group was found at post-90 minutes (5.53, 95% CI: 0.21, 10.84). This is the first HDT spaceflight analogue study, to our knowledge, in which dermal fluid shifts were evaluated using point-of-care noninvasive imaging modalities including Near Infrared Spectroscopy (NIRS), thermography and a subcutaneous edema monitor, with subsequent application of manual lymphatic drainage (MLD) techniques to stimulate dermal lymphatic function as a countermeasure and mitigation therapeutic for intervention in cephalad fluid shifts. Further validating studies are indicated for NIIDs to further quantify fluid shifts in both analogues and weightlessness.

## Background

Under the influence of standard developmental physiology on the Earth's surface (1 gravity equivalent known as "1G"), 70% of body fluids reside below the level of the heart (Fig. 1).[1] The lymphatic system has the capacity and capability to transport fluid from distal to proximal in an upward manner, against gravity and tissue pressure gradients, via lymphangion contractility, leg muscle contraction, respiratory and chest wall function, thus augmenting a "suction effect" for pumping lymphatic fluid within the subatmospheric pressure tissue distribution zones (the Guyton principle).[2] Lymphatic drainage of the head and neck must be assisted by gravity, since these regions are above the level of the heart. In the weightlessness of space and significant alterations of terrestrial 1G head-to-foot hydrostatic pressure gradients, astronauts experience a dramatic fluid redistribution of ~ 2 liters from the legs to the head and neck within the first 24–48 hours of flight, among other cardiovascular and physiologic system adaptations. After only 4 days in the weightlessness of low earth orbit (LEO), changes can be seen in baroreceptor responsiveness, causing orthostatic hypotension upon subsequent return to Earth. Fluid shifts may also result in headaches, congestion or facial puffiness that can contribute to deteriorating sleep patterns.[3] The ability to manage, mitigate or offset these fluid shifts is vital to maintain nominal health for short and long duration space flight and potentially improve readaptation to terrestrial gravity or other surface gravity fields such as the moon or Mars. During deep space missions, ground-based medical teams will no longer be easily accessible due to distance and communication delays, hence crew members must perform point of care functions independently to maintain preventative health measures along with urgent corrective and potentially curative countermeasures.[4] Fluid shifts towards the cephalic region during microgravity have also been speculated to contribute towards spaceflight associated neuro-ocular syndrome (SANS).[5] SANS is a distinct, microgravity-induced phenomenon of neuro-ophthalmic findings observed in astronauts following long-duration spaceflight including choroidal folds, optic disc edema, posterior globe flattening, refractive shift, and cerebral fluid shifts noted to be persistent at 6 month post-flight MRI scans. SANS has been identified as a "red" risk based on occurrence probability and its impact on mission performance and human health. Although a significant barrier to spaceflight, the underlying mechanism of SANS is not well understood, partially due to the spaceflight environment and limited medical capabilities including invasive diagnostic testing. Thus, non-invasive approaches to studying real-time fluid shifts in weightlessness could serve as critical areas of research to further SANS study and effective countermeasure protocol development. For continuous fluid shift monitoring and management, the goal is to establish baseline assessments utilizing real time point of care noninvasive imaging devices (NIID). NIID will first need to be quantified and validated through ground-based analogues, with subsequent acquisition of near continuous imaging from arrival in the weightlessness of LEO and during the time of adaptation, which may be variable among crew members.

## Specific Aims

The purpose of this study was to examine temperature differential alterations, superficial venous flow patterns (head, neck, upper torso), and venous flow patterns along the lymphatic ventromedial bundles of the medial calves and thighs. Imaging was obtained in three different positions using three standards of care medical NIIDs: SnapShot™ by Kent Imaging, Scout by WoundVision™ and LymphScanner™ by Delfin. Comparisons between devices were not made, as each is designed to capture different data. SnapShot was used to capture perfusion changes through total hemoglobin, total oxy- and deoxyhemoglobin, and superficial oxygenation saturation measurements, WoundVision Scout was used to capture thermal images measuring physiological temperature differentiation and the LymphScanner captured moisture readings (tissue dielectric constant or TDC) consistent with lymphatic flow patterns and interstitial fluid alterations related to positioning. The goal was to establish baseline assessments through a validated spaceflight analogue (head down tilt (HDT) and variable positioning) to simulate the impact of fluid shifts and associated temperature alterations experienced due to weightlessness and the inherent changes in lymphatic and venous flows. This is the best currently validated analogue that addresses fluid shifts in a timely manner.

# Specific Aim 1:

Evaluate changes in dermal lymphatic and venous blood flow and temperature differentiation using three different noninvasive devices in three different positions (sitting, supine, 6-degree head down tilt).

### Hypothesis

Dermal venous and lymphatic flow patterns of the head, neck, upper torso, and ventromedial bundle will vary depending upon position (sitting vs. supine vs. 6-degree head down tilt). It is hypothesized that flow patterns will shift to cephalad (dermal interstitial fluid increase) in the 6-degree head down tilt compared to the sitting and supine positions. With changes in flow patterns, it is hypothesized that temperature will increase in areas of increased lymphatic and venous flow.

# Specific Aim 2:

Assess the image and temperature changes and alterations of the head, neck, upper torso, and ventromedial bundle related to performance of MLD (Manual Lymphatic Drainage), during the 3 defined positions.

### Hypothesis

It is hypothesized that the use of MLD will alter lymphatic contractility flow patterns and interstitial fluid, in addition to temperature differentials, in the 6-degree head down tilt. MLD is a manual technique delivering light pressure through the skin to stimulate lymphatic vessel function. Specific changes in images after MLD during sitting and supine are not anticipated to be as significant compared to changes seen in head down tilt; however, tissue temperature is expected to reduce with the implementation of MLD in all positions.

## Significance

Within the context of space exploration, the lymphatic and venous systems and the related vascular endothelial glycocalyx (GCX) remain under-researched areas. Numerous biologic and physiologic pathologies are associated with exposure to extreme environments and may result from the response and adaptation of the venous and lymphatic systems as well as the GCX.[7–10] Research is required in ground-based analogues for spaceflight, then should progress to true weightlessness in low Earth orbit (LEO) as we begin to understand the impact on human physiology in the context of venous, lymphatic and GCX functioning during exposure to extreme environments.[11–19]

Lymphatic function/contractility is noted to be regionally, organ- and tissue-function specific.[20] The GCX lines the luminal surface of the 60–70,000 miles of the arterial, venous, and lymphatic vasculature.

