

**University Hospitals Cleveland Medical Center
Department of Dermatology
Research Protocol**

Protocol Title: Sleep, Circadian Rhythm and Skin Health

Principal Investigator: Kevin D. Cooper, M.D.

1. BACKGROUND/RATIONALE

Sleep is controlled by the circadian clock and is essential for growth and repair of the body's many biological and physiological systems. There is a central circadian clock in the anterior hypothalamus that coordinates the body with the Earth's 24 hour day-night cycle (1). Signals from the central clock are relayed to peripheral clocks which are in almost every cell and tissue in the body (2, 3) including the skin (4,5).

Inadequate sleep is a public health epidemic according to the United States Center for Disease Control (6). Inadequate sleep has been associated with motor vehicle crashes (7), medical errors and occupational mistakes (8). Modern day lifestyles, occupations and habits encourage people to be on irregular schedules that disrupt the sleep-wake cycle. Hence, many American adults report chronic inadequate sleep. It is well established that inadequate sleep is associated with health issues such as skin health and disease are not well established. This represents a knowledge gap in the field of dermatology. Bridging this gap may provide better ways of preventing and managing skin disorders in the future.

It is known that skin has variations in temperature, pH, barrier function and transepidermal water loss that are time-dependent (12). And, we know that skin has its own peripheral clock and skin cells have been shown to express clock proteins (13). Thus, it seems probable that sleep quality (or lack thereof) would affect skin function and integrity. Our pilot study of 60 normal female volunteers did show that poor sleep quality is associated with increased signs of intrinsic aging, poor skin barrier repair, and poor perception of appearance (14). However, the mechanisms behind these associations are not yet well understood. It is speculated that increased oxidative stress secondary to inadequate sleep may be the culprit (15, 16). More research is needed to better understand the role of oxidative stress and clock proteins in inadequate sleep induced skin dysfunction.

Also, little is known about the effects of chronic inadequate sleep on psoriasis and other inflammatory skin disorders. Acute sleep deprivation was found to worsen psoriasis inflammation in animals (17) but there is a lack of research in human subjects. Given that our pilot study (mentioned above) showed an association between poor sleep quality and skin function in normal volunteers, it is only fitting that we should expand the study population to look at not only normal volunteers but also those with inflammatory skin disease.

This study will be a cross-sectional study on normal volunteers and volunteers with inflammatory skin diseases to explore the mechanisms behind how sleep quality may affect skin aging, skin integrity and skin disease.

2. STUDY OBJECTIVES/HYPOTHESIS

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2.1. SCIENTIFIC AIM/PURPOSE The purpose of this study is to explore the mechanisms behind how sleep quality may affect skin aging, skin integrity and skin disease.

2.2. HYPOTHESIS We hypothesize that sleep disturbance or disruption of circadian rhythm results in disruption of normal skin physiology that could be manifested by enhanced inflammation, ineffective barrier function, etc.

2.3. SPECIFIC AIMS & OUTCOMES There is a knowledge gap in the field of dermatology regarding the interaction between sleep and skin function. Hence, we are looking at several parameters as we have no basis to focus on just one or two outcomes. The primary and secondary outcomes for this study will include:

2.3.1. Primary:

2.3.1.1. Analysis of Sleep Pattern via Actigraphy, polysomnography & questionnaires

2.3.1.2. Evaluation of skin (both in terms of skin aging and skin disease)

2.3.2. Secondary:

2.3.2.1. Sample analysis (from skin, blood, urine, saliva and hair follicle samples)

2.3.2.2. Skin barrier recovery (via TEWL measurement)

2.3.2.3. MED Testing & Recovery from UV-induced erythema

3. STUDY DESIGN

Subject Recruitment

One hundred adults who are either healthy, normal volunteers or have skin pathology including but not limited to eczema, psoriasis, acne, and other inflammatory dermatoses will be recruited through IRB-approved advertising. In a previous sleep study with 60 volunteers, we found significant results in the parameters of transepidermal water loss and intrinsic skin aging signs. The n=60 in that study only underwent a standard sleep questionnaire (the PSQI) for classification of their sleep quality. In this current study, we aim to characterize sleep quality more sensitively through actigraphy and polysomnography so an n=100 should be sufficient.

Informed consent process and eligibility screening will be performed.

