The Ability of an Oral Supplement to Improve Skin Microperfusion, Hydration, Elasticity and Barrier function

STUDY NUMBER: DCS-71-14

INVESTIGATOR: Gayle M. Gordillo, M.D.

STUDY SITE:
The Ohio State University
Columbus, Ohio

SPONSOR: Natreon Corporation

PRODUCT: Dietary Supplement

STUDY MONITOR: TBD

PROTOCOL NUMBER: DCS-71-14

PROTOCOL SYNOPSIS

Title of Study:	The Ability of an Oral Supplement to Improve Skin Microperfusion, Hydration, Elasticity and Barrier function
Study Period:	14 weeks
Active study product:	Oral dietary supplement PrimaVie® (Shilajit) 125mg or 250mg bid
Placebo:	Placebo dietary supplement - a control to compare the effectiveness of the active study product, PrimaVie®. Ingredients include: 1. Microcrystalline Cellulose, NF 2. Croscarmellos Sodium, NF 3. Silicon Dioxide, Fumed, NF 4. Magnesium Stearate, NF, and 5. Gelatin (from the capsule shell)
Background:	Natreon Inc.'s patented ingredient, PrimaVie® [US patents: US 6,969,612, and 6,440,436] is a purified and standardized shilajit extract for nutraceutical use. It has been well documented that many of the indigenous people of the Himalayas live well over one hundred years. Their longevity has been traced to a rare humic substance called shilajit, found in the high altitudes of Himalayan Mountains. Research indicates that PrimaVie® has targeted action for increasing energy, revitalization and antiaging. PrimaVie® is standardized to have not less than 60% fulvic acid and equivalents with high levels of dibenzo-α-pyrones and dibenzo-α-pyrone chromoproteins. PrimaVie® also works at the cellular level, playing a direct role in the synthesis of ATP, a compound that supplies the body with energy, and healthy mitochondrial function.
Objective:	To demonstrate the ability of the oral supplement, PrimaVie®to improve skin microperfusion, hydration, elasticity and barrier function.
Study Population:	Female subjects 30 – 65 years old
Number of Patients:	45 subjects will be enrolled in this study (enrollment: Arm 1: 15 subjects to take Primavie® 125 mg BID, Arm 2: 15 subjects to take PrimaVie® 250 mg BID and Arm 3: 15 subjects to take placebo BID. Enrollment will take place at The Ohio State University Wexner Medical Center.
Inclusion Criteria:	1. Subjects willing to discontinue any dietary or nutritional supplements, other than a general multivitamin, starting two weeks before onset of study and also during the study. 2. Subjects must be willing to maintain their present diet with no major changes throughout the study. 3. Subjects must be willing to take the dietary supplements as required by

	the study protocol twice daily. 4. Female subjects must be between the ages of 30 to 65 years of age 5. Subjects must provide written informed consent and are willing to comply with all study procedures.
Exclusion Criteria:	1. Any dermatological disorder that may interfere with the accurate evaluation of the subject's skin. 2. Subjects who are pregnant, breast feeding, or planning a pregnancy. 3. Subjects with clinically significant unstable medical disorders. 4. Subjects who have history of, diabetes, heart or kidney disease. 5. Subjects who have history of a psychological illness or condition that would interfere with their ability to understand and follow the requirements of the study. 6. Subjects who have any skin disease in the area of the upper inner arm where the biopsies will be obtained. 7. Currently taking the following medications: a. Steroids b. Beta-blockers c. Immunosuppressant's d. Hydochlorothiazide, e. Statins f. Aspirin g. ACE Inhibitors h. Accutane i. Muscle relaxants j. stimulants 8. Prisoners 9. Males
Study Design:	Subjects with any Fitzpatrick skin type are eligible for this study to evaluate the effect of the oral dietary supplement, PrimaVie® on skin parameters including microperfusion, hydration, elasticity and barrier function. Female subjects between the ages of 30 to 65 years old will be enrolled in this study. At the baseline visit, inclusion and exclusion criteria will be reviewed, informed consent, demographics, a brief medical history, and medication list/ history will be obtained. Subjects will be evaluated visually (investigator assessments) and photographed. A combination of objective non-invasive assessments including trans-epidermal water loss (TEWL), skin hydration, and elasticity will be completed using the DermaLab combo instrument (representative images from one male and one female subject shown in Fig. 1). Laser speckle imaging of micro perfusion (representative images from a male and a female subject shown in Fig.2.), subjective assessment, and a rated utilizing ordinal scale (Glogau scale), will define the parameters that evaluate the effect of supplement on the skin. Subjects will be randomized into one of the 3 arms of the study including, the placebo supplement, PrimaVie® 125mg or PrimaVie® 250mg to take orally twice daily for 14 weeks. Subjects will record their consumption in a diary and the supplements given and