On Earth, body fluid compartments are maintained and balanced by the nano-scaled architectural integration of the endothelial GCX, the vasculature, the integument, and the lymphatic system. The lymphatic system's capability to transport fluid from the lower extremities and torso, against gravity and soft tissue gradients, occurs via a combination of lymphangion contractility, leg muscle contraction, and respiratory/chest wall function in the setting of "primed" local/regional subatmospheric tissues. This creates a "suction effect" within the subatmospheric tissue distribution zones for lymphatic fluid movement within the lymphatic vasculature, which is lined with lymphatic endothelial cells and the GCX (the Guyton principle).[22–23] The head, neck, and upper torso, however, are gravity gradient-dependent for venous and lymphatic drainage, and must be balanced with arterial in-flow to this region.[24] In the weightlessness of space, astronauts experience a dramatic fluid redistribution of ~ 2 liters from the legs to the head, neck and upper torso within the first 24–48 hours of flight;[25] this may be due in part to the persistence of pedal to cephalad lymphatic fluid flow due to "Guyton forces" and the loss of gravity induced head and neck lymphatic drainage, resulting in an imbalance of soft tissue fluid gradients.

Research leading to countermeasure development to support the venous and lymphatic systems at pre-launch, in-flight and post-flight have the potential to support nominal human health in space. Additionally, an improved understanding of venous, lymphatic and GCX functional restoration and maintenance will support improved human health on Earth for the vast number of patients dealing with the many associated diseases and conditions related to dysfunction and dysregulation of these integral systems.

## Innovation

Studies upon astronauts and cosmonauts in true weightlessness have been performed in all spaceflight eras, from Mercury to Apollo,[26] to ISS, including the 25-month long NASA Twins Study,[16] that compared monozygotic twin astronauts through extensive integrated longitudinal, multidimensional measures of physiological, telomeric, transcriptomic, epigenetic, proteomic, metabolomic, immune, microbiomic, cardiovascular, vision related and cognitive data, as one spent 340 days in space on ISS and his identical twin remained Earthbound. Ground based analogues for simulation of the impact of weightlessness on human physiology, providing for extensive research opportunities, exist in several forms including days/weeks of strict 6-degree head tilt down bed rest, dry immersion, and brief periods (20–40 seconds) of weightlessness during parabolic flights. [11, 27–28]

Lymphatic research has experienced a renaissance in the past decade with the recognition and increasing knowledge of the dynamic nature of the endothelial glycocalyx and the resulting modification of the classical Starling function to recognize the true critical nature of lymphatic integrative function with arterial, venous, integument and immune health. The modified Starling principle is a recognition that most interstitial fluid resulting from arterial perfusion re-enters the central venous system via the vast lymphatic network, and not via venules.[8–12, 29–33] Despite new significant milestones in research and clinical application, lymphatic education and recognition remains “paradoxically and unnecessarily ignored” at the medical school and residency levels.[34] Lymphatic research in space and astronaut health may “paradoxically” elevate the subject on terra firma. Given that all venous ulcerations and venous hypertension have associated glycocalyx thinning or shedding,[35] and associated lymphedema of venous etiology/phlebolymphedema is a common though vastly underrecognized and undertreated component,[36–37] the potential for patient care improvement may be elevated with the continued focus on astronaut health, countermeasure development, the near-term space tourism era, and the ambitious near-term goals of moon habitation and Mars human exploration.[6]

Lymphatic research in low Earth orbit (LEO) is in the initial phases of development. Current published data has used mice and rats in studies involving tail elevation (simulates effects of weightlessness), space shuttle and satellites (Bion-M unmanned automated 30-day satellite mission with mice).[38] A 2-week head down, tail suspension model simulating microgravity, was found to be a potent inhibitor of pressure/stretch-stimulated pumping in cervical, thoracic and to a lesser degree, mesenteric lymphatics. The greatest inhibition was found in cervical lymphatics. These findings presumably are correlated to the cephalic fluid shifts that occur in HDT suspended rats as well as those observed during spaceflight.[39, 24]

The opportunity to further the understanding and develop noninvasive imaging methods to assess fluid shifts may contribute to the development of restorative countermeasures for lymphangion contractility, overall lymphatic function, and associated immune function in the extreme environment of space. This carries high potential to improve our understanding and treatment of patients that we care for in 1G clinics, who have lymphedema and venous disease that negatively affects quality of life, outcomes, and economics. The consequences on crew behavior and performance due to these fluid shifts in microgravity has not yet been fully determined for long duration missions.[6]

This novel study will evaluate four non-invasive medical devices to assess venous and lymphatic flow patterns as well as temperature differentiation, during performance of a validated spaceflight analogue. Fluid shifts experienced in microgravity can contribute to alterations in the cerebral venous system and are hypothesized to increase susceptibility to ophthalmic pathologies experienced in weightlessness, such as spaceflight associated neuro ocular syndrome (SANS).[6] Imaging in true weightlessness may contribute to an enhanced understanding of how to manage cephalad fluid shifts and the contribution to SANS development in astronauts, which has not been fully assessed in space medicine to date.

## Research Plan

Thirty healthy college students (all within a healthy BMI and without lymphedema or known lymphatic dysfunction) were recruited for this study. There were 12 males and 18 females, ranging in age from 21 to 36. Each student was assessed for temperature and lymphatic/venous flow using three different non-invasive devices that can measure temperature, perfusion, and image the lymphatic and venous structures. Students were assessed in the sitting, supine, and 6-degree head down tilt (HDT) positions, with pre/post assessments for the HDT position. Venous and lymphatic flow patterns may vary upon position changes (sitting vs. supine vs. 6-degree head down tilt), and in response to MLD performance in the treatment group.

## Sample Size and Randomization

The sample size for this exploratory pilot study was 30 participants (15 participants in the control group and 15 participants in the treatment group who received MLD). Participants were recruited and data collected over a 3-month period. Students were randomly assigned to either the control or treatment group, using a random number generator.

## Overall Strategy

Participants were recruited to take part in this study through flyers and email announcements throughout the Health Professions Division at Nova Southeastern University. Once consented and randomized to a group, participants were sent a link to Zcal (an online scheduling system) where they could sign up for data collection days and times according to their individual schedules to accommodate duration of position time, the longest being up to 4 hours (Treatment group, HDT position). Participants arrived at a predesignated room for assessment and data collection. Participants acclimated to the room and ambient temperature by sitting quietly for 15 minutes prior to data collection. Baseline vitals (heart rate, blood pressure, respiration rate, and oxygen saturation) were taken and monitored throughout the data collection process. All participants were assessed in the sitting position for 30 minutes (gravity dependent), supine for 1.5 hours (gravity neutral) and HDT for 3 hours (validated simulated weightlessness). Each position was assessed on a different day to reduce any position-related carryover effects. Baseline image assessments using the devices were taken 1 minute after assuming the designated position. Subsequent image assessments were taken every 30 minutes thereafter. Each image acquisition requires ~ 15–30 seconds and was performed by 1 of 4 trained imagers. At the end of the established timepoints, reassessment using the devices was taken prior to moving out of designated position. Participants in the MLD group received 15 minutes of an established MLD protocol to the head, neck, and upper torso prior to moving out of position. Immediately post MLD, reassessment with the devices was taken. A follow up reassessment was taken with the devices 30 minutes after MLD to assess potential changes and resolution of any symptoms experienced in any of the positions. Our observations were designed to detect differences in flow patterns and temperature based upon position and with the implementation of MLD pre- and post HDT.