3.1. Study Population

Inclusion Criteria:

- Males or females ages 18 years and above
- Capable of giving informed consent

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- Adults who are either healthy, normal volunteers or those with a skin disease including but not limited to eczema, psoriasis, acne or other inflammatory dermatoses
- Fitzpatrick Skin Type I-VI
- In good general health
- Willing to cooperate with study instructions

Exclusion Criteria

- Volunteers younger than 18 years
- Any serious medical or physical condition that would cause the subject significant discomfort with study procedures
- Women who are pregnant, nursing, or who may become pregnant in the next 3 months

3.2. Size and Source of Population

100 subjects will be recruited from Northeast Ohio via IRB-approved advertisements placed in local newspapers and/or TV, through hospitals and university bulletins and posters, and by referral from other participants. Subjects will also be recruited from the Skin Study Center Database.

3.3. Study Procedures

As mentioned above, there is a knowledge gap in the field of dermatology regarding the interaction between sleep and skin function. Hence, we are looking at several parameters and will utilize a series of procedures related to sleep quality, skin integrity & recovery, skin aging and skin disease. Some of the procedures (such as TEWL, SCINEXA scoring, photography etc) were used in our pilot study. Other procedures (such as body fluid and tissue sample collection) were added to help explain the mechanisms behind the sleep quality and skin function association. More sensitive sleep-related procedures such as actigraphy and polysomnography will be used to better assess quality of sleep and identify any underlying sleep disorders (as opposed to the PSQI questionnaire used in our pilot study). In addition, questionnaires related to specific skin diseases (such as the PASI and sPGA) were added to assess the severity of disease for those volunteers with psoriasis. Not all procedures may be required of all subjects. Investigators will explain and discuss which specific steps each subject will undergo. For instance, only subjects with psoriasis will undergo PASI scoring. Also, given that actigraphy is a simple, non-invasive method to assess rest and activity cycles, it will be the primary method for initial sleep quality assessment. If abnormalities are seen on actigraphy, subjects may then be asked to undergo a portable or standard sleep study. In patients who have a known, diagnosed sleep disorder, actigraphy may be bypassed going straight to a portable or standard sleep study and/or if a recent sleep study has been performed, another study may not be necessary. As more data is obtained, some assays may be favored over others. For example, since so little is known about this topic, we may discover that oxidative stress and/or clock proteins can be better assessed in one biospecimen versus another (e.g. hair follicle sampling versus urine sampling). Or, certain volunteer groups (e.g. those with skin disorders versus normal volunteers) may have one biospecimen that is of interest as opposed to others. Also, given that our pilot study only looked at normal females, gender differences may also play a role in study procedure choice. Prior to undergoing study procedures, volunteers may be asked information

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about their medical and dermatological history and may be asked to undergo a physical/dermatological exam including vital signs.

3.3.1. MED Testing/UV Irradiation (Single or repeated exposures):

3.3.1.1. **MED Testing:** The MED testing procedure will require 2 visits to the Skin Study Center. It involves exposing eight 1 square cm areas of skin over increasing doses of SSR, while the rest of the body is draped. Doses for MED testing will be determined based on the individual's Fitzpatrick Skin Type. SSR is delivered by a 1,000-Watt xenon arc lamp, which emits ultraviolet wavelengths from 290-400nm, closely resembling natural sunlight. Approximately 24 hours later the researcher will examine these sites to determine the lowest exposure time [Minimal Erythema Dose (MED)] that causes sunburn to occur.

3.3.1.2. **UV Irradiation:** Following the MED Testing, volunteers may receive a predetermined amount of ultraviolet light based upon their skin type and/or the sun protection factor (SPF) of the "Sunscreen" product being tested. The UV dose will be 1-3 times the MED which will cause either no sunburn, mild sunburn, or moderate sunburn, which means that the skin may turn red but typically without any blister. However, there is a small risk that this procedure can cause blistering of the skin. To help avoid blistering, doses above 3MED will not be used. There will be at least two visits with this procedure. At the first visit, the subject will receive a pre-determined amount of UV light. The subject will be asked to return at least once to have the area examined.

3.3.2. Sleep monitoring

3.3.2.1. **Actigraphy:** The participant may be asked to wear a wrist actigraph to measure movement. This watch would be worn for 24 hours per day for 7-14 days. This procedure will involve two visits. At the first visit, the subject will receive the actiwatch. At the second visit, the subject will return the actiwatch.