	received will be counted for compliance at each study visit. Subjects will return 2 weeks later where the non-invasive assessments, laser speckle perfusion imaging, subjective assessment, supplement tolerability assessment, photography, and adverse event review will take place. Subjects will undergo a 3mm biopsy from the upper inner left arm. Local anesthetic will be used to numb the area where the biopsy will be obtained. The biopsies will be processed according to laboratory instructions for gene chip array analysis at Ohio State University. Subjects will have their diaries reviewed and supplements counted for compliance. Subjects will continue to record their daily consumption in a diary and the supplements given and received will be counted for compliance. Subjects will return for follow-up visits at week 4, 8, 12, and 14 where investigator, subjective, supplement tolerability/compliance assessments, photography, non-invasive assessments, and adverse event review will be obtained. One 3 mm biopsy will be obtained during week 14 visit from the same location that the first biopsy was obtained (inner left arm) at the week 2 visit.
Endpoints:	<u>Primary Efficacy Endpoint:</u> The primary efficacy endpoint is the improvement in noninvasive skin assessment of objective measurements such as skin microperfusion (laser speckle contrast imaging), elasticity, hydration, barrier function (Dermalab combo) after 12 weeks of oral supplementation. <u>Secondary Efficacy Endpoint:</u> The secondary efficacy endpoint is the up or down regulation of key youthful skin markers (gene expression) produced by the supplement over the placebo as identified by gene chip analysis after 12 weeks of oral supplementation. <u>Tertiary Efficacy Endpoint:</u> The tertiary efficacy endpoint is the subjective visual assessment of the effect of supplement on skin appearance by the investigator at the end of 12 weeks of treatment from photographs taken at each time point.
Phone follow-ups and Supplement Compliance:	Subjects will receive phone call follow-ups while they are in the study to monitor supplement compliance and to review any AEs that the subject may experience. Subjects will also be asked if they have experience any changes since beginning each supplement. If the subject has any serious or serious non-expected AEs, a PRN visit will be scheduled. Phone calls will occur at weeks 3, 6, 10, and 13. Subjects will also be asked to record in a subject diary at what time points each day they take the supplement and will include a notes section to document any changes they experience supplement intake. If subjects are

	non-compliant 80% of the days asked to take the supplement at each scheduled visit, they will be withdrawn.
Compensation:	Subjects will be compensated $30 for completed visits where non-invasive imaging performed (baseline, visits 3, 4, 5), and subjects will be compensated $100 for completed visits with biopsies included (visits 2, 6).
Measurements:	Photography: Photographs will be taken of the face from the front, 45 degrees to the right, and 45 degrees to the left. Subjects will have their hair pulled back in a headband and a drape placed around their neck to cover clothing and maintain anonymity. This evaluation will occur at all study visits. Noninvasive Assessment: TEWL, skin hydration, elasticity measurements will be taken from the right and left cheek at baseline and weeks 2, 4, 8 and 12 using the Dermalab combo instrument. Additionally, laser speckle imaging of microperfusion in the same areas of the face will be performed. Data from both sides of the face will be averaged for final results. These measurements can be obtained rapidly with minimal discomfort to the subjects. They will inform the objective assessment of skin parameters that define the quality of the skin. Biopsy: Biopsy specimens will be taken with a 3mm punch from the upper inner left arm at week 2 and at week 14 from all subjects. Wound care materials and care instructions will be provided to the subjects. Biopsy specimens will be processed for gene chip array analysis. *Note: The biopsies can also be obtained from the upper inner right arm if they are not able to be obtained from the left and will be noted within data collection. Subjective assessments: Physician Appearance Assessment: Lack of softness, lack of smoothness, fine lines, wrinkles, rough skin texture, lack of firmness, lack of skin tone, lack of luminosity, lack of radiance, and overall appearance issues. Ratings will be performed on the following ordinal scale: 0=none, 1=minimal, 2=mild, 3=moderate, 4=severe. This evaluation will occur at baseline, week 2, week 8 and week 14. Tolerability Assessment (Supplement tolerability): Subjects will be queried for stomach upset, indigestion, cramping, or flatulence associated with the assigned supplement. This evaluation will occur at baseline, week 2, week 4, week 8, week 12 and week 14. Subject Appearance Assessment: Lack of softness, lack of smoothness, fine lines, wrinkles, rough skin texture, lack of firmness, lack of skin tone, lack of luminosity, lack of radiance, and overall appearance issues. Ratings will be performed on the following ordinal scale: 0=none,