The devices being used include SnapShot to assess perfusion/flow by capturing total hemoglobin, total oxy- and deoxyhemoglobin, and superficial oxygenation levels as a near-infrared spectroscopy device, WoundVision Scout to assess physiological thermal temperature levels using long-wave infrared thermography, and LymphScanner to measure the tissue dielectric constant (TDC) which is representative of localized percent water content.

## Data Analysis Plan

Summary statistics were calculated for study variables. We employed random effects, generalized linear models, to look for differences in Left and Right Temple TDC, Left and Right (sternocleidomastoid) SCM TDC, Left and Right SUBC (sub-clavicular) TDC, and Left and Right Ventromedial Bundle TDC and temperature. First, we looked for changes within the treatment group only. Second, we examined differences between the treatment and control groups for each visit. The fixed effects were visit (baseline, post-30-minutes, post-60-minutes, post-90-minutes, post-120-minutes, post-180-minutes, post-MLD, and post-30 minutes-MLD). The random effect was the subject. For all post-modeling comparisons, a false discovery adjustment (FDR) was used. The FDR method has higher power than the Bonferroni and Tukey HSD method and controls the type I error as well. This is important considering the project's exploratory nature. R version 4.2.2 was used for all statistical modeling and statistical significance was found at $p < 0.05$.

## Results

Fifteen subjects per group ($N = 30$) completed all visits and were used in the full analysis. The sitting and supine position data did not show statistical significance at any of the time points and with the devices used. The HDT data from SnapShot (looking at perfusion, deoxy and oxyhemoglobin) and Wound Vision Scout (thermal imaging) did not show statistical significance between the control and treatment groups at any time points. No significant difference for any of the within-group comparisons were found with the Lymphscanner. Please refer to Figs. 2–6. However, between the control and treatment group, differences were found with the Lymphscanner (TDC).

A significant reduction in left temple TDC for the treatment group was found at post-30-minutes (5.77, 95% CI: 1.24, 10.30) and post-180-minutes (6.42, 95% CI: 1.71, 11.13). A significant reduction in right temple TDC for the treatment group was found at post-30-minutes (5.35, 95% CI: 0.25, 10.44) and post-120-minutes (6.42, 95% CI: 1.32, 11.51).

A significant reduction in left (sternocleidomastoid) SCM TDC for the treatment group was found at baseline (4.15, 95% CI: 0.40, 7.90), post-120-Minutes (4.26, 95% CI: 0.51, 8.01), post-150-Minutes (4.78, 95% CI: 1.03, 8.53), and post-180-Minutes (4.98, 95% CI: 1.21, 8.74). A significant reduction in right SCM TDC for the treatment group was found at baseline (4.93, 95% CI: 0.76, 9.10), post-30-Minutes (5.56, 95% CI: 1.39, 9.73), post-60-Minutes (4.37, 95% CI: 0.20, 8.53), post-90-Minutes (4.75, 95% CI: 0.58, 8.92), post-120-Minutes (5.49, 95% CI: 1.32, 9.66), post-150-Minutes (5.35, 95% CI: 1.18, 9.51), and post-180-Minutes (8.35, 95% CI: 4.18, 12.51).

A significant reduction in right subclavicular TDC for the treatment group was found at post-180-Minutes (5.09, 95% CI: 0.16, 10.03).

A significant increase in the left ventromedial bundle for the treatment group was found at post-90-Minutes (5.72, 95% CI: 0.34, 11.10) and post-120-Minutes (5.85, 95% CI: 0.47, 11.23). A significant increase in the right ventromedial bundle for the treatment group was found at post-90 minutes (5.53, 95% CI: 0.21, 10.84).

Lastly, a significant increase in body temperature for the treatment group was found at post-30 minutes (0.18, 95% CI:0.01,0.36).

## Discussion

As performed in this time limited HDT analogue study, NIID has been demonstrated to have high potential for continuous monitoring for terrestrial models and potentially in true weightlessness. The reduction in Tissue Dielectric Constant (TDC) at the temple, post MLD (post 30 minutes after 180 minutes in HDT) suggests enhanced lymphatic function resulting in decreased subcutaneous edema induced by the techniques of manual lymphatic drainage. This requires further testing and validation to confirm these results, potentially with near-infrared fluorescence imaging. If confirmed to have reproducible results, MLD may serve as another potential countermeasure for fluid shift mitigation by any crew member in any location without requiring additional equipment. MLD could be taught by crew members by Certified Lymphedema Therapists as an easily employable therapeutic. Further, MLD pre-flight, during flight and post-flight should also be further investigated for reduction of fluid shift consequences.

An increase of Tissue Dielectric Constant (tissue edema) at ventromedial bundle (medial knee) may be related to fluid shifts while in the HDT position, as the calf dermal venous and lymphatic microvasculature became distended. This is a novel finding not previously reported to our knowledge, necessitating further validation. The potential significance of a change in the TDC may be enhanced by more prolonged monitoring beyond 3 hours. This should be assessed in other HDT studies, specifically looking at Space Associated Neuroocular Syndrome (SANS), ideally at multiple locations along the VM bundle, could further enhance understanding of fluid shift in prolonged HDT studies (days to weeks). Ultimately this could serve as baseline measurements to compare similar measurements in true weightlessness.

Anecdotally, increased heat signals on the right side of the neck compared to left, though not quantifiable (Figs. 7 and 8) were visually noted with both thermography and NIRS, as VM bundles did not increase in thermal patterns throughout the data collection, as expected, due to decreasing lower extremity venous pooling based on non-gravity assisted positions. While our infrared images concentrated on gathering data on specific points of interest, the visual color images of larger areas demonstrated thermal changes affected by position, time, and manual lymphatic drainage (MLD) techniques. The long wave infrared thermography scale (Fig. 7) represents a color thermal energy gradient measured in degrees Celsius. As a reference, values above 0 indicate hyperperfusion while values below 0 indicate hypoperfusion. The color scale for NIRS (Fig. 8) represents tissue oxygenation saturation levels in percentages. A decrease in perfusion is noted as the value moves towards 0. Further quantification of these perceived changes will require advanced software for definitive validation.

A report by Whittle et al in 2023, noted the right-side dominance of venous drainage, and this may correlate with the non-validated visual findings noted during this study.[40] A further investigation could correlate these findings with venous duplex ultrasound to determine venous distention, which has been performed in both space flight analogues and true weightlessness.[41]

This is the first HDT spaceflight analogue study, to our knowledge, in which dermal fluid shifts were evaluated using point-of-care noninvasive imaging modalities including Near Infrared Spectroscopy (NIRS), thermography and a subcutaneous edema monitor, with subsequent application of manual lymphatic drainage (MLD) techniques to stimulate dermal lymphatic function as a countermeasure and mitigation therapeutic for intervention in cephalad fluid shifts.