3.3.2.2. **Portable Sleep Device:** A portable polysomnogram can be used at home to capture data regarding sleep patterns. It consists of an apparatus worn over the face like a small mask. It is worn overnight to monitor sleep. A study investigator will instruct the subject as to how to correctly use this device at home. This procedure will be conducted at home but the subject will be asked to come to the Skin Study Center to pick up and drop off the device.

3.3.2.3. **Standard Sleep Study:** An overnight, one visit stay in the hospital or sleep center or Dahms Clinical Research Unit may be required for a standard sleep study/polysomnography. Equipment is placed on the subject's digits, scalp, face and other areas of the skin to detect signals relevant to sleep patterns. The data will be downloaded after the study and analyzed using specific software/programs. *(Note: if abnormalities are found during the portable or standard sleep test, the subject will be referred for further workup).*

3.3.3. **Questionnaires:** The questionnaires will require at least one visit to either the Dermatology outpatient clinics or the Skin Study Center. Most questionnaires will be

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electronic and can be completed at home by the study participant. If any questionnaire is on paper and completed at home, the subject may be asked to return the questionnaire at a second visit.

3.3.3.1. Quality of life: We will utilize one of the most common quality of life questionnaires used in Dermatology studies (DLQI) or a similar tool. Formal permission has been granted to use the Dermatology Life Quality Index (DLQI).

3.3.3.2. Nutrition Questionnaire: The participant will be asked to complete a survey about their nutritional habits.

3.3.3.3. Sleep related questionnaires: The participant may be asked to complete a series of questionnaires regarding their medical conditions, medications, sleep patterns and/or daytime functioning. These could be in the form of the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), or similar materials.

3.3.4. Photography/Imaging: The participant may be asked to have a photographic record or imaging made of their skin. Photographic records and imaging provide objective data on skin lesions of persons with skin disease and potential changes that may occur over time in the disease. Photography and imaging gives us a tool to compare clinical appearance with laboratory and research findings. Clinical images will be stored as digital images electronically. Depending upon the extent of skin lesions, the subject may be asked to participate in whole body or specific lesion photography or imaging. Subject identity will be masked should facial photographs be necessary however; in most cases only body shots or lesion close-ups are recorded. Should the subject wish to decline, they may still participate in the study, without photographs or images. Photographs will be taken throughout the study at the discretion of the investigators.

3.3.5. Transepidermal Water Loss (TEWL): TEWL is an indicator of the water vapor pressure gradient between the boundary layer of the surface of the skin and the ambient air. It shows the skin's ability to prevent water loss from interior layers. Increased TEWL indicates decreased barrier function. Decreasing TEWL after disruption reflects increasing barrier recovery. TEWL is a sensitive marker in assessing irritant effect on barrier function. The TEWL procedure will be performed using evaporimetry, according to published guidelines, and the mean will be calculated (18). The subject will rest for 10 minutes in a room free of excessive draughts and at a stable temperature. The TEWL will be calculated from three readings (g/m²h) taken 30 seconds after application of the probe to designated skin areas. This procedure will involve at least one visit to the Skin Study Center.

3.3.6. Clinical evaluation of skin and skin disease including standard scoring systems when applicable (e.g. PASI, Scinexa, etc) Skin assessments may be performed only once during the study or at specific time points depending on what other procedures are being performed. A study investigator will inform the subjects as to how often they will be receiving skin evaluations and which skin assessments they will receive.