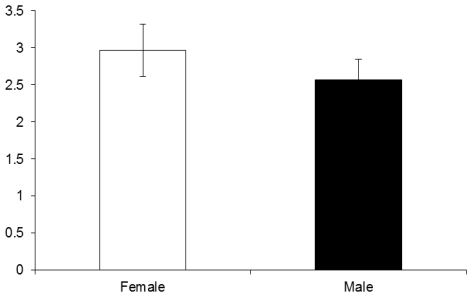
	1=minimal, 2=mild, 3=moderate, 4=severe. This evaluation will occur at baseline, week 2, week 4, week 8, week 12 and week 14.
Statistical Methods:	Statistical significance is defined as p less than or equal to 0.05. A student Mann Whitney test for nonparametric data will be used to analyze the investigator and subject ordinal assessments of skin appearance and tolerability. A one-way ANOVA test will be used to assess the noninvasive data. Gene chip arrays on biopsies for markers defining youthful skin will be analyzed as expected.

STUDY VISIT Schedule

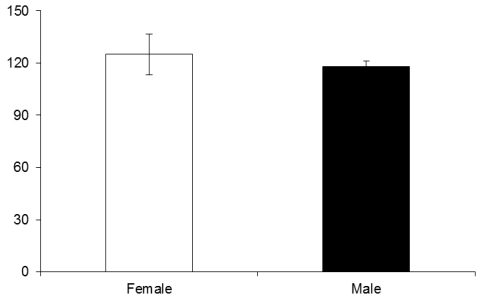
Procedures	**Visit 1 Baseline**	**Visit 2 Week 2**	**Visit 3 Week 4**	**Visit 4 Week 8**	**Visit 5 Week 12**	**Visit 6 Week 14**	***PRN visit (s)**
	- Placebo supplement, PrimaVie®125mg or 250mg						
Consent, exclusion and inclusion criteria	X						
Brief medical/dietary history	X						X
Medication list/ history	X						X
Tolerability assessment		X		X		X	X
Investigator appearance assessment	X	X		X		X	
Subject appearance assessment	X	X	X	X	X	X	
Photography	X	X	X	X	X	X	
Noninvasive assessment (TEWL, hydration, elasticity, laser speckle perfusion)	X	X	X	X	X	X	
Skin biopsy (left inner upper arm)		X				X	
Adverse Event Review		X	X	X	X	X	X
Supplement randomization	X						
Supplement distribution	X	X	X	X	X		
Supplement count/compliance review		X	X	X	X	X	

***PRN Visits –An additional medical/dietary history, medication list/ history review and adverse event review will be completed at these visits. All other activities/ assessments will be performed based on need.**

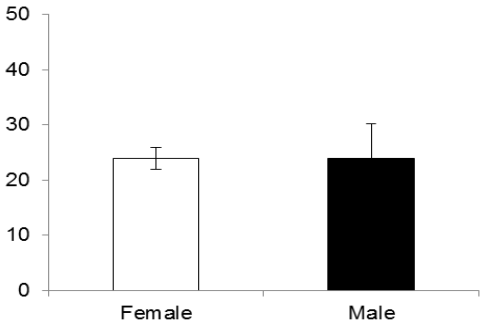
Figure 1. Assessment of trans epidermal water loss (TEWL), skin hydration and elasticity using Dermalab combo. Skin on the right cheek of one male and female subject were assessed using the Dermalab combo instrument for (i) elastic modulus, (ii) hydration, (iii) trans-epidermal water loss.



(i) Elasticity modulus (MPa)