The imaging modalities are light weight/low mass units that can acquire repeatable imaging in a short period of time, and do not require dye injections, radiation exposure, or significant training. Similar to ultrasound techniques already used by crews in low Earth orbit, ground based crews would be able to assist in techniques and interpretation of image acquisition. Another significant potential is the ability to obtain images beginning shortly after entering weightlessness. Noninvasive imaging also allows repeatable imaging over the initial 48 hours to 7 days during individual crew member acclimation to fluid shifts. Differentiating relative differences between individuals then correlation with single nucleotide polymorphism (SNPs) such as MTRR A66G and SHMT1 C1420T. SNP status may improve the predictive potential of SANS development and allow for a more personalized, precision, and prescriptive application of countermeasures (i.e., MLD frequency, B vitamins, pre-flight lymphatic preconditioning, post-flight rehabilitation to enhance recovery rates and return to full 1 G functional status).

In the NASA Twins study, the internal jugular vein cross-sectional area increased, and forehead tissue thickness increased in the twin on International Space Station when compared with values associated with the seated position on Earth and compared to the earthbound twin.[16] For the twin in low Earth orbit, increases in subfoveal choroidal thickness (primary vascular supply of the outer retina) and peripapillary total retinal thickness were also observed, indicating retinal edema formation. The ocular changes are consistent with SANS development. Risk alleles in five SNPs are predictive of the incidence of ophthalmic changes, and six of the nine risk alleles are present in the twins.[16] Head down tilt study results support genetic traits and potentially SNPs such as methionine synthase reductase 66 (MTRR 66) and serine hydroxymethyltransferase1 1420 (SHMT1 1420) as risk factors for SANS development.[42] HDT itself may be a practical way to screen for risk of SANS and response to MLD and other countermeasure responses such as lower body negative pressure, artificial gravity, dietary supplementation (B vitamins)[42] or diosmin,[43] varying training, prehabilitation regimens, and dermal electrical stimulation.[44] HDT may be helpful to identify the best in-flight measurements with which to diagnose and monitor fluid shifts associated with SANS, as demonstrated in this study. Further HDT studies employing these techniques for POC imaging and variations of mitigation strategies will help to further refine best alleviation strategies.

Utilizing ultrasound dermal thickness determination is a validated and quantified method,[45] and future studies comparing NIRS and thermography to evaluate zonal gradient changes over larger surface areas in a noninvasive non- contact method, may further complement research for both fluid shifts and response to mitigation methods. Another technology that would be of significant correlative benefit for determining dermal lymphatic function is Near-infrared fluorescence lymphatic imaging (NIRF-LI). NIRF-LI collects NIR fluorescent signals emanating from the lymphatics following the off-label administration of indocyanine green (ICG). Using this approach, the peripheral lymphatic vessels can be visualized in both health and disease.[46] This method was used in a HDT model to evaluate the potential contributions of fluid shift in SANS.[47] A pilot study was initially performed in human volunteers to evaluate the effect of gravity on deep cervical lymphatic flow via palatine tonsil injection of indocyanine green (ICG).[47] The ICG imaging was noted to drain into deep jugular lymphatic vessels and subsequent cervical lymph nodes. NIRF-LI was then performed under HDT, sitting, and supine positions. NIRF-LI demonstrated that lymphatic drainage shared pathways with CSF outflow that are dependent upon gravity and are impaired when subjected to short-term HDT. Of significance, NIRF-LI determined lymphatic contractile rates following intradermal ICG injections of the lower extremities were slowed in the gravity-neutral and supine positions but increased under the influence of gravity, regardless of whether the force direction opposed (sitting) or favored (HDT) lymphatic flow towards the heart. The implications of this study outcomes provided evidence for the role of a lymphatic contribution in SANS.[47] Performance of NIRF-LI in true weightlessness would be an ideal “stretch goal,” though dermal injections and the specialized equipment needed for visualization may be prohibitive in the near term.

To ultimately determine if findings in HDT position are comparable to what we may see in microgravity, we would need imaging capabilities on the ISS. A technique known as ‘Near-infrared spectroscopy’ (NIRS) was recommended by Myllylä and colleagues as a method to measure long-term water dynamics in the human brain that may correlate with the dynamics of glymphatic circulation.[48] Dysfunction of the ocular glymphatic system during microgravity fluid shift has been analyzed and proposed as an etiology of SANS pathogenesis.[49] A specialized NIRS device that is portable, compact, and noninvasive can potentially be used to look through “bone windows” at CSF flow. NIRS imaging could allow for the ability to measure fluid dynamics on the International Space Station during long-term space travel and determine impacts on the head and neck.[48,49]

There are a multitude of proposed countermeasures that could be used in conjunction with MLD to reduce lymphatic/venous flow to the head and neck during spaceflight. Wostyn and colleagues suggested those such as thigh cuffs and lower body negative pressure (LBNP) used by Russian cosmonauts to restrict fluid movement upward from the lower extremities.[50]

Limitations for this study include activities outside of the lab that may influence lymphatic function such as exercise, which can activate lymphatic function that may then persist for 1–4 hours. Genetic influences such as single nucleotide polymorphisms (SNPs) or the presence of Raynaud's phenomenon may impact thermal monitoring. Additionally, entering a cool building from a warm humid external environment (study performed in Ft. Lauderdale, Florida) may also potentially impact imaging results. To control for these possibilities, the participants were young college students screened by a self-report of their known medical history. In addition, study participants were asked to sit for 10 minutes upon arrival to the study room to acclimate to the ambient temperature prior to positioning and imaging.

Another notable limitation is the relatively small sample size of HDT studies. The necessity of volunteers to participate for days to months while maintaining a strict position that may be uncomfortable, induce headaches, and severely limit mobility, often restricts the available volunteer pool. Generalizing HDT observations to highly trained astronauts may also be a significant limitation. Two years before a spaceflight mission, astronauts typically undergo a minimum of 2–3 years of intensive strength and aerobic conditioning prior to an anticipated launch and mission date. Having volunteers match this 2–3-year training regime is not practical.

For this study, the positions were not sustained over days, weeks or months; rather, sitting, supine and HDT were scheduled on different days for 30, 90 and 180 minutes respectively. It is possible these time points were not as sensitive to imaging changes as anticipated. Additionally, the data points or areas of interest (AOI) for the SnapShot and WoundVision Scout were hand selected for analysis which may have led to subtle variation in AOI or inter-rater error.