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- 3.3.6.1. **PASI - Psoriasis volunteers ONLY:** The PASI is a measure of psoriatic disease severity taking into account qualitative lesion characteristics (erythema, thickness, and scaling) and degree of skin surface area involvement in defined anatomical areas. The PASI is a validated instrument that has become standard in clinical trials for psoriasis. Psoriasis Area Severity Index scores range from 0 to 72, with higher scores reflecting greater disease severity. Erythema, thickness, and scaling are scored on a scale of 0 (none) to 4 (very severe) on 4 anatomic regions of the body: head, trunk, upper limbs, and lower limbs. Degree of involvement on each of the 4 anatomic regions is scored on a scale of 0 (no involvement) to 6 (90-100% involvement). The total qualitative score (sum of erythema, thickness, and scaling scores) is multiplied by the degree of involvement for each anatomic region and then multiplied by a constant. These values for each anatomic region are summed to yield the PASI score.
- 3.3.6.2. **Physician Global Assessment (PGA) – Psoriasis volunteers ONLY:** This psoriasis assessment is a scale from 0 (clear) to 5 (severe). It is a global assessment of the subject's psoriasis based on the severity of induration, scaling and erythema.
- 3.3.6.3. **Score for Intrinsic and Extrinsic Skin Aging (SCINEXA):** The *SCINEXA* is a validated tool used in dermatology for assessing skin aging by taking into account the intrinsic or chronological and extrinsic factors that contribute to skin aging (19). A trained study staff will perform a focused clinical examination to score subjects on the 5 intrinsic and 18 skin aging symptoms as outlined in published studies (19).
- 3.3.7. **Punch Biopsy:** Primarily used for the purpose of immunohistochemical detection and/or preparation of RNA samples to be used in quantitative PCR experiments to determine messenger RNA levels of skin specific cytokines, chemokine, growth factors or matrix components. This procedure involves the removal of a small circle of skin (pencil eraser-sized) using a cookie cutter-like instrument. Lidocaine is used to numb the area to be removed before obtaining the biopsy and then 1-6 stitches are used per biopsy site to prevent bleeding, speed healing and improve the appearance. This procedure may last up to an hour depending on the number of biopsy sites. The stitches are then removed 7-14 days later. This procedure will involve at least two visits. One visit will be when the biopsy is performed. There will also be another visit required to remove the sutures from each biopsy. A study investigator will explain to the participant how many biopsies will be performed throughout the study and at which time points. If multiple biopsies are being performed throughout the study and at different time points (e.g. different days), this procedure may require more visits. The subject will be compensated for the total amount of biopsies performed throughout the study. Biopsies are optional. If the subjects agreed to have the MED testing done, then they were also asked to consent to biopsies. The biopsies were done on the buttocks after UV light exposure. The biopsies were up to 8mm and there could be up to four biopsies performed. The subjects would be burned and then biopsies taken one from each cheek in the morning and then again in the late afternoon. For a total of four for this procedure. Similarly, there could also be up to four

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biopsies performed before and after sunscreen product application with UV exposure. The total amount of possible biopsies for the study could be eight (8).

- 3.3.8. **Blood Samples:** Blood samples (4-30 tablespoons) will be taken by venipuncture to provide samples for analysis of peripheral blood. Blood may be taken from normal participants or participants with a skin disease. If the participant is asked to donate only one blood sample, this procedure will require only one visit. If the participant is asked to donate more than one blood sample, this procedure may require multiple visits. A study investigator will explain to the participant how much blood will be drawn throughout the study. The participant will be compensated at the end of the study for the total amount of blood drawn.
- 3.3.9. **Salivary assay for melatonin and other markers:** Saliva samples are provided for the purpose of measuring melatonin, cortisol and other markers. Volunteers may be asked to donate saliva either by spitting into a sterilized tube or rinsing with a salt water solution for at least one minute and then spitting the contents into a tube. If the subject is asked to donate only one sample, this procedure will require only one visit. If the subject is asked to donate more than one sample, this procedure may require multiple visits. A study investigator will explain to the participant how much and how many times saliva will be collected throughout the study. As an alternative to multiple visits, the study investigator may show the participant how to perform the procedure so the participant can collect the samples at home at the time points specified by the study investigator. If the samples are collected by a participant at home, the participant will be asked to bring the samples to the Skin Study Center after collection.
- 3.3.10. **Urine collection for biomarkers:** Urine samples will be collected and analyzed for cortisol and biomarkers. If the subject is asked to donate only one sample, this procedure will require only one visit. If the subject is asked to donate more than one sample, this procedure may require multiple visits. A study investigator will explain to the participant how much and how many times urine will be collected throughout the study.
- 3.3.11. **Hair follicle sampling for clock genes and proteins:** Hairs will be plucked for analysis of clock genes and proteins. If the subject is asked to donate only one sample, this procedure will require only one visit. If the subject is asked to donate more than one sample, this procedure may require multiple visits. A study investigator will explain to the participant how much and how many times hair follicle samples will be collected throughout the study. As an alternative to multiple visits, the study investigator may show the participant how to perform the procedure so the participant can collect the samples at home at the time points specified by the study investigator. If the samples are collected by a participant at home, the participant will be asked to bring the samples to the Skin Study Center after collection.

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3.3.12. **Product Application:** The topical products used in this study are commercially available “suncscreens” . The ingredients in the product will be reviewed with the patient prior to use. The Suncreen product may be applied before another procedure in this protocol (such as ultraviolet light) to study the effects the product has on the skin and how they relate to skin diseases and sleep. This procedure will require at least one visit which will involve the initial product application. The participant may be asked to return at time points specified by a study investigator to evaluate the area to which the product was applied.