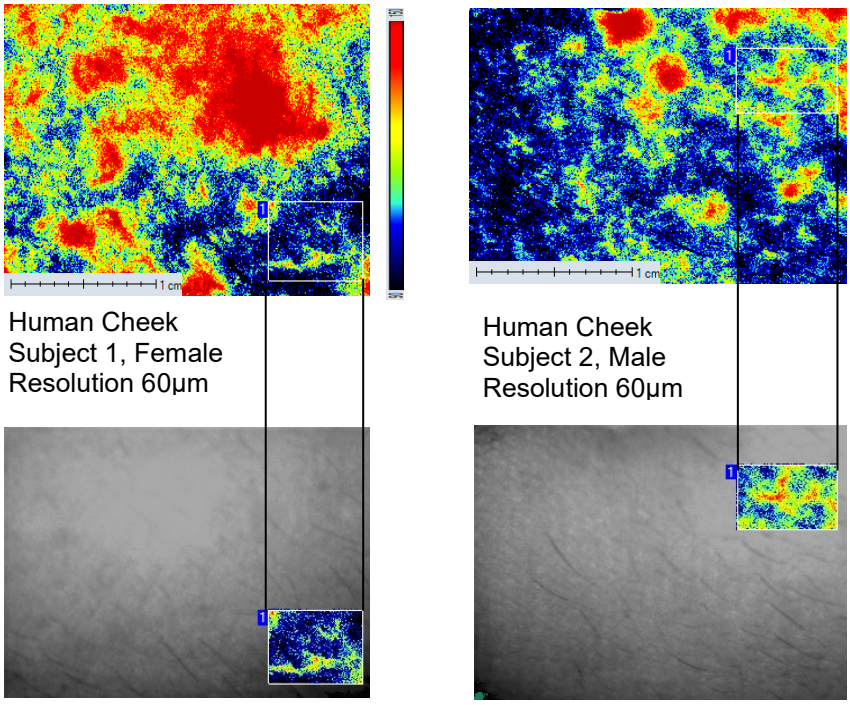


(ii) Hydration (uS)



(iii)Trans-epidermal water loss (g/m2/h)

Figure 2. Laser speckle imaging of skin microperfusion. Microperfusion of skin on right cheek of one male and one female subject were assessed using laser speckle imaging.



Addl. information:
Hydration: A commonly used method to determine the skin surface moisture by capacitance. An essential tool for the promotion of all kinds of facial and body care products.

Visco-elasticity: The viscoelasticity of skin surface is determined by the elastine and collagen fibres. In younger skin these fibers are dispersed beneath the skin surface keeping it firm, supple and elastic. With skin ageing, and additional influences like UVA light, mechanical and chemical strain, nicotine, alcohol, genetic predispositions, diseases and many others, the network of fibers gets more stiff and clustered, thus leading to skin slackening and wrinkle formation. The measurement of the skin's viscoelastic properties are therefore a true indicator for the real biological age of the skin.

TEWL: The measurement of the water evaporating index from the skin is an indication of the quality of the barrier function of the skin. The measurement is performed in an open measuring chamber with two sensor pairs: temperature and humidity to exactly calculate the water gradient without influencing the skin and its micro climate. Even slightest damages in its barrier (not necessarily visible to the human eye) can easily be determined by this measurement.

Laser speckle contrast imaging: The PeriCam PSI System is a blood perfusion imager based on the Laser Speckle Contrast Analysis (LASCA) technology. It is a method that visualizes tissue blood perfusion in real-time. LASCA provides new means to study the microcirculation in ways that were not possible in the past. The PeriCam PSI System combines dynamic response and high spatial resolution in one instrument, providing both real-time graphs and video recordings of the area of interest. (http://www.perimed-instruments.com/products/pericam-psi#instrument-versions)

Fitzpatrick Scale for Skin-Type classification

Skin type	Skin color	Hair color (darkest)	Eye color (most common)	Description
I	White or very pale	Blonde	Blue, grey, green	Always burns, never tans
II	Pale white with beige tint	Chestnut or dark blond	blue	Always burns, sometimes tans
III	Beige to light brown (olive)	Dark brown	Dark brown	Sometimes burns, always tans
IV	Light to moderate brown	Black	Brown	Rarely burns, always tans
V	Medium to dark brown	Black	Brownish black	Rarely burns, tans more than average
VI	Dark brown to black	Black	Black	Never burns

Glogau scale for photo aging

GROUP	CLASSIFICATION	TYPICAL AGE	DESCRIPTION	SKIN CHARACTERISTICS
I	Mild	28-35	No Wrinkles	Early photo aging: mild pigment changes, no keratosis, minimal wrinkles, minimal or no makeup
II	Moderate	35-50	Wrinkles in Motion	Early to moderate photo aging; early brown spots visible, keratosis palpable but not visible, parallel smile lines begin to appear, wears some foundation
III	Advanced	50-65	Wrinkles at Rest	Advanced photo aging: obvious discoloration, visible capillaries, visible keratosis, wears heavier foundation
IV	Severe	60 & up	Only Wrinkles	Severe photo aging: yellow/grey skin color, prior skin malignancies, wrinkles throughout - no normal skin, cannot wear make-up because it cracks and cakes