## Conclusion

Point of Care imaging involves emerging technologies that offer low mass/weight handheld non-contact devices with clinical applications for CVI, lymphedema and early detection of skin temperature thermal pattern abnormalities. POC has high potential for application and use in spaceflight analogue testing, on parabolic flights, ISS, and other spacecraft for fluid shifts (acute and chronic), SANS, and crew core body temperature (CBT) evaluations. Ideally, these devices will help guide or establish countermeasures specifically to address fluid shifts pre-flight, during early and late space flight, and post-flight conditions, with the potential to have significant “spinoff” applications for fluid shift recognition in wound care and lymphedema clinics to improve outcomes through recognition and improved treatment protocols. Lower body negative pressure (LBNP) has been validated as a mechanism for altering fluid shifts in low Earth orbit, manual lymphatic drainage as an adjunct may also prove to be an effective countermeasure used pre-flight, during flight and post flight to manage and/or restore fluid shifts experienced during weightlessness.

## Declarations

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## Figures

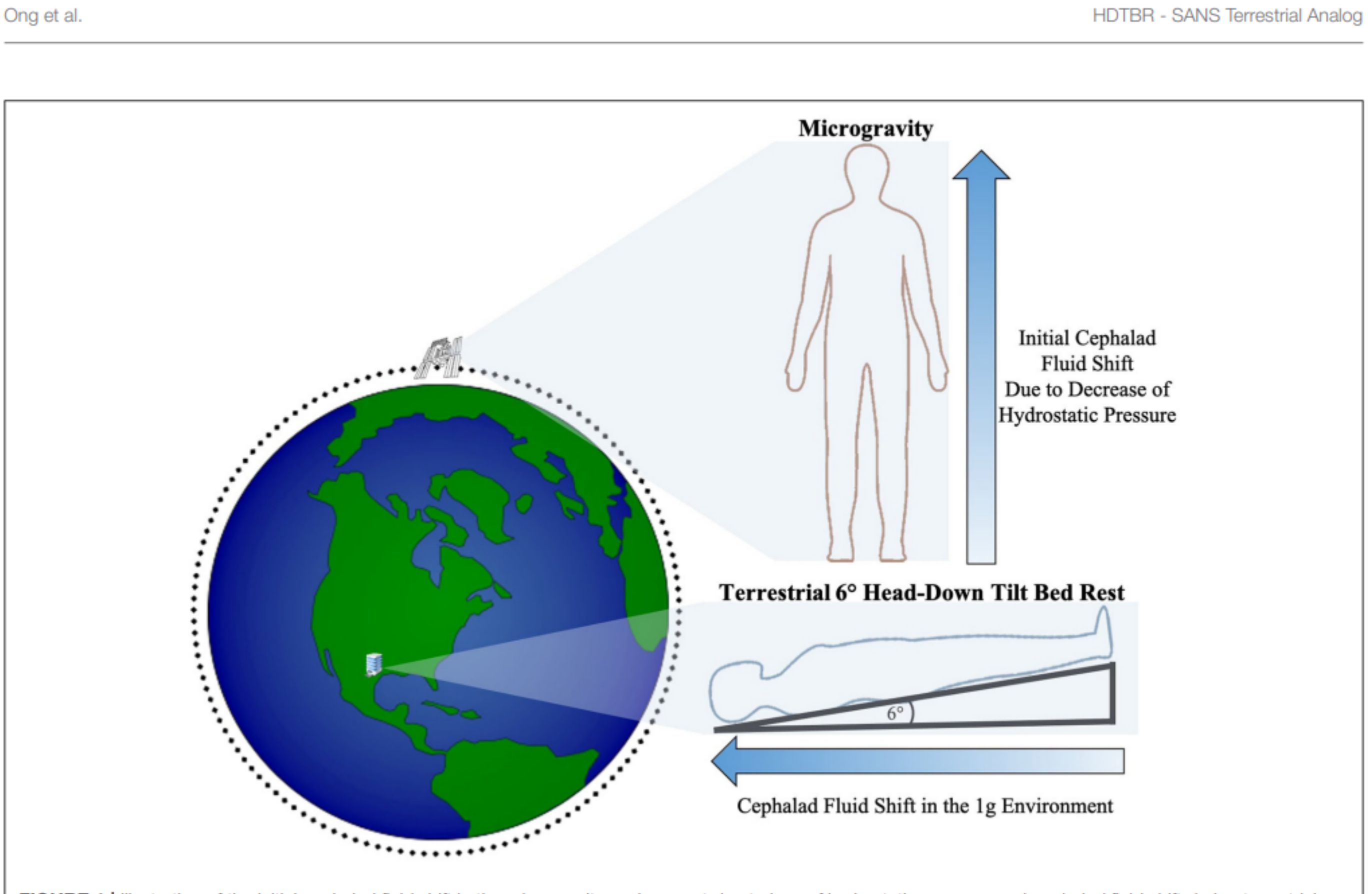


**FIGURE 1** | Illustration of the initial cephalad fluid shift in the microgravity environment due to loss of hydrostatic pressure and cephalad fluid shift during terrestrial HDTBR studies (not drawn to scale).

Figure 1

**Body Fluids at 1G on Earth in spaceflight analog head-down tilt bed rest compared to the weightlessness environment on the International Space Station.** (Used with permission from author and journal)