4. RISKS AND DISCOMFORTS AND HOW MINIMIZED

4.1 **MED Testing /UV Irradiation:** The risks associated with the sunburn component of this study are minimal. The consequences of the sunburn are short-term, and primarily involve mild tenderness at the exposure site followed by minor itching and dryness of the skin several days later. The degree of the sunburn is typically mild to moderate but, less commonly, can cause blistering of the skin. Prior to the administration of the primary dose of ultraviolet light, all study volunteers will be asked questions to determine their skin type (Fitzpatrick Skin Type) and undergo MED testing to determine how much UV light their skin can tolerate. This will decrease the risk that the participants will develop severe blistering sunburn.

4.2 **Actigraphy:** Some participants may find the watch mildly uncomfortable to wear.

4.3 **Portable Sleep Device:** Some participants may find the device to be slightly inconvenient to use.

4.4 **Standard Sleep Study:** There is minimal risk with this procedure. There is a risk of skin sensitivity to the tape which may be used to secure some of the equipment to the skin.

4.5 **Questionnaires:** There are no known risks to filling out questionnaires. However, in rare instances, some participants may feel uncomfortable with answering or applying responses to some questions.

4.6 **Photography/Imaging:** There are no known risks associated with digital photography or imaging. In the event that a photograph or image is made of the face or any other identifiable feature, the subject’s identity may become known.

4.7 **Transepidermal Water Loss (TEWL):** There are no known risks from measuring water loss.

4.8 **Skin Evaluations:** There are no known risks from a skin evaluation.

4.9 **Punch Biopsy:** Punch biopsies pose minimal risk, and volunteers are instructed as to the proper care of the biopsy site. Lidocaine may cause a tingling or burning sensation when injected into the skin. Potential problems localized to the biopsy site include bleeding or oozing, discomfort, or infection. The biopsy site will heal with a small, flat scar, which may

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be red for several months. The scar may also turn lighter or darker than the surrounding skin after some time.

- 4.10 Blood Sample:** The risks of simple blood draw may commonly include the occurrence of discomfort and/or bruise at the site of puncture and, less commonly, the formation of a small clot, swelling of the vein, or bleeding from the puncture site. There is also a small risk of infection.
- 4.11 **Saliva Sample:** There are no known risks from donating saliva into a sterilized tube or rinsing with a salt water solution.
- 4.12 **Urine Sample:** There are no known risks from providing a urine sample.
- 4.13 **Hair Follicle Sample:** Some participants may have slight discomfort when the hairs are being plucked.
- 4.14 **Product Application:** There are no known side effects other than the remote chance of the development of an allergic reaction.

5 COMPENSATION FOR INJURIES

Every effort to avoid side-effects of participating in this research study will be made, but there can be no guarantee that study subjects will not experience some physical damage. As there is no automatic compensation available for physical injury for a research study, study subjects will not be automatically reimbursed for the costs of time lost from work or the costs of extra hospital days, doctor's fees, and medicine.

6 BENEFITS TO SUBJECT

There are no direct benefits to the study subject for participating in this research. There is, however, a clear benefit to society by improving our understanding of the role of sleep in skin disease.

7 COSTS TO THE SUBJECT

Study volunteers will be responsible for all costs associated with transportation to and from their research study appointments.

8 ALTERNATIVES TO PARTICIPATION

The only alternative for potential subjects is to not participate.

9 WITHDRAWAL FROM STUDY PARTICIPATION

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The participants are entitled to withdraw from the study at any time and for whatever reason without this having an effect on their access to treatment via the investigator.

10 PAYMENT TO THE SUBJECTS (REIMBURSEMENT AND INCENTIVES)

Compensation will be determined by the procedures that are done, listed in the table below. It is explained to each volunteer what procedures will be done and the amount of compensation prior to signing the consent form. Payment is made after procedure is complete, usually at the end of a study visit. If subject declines ½ way through, they will be paid for procedures they have completed. In the case of actigraphy and journals, subjects are paid after they return the equipment and journals.

Patient Compensation:

| Procedure | Size | Compensation |
|-----------------------------|-------------|---------------------|
| MED Testing | N/A | \$20.00 |
| UV Light Exposure | N/A | \$20.00 |
| Saliva Sample | N/A | \$10.00 |
| Photography | N/A | \$10.00 |
| Punch Biopsy (each) | N/A | \$50.00 |
| Blood Draw | Per 60 ml | \$20.00 |
| TEWL Measurement | N/A | \$10.00 |
| Actigraphy | N/A | \$50.00 |
| Portable Sleep Study Device | N/A | \$30.00 |
| Standard Sleep Study | N/A | \$150.00 |
| Questionnaires | N/A | \$10.00 |
| Skin Evaluation | N/A | \$10.00 |
| Urine Sample | N/A | \$10.00 |
| Hair Follicle Sample | N/A | \$10.00 |
| Product Application | N/A | \$10.00 |

Study volunteers will be responsible for all costs associated with transportation to and from their research study appointments. Subjects parking at University Hospitals Cleveland Medical Center for a Skin Study Center procedure will receive a parking voucher.

11 PLAN FOR OBTAINING INFORMED CONSENT (INFORMED CONSENT PROCESS)

Potential subjects recruited for study participation will be invited to the Department of Dermatology Clinical Research Unit for the formal consent process that will take in a private exam room. Each participant will have the study explained in its entirety with special attention to the length of the study, the actual study procedures, financial obligations, risks, benefits and

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alternatives to participation. Participants will be given ample time to consider study participation and encouraged to discuss with significant others, have any questions answered by the study staff. Only participants willing to undergo all study procedures will be asked to affirm participation by signing the consent form. Only study staff that have been trained on the study and having IRB required Human Subject Protection Training and authorized by the investigator will obtain consent. Subjects will be reminded the importance of adhering to all study requirements and their right to withdrawal at any time without affecting the quality of their individual medical care.

12 PROVISIONS FOR SUBJECTS FROM VULNERABLE POPULATIONS

No special subject populations will be used.

Illiterate participants will be eligible to participate in this study. To enroll these subjects, the approved consent will be read aloud to them in the presence of a witness, and the subjects will be asked to acknowledge their understanding by making a personal mark on the signature space of the consent form. Both the person obtaining the consent (either the PI or his/her official delegate) and the witness will co-sign the consent form.

Non-English speaking study subjects presenting themselves as potential study participants and having met all inclusion/exclusion criteria will be considered for the study. Consent documents will be translated to the native language if such written translation assistance is available. When the translated documents become available, they will first be submitted to the IRB office for approval. Study staff at the Skin Study Center will locate a reliable translator to be present as witness to the informed consent process as well as all study visits. Both participant and translator will sign and date the informed consent documents.

House staff and students, medical students on a clerkship, and employees of Case Western Reserve University (CWRU) or UHCMC are not excluded from the study, but their participation will be entirely voluntary. Their scholastic or employment evaluations will not be affected by participation in this study.

13 SUBJECT PRIVACY AND DATA CONFIDENTIALITY

Study participant information collected in the research process may include: contact information, consent documentation, demographics, social security number (necessary for reimbursement), and personal medical history. All data will be stored on the secure UH or REDCap[®] server, or as paper records stored in a locked cabinet. Data collected in this study will include: Patient records collected electronically and on paper, consent forms, and samples. Each class of data will be handled in the following manner:

13.1 Patient Records and Consent Forms: Patients enrolled into the study will complete informed consent and provide medical history. All patients upon entering the study will be assigned a unique identifier by REDCap[®] that will be used throughout the study to de-

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identify their information. Consent forms and paper records will be filed in study binders, stored in a locked room within the Department of Dermatology. Only authorized research personnel will have access to these records.

Patient records will be created and stored electronically on both the secure and encrypted University Hospitals server and the secure REDCap[®] server. REDCap[®] has been adopted and adapted specifically for our use on the CTSC under licensing terms with Vanderbilt University and has been approved for Vanderbilt, in collaboration with a consortium of leading research institutional partners who were funded by both Vanderbilt and the NCCR, has developed a software toolset and workflow methodology for electronic collection and management of research and clinical trial data. REDCap[®] data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the CTSC Informatics Core resources. The iterative development and testing process results in a well-planned data collection strategy for individual studies. Whether a study is submitted for scientific review on one of the three CTSC Clinical Research Units, each with a full-time dedicated informatics resource available to support investigators with REDCap[®], or an institutional request outside of the controlled environment of the CWRU, our CTSC has made the REDCap[®] tool and associated resources available for use.