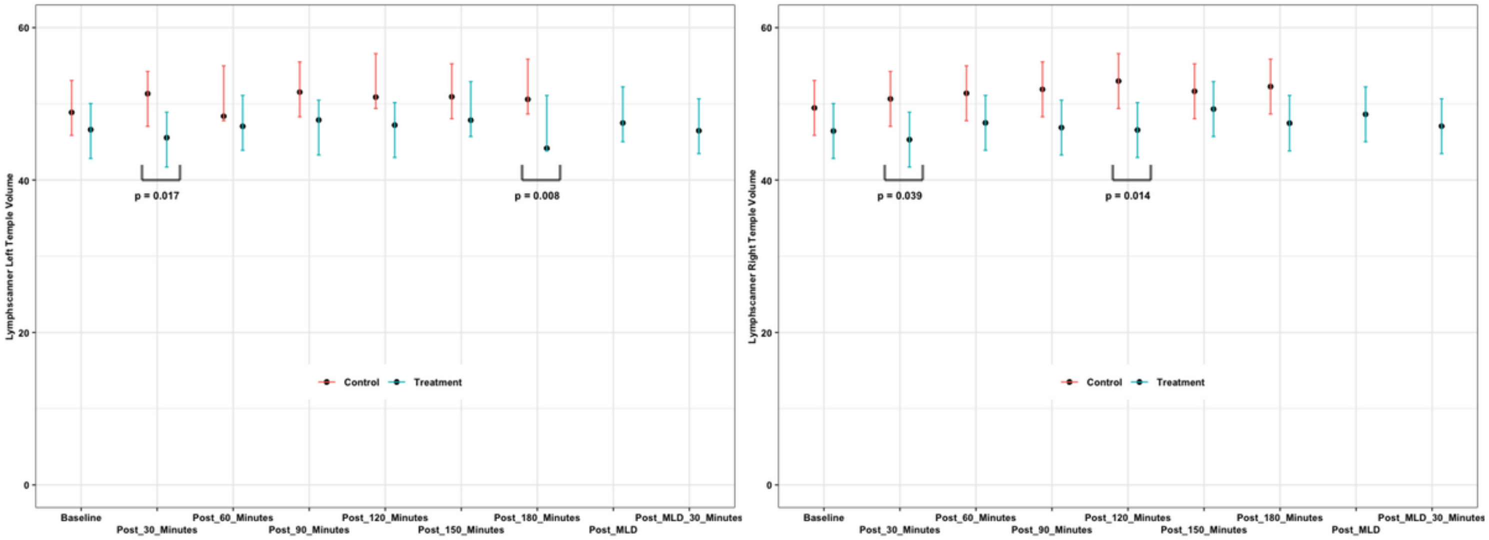


Figure 2

**Between Group Comparisons.** A significant difference in *left temple volume* between the treatment and control groups was found at post-30-minutes (5.77, 95% CI: 1.24, 10.30) and post-180-minutes (6.42, 95% CI: 1.71, 11.13). A significant difference in *right temple volume* between the treatment and control groups was found at post-30-minutes (5.35, 95% CI: 0.25, 10.44) and post-120-minutes (6.42, 95% CI: 1.32, 11.51).

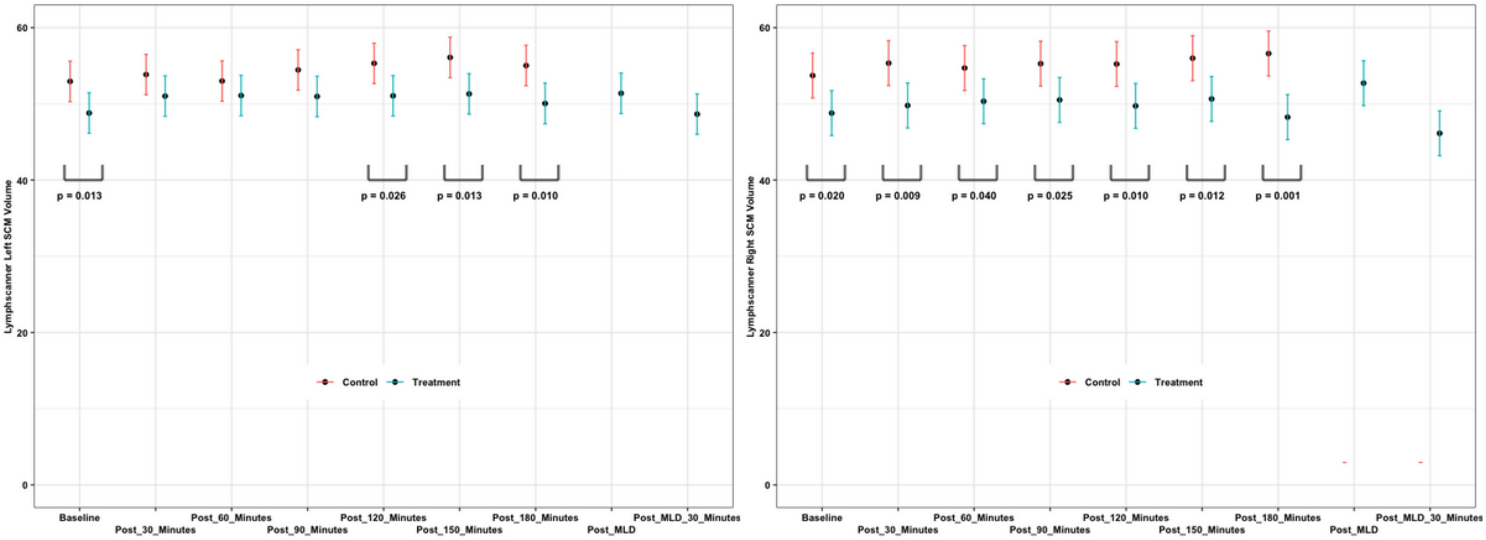
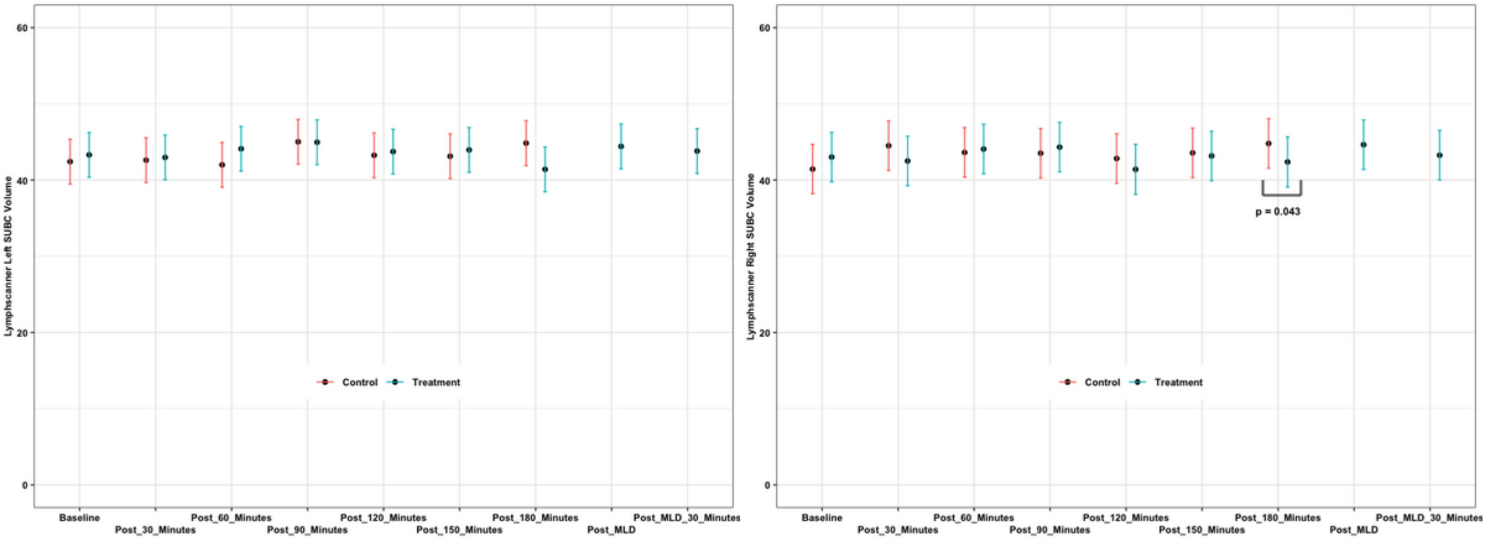