The REDCap[®] survey, a powerful tool for building and managing online surveys as the research team can create and design surveys in a web browser and engage potential respondents using a variety of notification methods. Both REDCap and REDCap Survey systems provide secure, web-based applications that are flexible enough to be used for a variety of types of research, provide an intuitive interface for users to enter data and have real time validation rules (with automated data type and range checks) at the time of entry. These systems offer easy data manipulation with audit trails and reporting for reporting, monitoring and querying patient records, and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R), and are managed in-house by dedicated resources, including for backup/recovery to prevent data loss due to equipment failure.

The PI will ensure protocol adherence, proper protocol conduct, data quality, and subject safety and efficacy by conducting regular meetings with the entire study staff. The adverse events submitted by subject phone inquiry or email will be reviewed and adjudicated with all research team members. In addition, any adverse events or deviations to the protocol or other issues will be discussed at the Regulatory Meeting held monthly in the department of Dermatology.

- 13.2 Blood Samples:** Study personnel will acquire the specimen from each patient via needlestick. Blood specimens will be de-identified before being transported to the lab for analysis. For those samples requiring the University Hospitals Laboratory for processing, the samples will be de-identified upon data entry and analysis.

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- 13.3 Tissue Samples:** Tissue samples will be de-identified before being transported to the lab for analysis.
- 13.4 Saliva Samples:** Saliva samples will be de-identified before being transported to the lab for analysis. For those samples requiring the University Hospitals Laboratory for processing, the samples will be de-identified upon data entry and analysis.
- 13.5 Urine Samples:** Urine samples being processed for cortisol levels or other biomarkers will be de-identified prior to being sent to the lab for processing. For those samples requiring the University Hospitals Laboratory for processing, the urine samples will be de-identified upon data entry and analysis.
- 13.6 Hair follicle sampling:** Hair follicles samples will be de-identified before being transported to the lab.
- 13.7 Photographs:** All photographs will be de-identified and stored on the secure UH server, only accessible to authorized study personnel. In the event a photograph is taken of the face or any other identifiable feature, the patient's identity may become known.
- 13.8 Data Transfer/Transmission:** Research data will be sent to Pingfu Fu, PhD, Biostatistical Core, CWRU for statistical analysis after finishing this research study: however, all of the data will be stripped of patient identifiers prior to transfer.

In order to comply with federal regulations, records identifying participants in this study may be reviewed by authorized study personnel, authorized representatives of the Institutional Review Board, or other federal regulatory officials responsible for oversight of human subject protection. Any data linking participants to their sample will be de-identified. A code will be used to link participants to identifying information and only the investigators and the research study coordinators will have access to this information, which will be stored on a server at University Hospitals Cleveland Medical Center with a separate encryption code. Upon enrollment, each patient record will be stripped of all identifying information and assigned a unique numerical identifier that has no reference to any identifiable patient information.

14. DATA ANALYSIS PLAN

As previously mentioned, this study will look at several parameters and will utilize a series of procedures related to sleep quality, skin integrity & recovery, skin aging and skin disease to help explain the mechanisms behind how sleep quality may affect skin.

- 14.1 Sample Size Rationale:** In a pilot study with 60 volunteers, we found significant results in the parameters of transepidermal water loss and intrinsic skin aging scores. The n=60 in that study only underwent a standard sleep questionnaire (the PSQI) for classification of their sleep

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quality. In this current study, we aim to characterize sleep quality more sensitively through actigraphy and polysomnography so an n=100 should be sufficient.

14.2 MED Testing/UV Irradiation Exposure: The MED will be calculated via Linear Regression analysis. The MED may be used to give volunteers a predetermined amount of UV exposure. The MED may also be compared at different time points throughout the day or to determine recovery from UV-induced erythema. The MED Testing will fulfill one of the secondary outcomes. The MED testing and/or UV exposure results may be compared to other procedures and to the sleep data obtained.

14.3 Sleep Monitoring: Actigraphy and polysomnography are more sensitive procedures to assess quality of sleep than the PSQI questionnaire used in our pilot study. Actigraphy and/or polysomnography may be used to capture data regarding subject's sleep pattern. The data will be downloaded after the study and analyzed using specific software. This will fulfill one of our primary outcomes. The sleep data may be compared to findings from other procedures.

14.4 Questionnaires: Data obtained from the questionnaires will be analyzed via statistical and other methods. Data may also be compared to other procedures undergone by the participants such as clinical evaluations of the skin, MED results, etc. The sleep-related questionnaires will fulfill the primary outcome of "Analysis of sleep pattern".

14.5 Photography/Imaging: Photography and imaging may be used to compare clinical appearance with laboratory and research findings. This will help fulfill the primary outcome of "Evaluation of skin".

14.6 TEWL Measurement: TEWL measurement may be used to assess skin barrier function. This will fulfill one of the secondary outcomes. Skin barrier recovery results may be compared to other procedures and/or to the sleep pattern data.

14.7 Clinical Evaluation of Skin: The various items of the SCINEXA may be analyzed and compared to other data obtained in the study. The PASI and PGA scores may also be analyzed for those subjects with psoriasis. This will fulfill one of the primary outcomes. This data may be compared to data obtained from sleep pattern analysis and other procedures.

14.8 Punch Biopsy: Punch biopsy tissue sample will primarily be used for the purpose of immunohistochemical detection and/or preparation of RNA samples to be used in quantitative PCR experiments to determine messenger RNA levels of skin specific cytokines, chemokine, growth factors or matrix components. The data obtained from the punch biopsies will contribute to the secondary outcome of "Sample analysis". This data may be compared with data from the sleep analysis or other procedures.

14.9 Blood Samples: Blood samples will provide samples of peripheral blood to be analyzed. The data obtained from the blood samples will contribute to the secondary outcome

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of “Sample analysis”. This data may be compared with data from the sleep analysis or other procedures.

14.10 Urine Samples: Urine samples will be analyzed for biomarkers and levels of cortisol. The data obtained from the urine samples will contribute to the secondary outcome of “Sample analysis”. This data may be compared with data from the sleep analysis or other procedures.

14.11 Hair Follicle Samples: Hair follicles will be analyzed for clock genes and proteins. The data obtained from the urine samples will contribute to the secondary outcome of “Sample analysis”. This data may be compared with data from the sleep analysis or other procedures.

14.12 Statistical Analyses: Statistical analyses will be completed under the supervision of Dr. Pingfu Fu, PhD, Biostatistical Core, CWRU.

14. DATA AND SAFETY MONITORING PLAN

15.1 Procedures for analysis and interpretation of data: The PI has meetings with the staff performing both the clinical and laboratory based assessments. These meetings occur on a minimum of a monthly basis, but often occur more frequently. These meetings are held for two primary functions: The meetings are, first, to discuss primary data and progress of the study. Data include both clinical observation and laboratory measurements. The secondary function of these meetings is to monitor clinical outcomes for potential adverse events associated with the study. The PI has put into place an action plan should adverse events occur during the course of the study. Adverse events that could be envisioned are outlined below:

- A. Responsible party for safety monitoring:** In addition to the PI, safety monitoring will also be performed by the designated medical study coordinator.
- B. Safety Monitoring Methods and Intervals:** Grading method and attribution for adverse event reporting AE will be graded according to the following 0-5 scale:

| Score | Description |
|--------------|--|
| 0 | No Adverse Event or within normal limits or not clinically significant |
| 1 | Mild AE did not require treatment |
| 2 | Moderate AE resolved with treatment |
| 3 | Severe AE resulted in inability to carry on normal activities and required medical attention |
| 4 | Life threatening or disabling AE |
| 5 | Fatal AE |

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The PI will determine the relationship of AE's to the test procedure/agent/device as; not related, possibly related, or definitely related, using standard criteria for clinical trials.

- All reported AEs will be followed until the event resolves or stabilizes OR
- The event returns to baseline OR
- The event can be attributed to agents other than the study agent(s) OR
- The subject withdraws informed consent OR
- The subject is lost to follow up.

- C. Implementation of the data and safety monitoring plan:** Adverse event reports for this protocol will be submitted to the approved IRB. Adverse Event Report Form sent in a timely manner consistent with the approved IRB policy.

In addition, any serious adverse event (grade 3 or greater), with the exception of those reported in the protocol, must be reported to the sponsor by the investigator by telephone, fax or email within 24 hours. A notification form will then be sent to the sponsor by fax or email with acknowledgement of receipt within 48 hours of the event.

The investigator will also inform the sponsor of adverse events and abnormal results of the analysis, which are defined as decisive for the evaluation of safety of persons taking part in biomedical research.

15. PLANS FOR THE SUBJECTS AT THE END OF THE PROTOCOL

Subjects will be instructed to resume normal activities at the conclusion of the study.

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