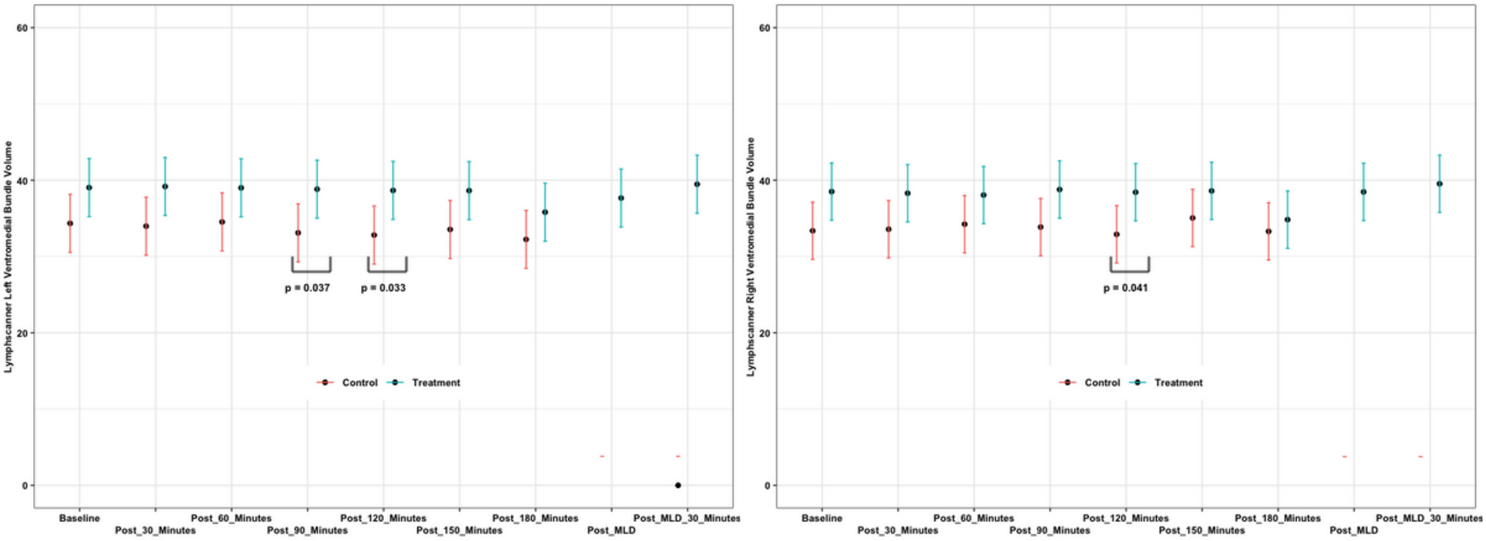
**Figure 3**

**Between Group Comparisons.** A significant difference in *left SCM* volume between the treatment and control groups was found at baseline (4.15, 95% CI: 0.40, 7.90), post-120-Minutes (4.26, 95% CI: 0.51, 8.01), post-150-Minutes (4.78, 95% CI: 1.03, 8.53), and post-180-Minutes (4.98, 95% CI: 1.21, 8.74). A significant difference in *right SCM* volume between the treatment and control groups was found at baseline (4.93, 95% CI: 0.76, 9.10), post-30-Minutes (5.56, 95% CI: 1.39, 9.73), post-60-Minutes (4.37, 95% CI: 0.20, 8.53), post-90-Minutes (4.75, 95% CI: 0.58, 8.92), post-120-Minutes (5.49, 95% CI: 1.32, 9.66), post-150-Minutes (5.35, 95% CI: 1.18, 9.51), and post-180-Minutes (8.35, 95% CI: 4.18, 12.51).



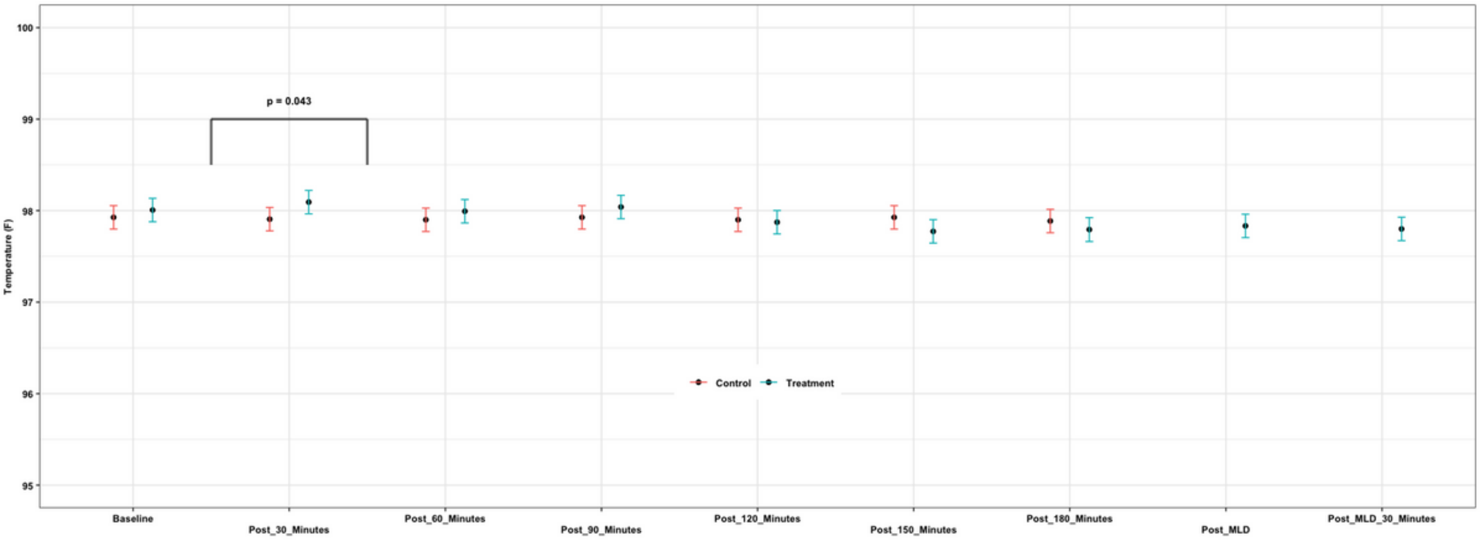
### Figure 4

**Between Group Comparisons.** A significant difference in right *subclavicular* volume between the treatment and control groups was found at post-180-Minutes (5.09, 95% CI: 0.16, 10.03).



### Figure 5

**Between Group Comparisons.** A significant difference in right *left ventromedial bundle* between the treatment and control groups was found at post-90-Minutes (5.72, 95% CI: 0.34, 11.10) and post-90-Minutes (5.85, 95% CI: 0.47, 11.23). A significant difference in right*ventromedial bundle* between the treatment and control groups was found at post-90-Minutes (5.53, 95% CI: 0.21, 10.84).



### Figure 6

**Between Group Comparisons.** A significant difference in body temperature between the treatment and control groups was found at post-30 minutes (-0.18, 95% CI: -0.36, -0.01).

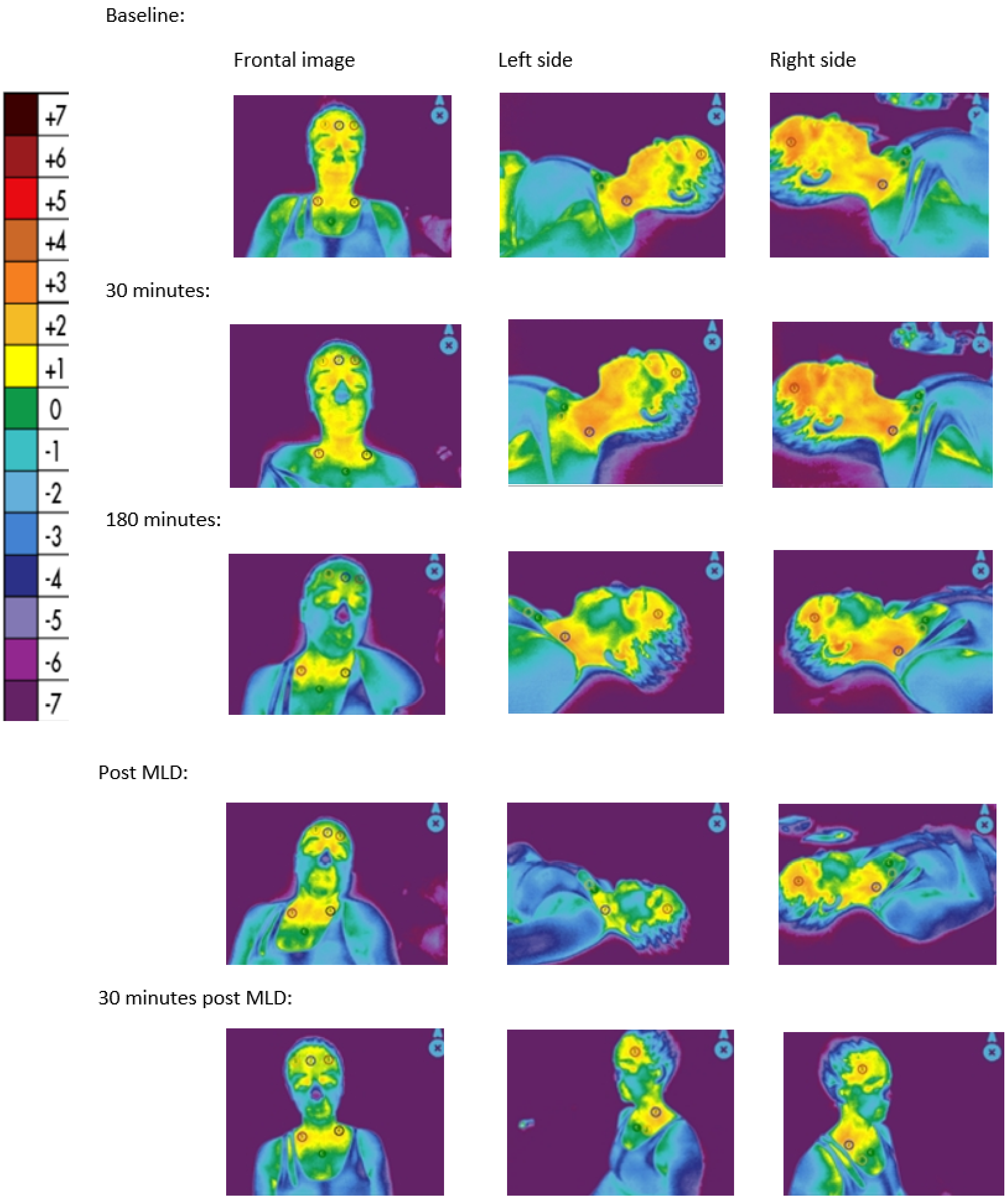


**Figure 7**

**HDT Long Wave Infrared Thermography (LWIT) Images.** Reference scale measuring thermal energy in degrees Celsius.

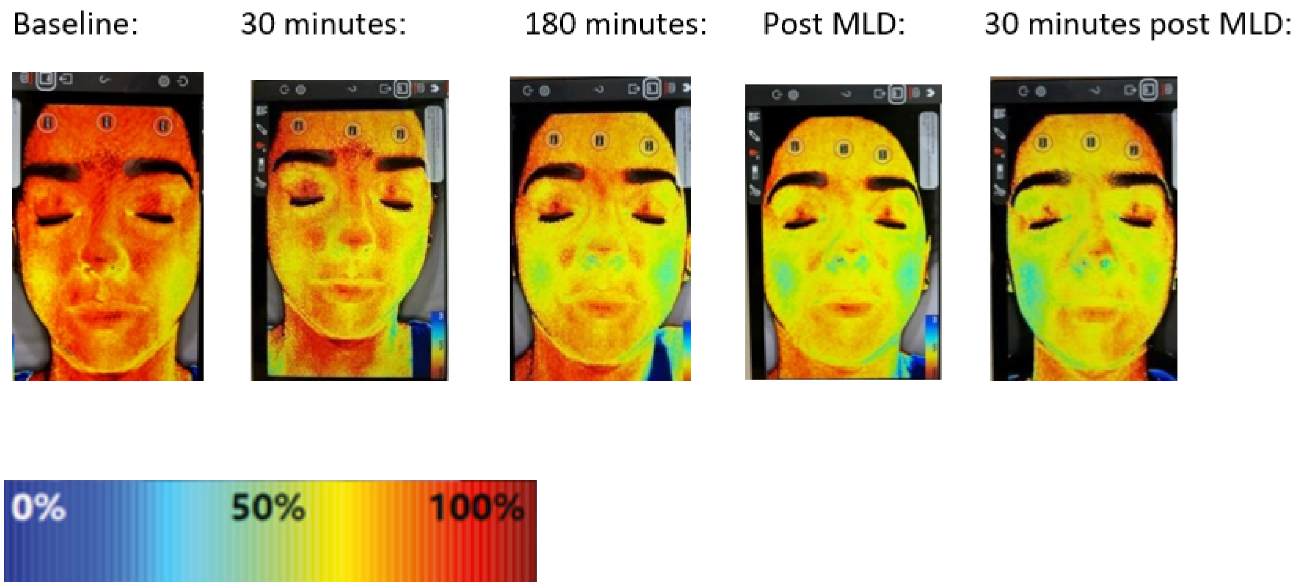


**Figure 8**

**Near Infrared Spectroscopy Images (NIRS).** Reference scale indicates tissue oxygenation saturation in percentages.