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Phototherapy Induced Metabolism Change Produced by the LifeWave X39 Non-transdermal Patch

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ABSTRACT

Purpose: *To determine X-39 patch impact in stimulation of Copper peptide biosynthesis and bio-available amino acid levels, neurotransmitters production, memory, sleep quality, vitality, muscle relaxation and blood pressure.*

Materials: *Biography Infinity physiology suite: Heart rate variability (HRV), GSR, EMG, EKG, blood volume pulse (BVP), temperature and respiration. Questionnaires: Marlow-Crowne, Global Mood Scale, Pittsburg Sleep Quality Index, Arizona Integrative Outcomes scale. WAIS III memory test. Amino acid and neurotransmitters testing of urine.*

Method: *Subjects were recruited (age 40 - 81), consented, randomized and scheduled. Data taken day 1, day 2, and day 7 except Marlow-Crowne taken day 1 and day 7.*

Results: *Improvements in short term memory $p<0.001$, sleep quality $p<0.04$, vitality $p<0.03$ day 2 and $p<0.08$ at day 7. Blood pressure change in VLF on day 7 at $p<0.02$, respiration on day 7 at $p<0.04$. Increase in amino acids: Creatinine, Normetanephrine, methionine, homocystine, isoleucine, glutamine, cysteine, 5-hydroxytrytophane, β-aminobutyric-acid.*

Conclusion: *The results of the double blind randomized controlled trial of 50 subjects with mean age 63 years, using the LifeWave non-transdermal X-39 phototherapy patch worn 8-12 hours per day for seven days produced an increase in 8 amino acids at significant levels. The study showed, there was an improvement in short term memory as measured by the WAIS III memory test at significant levels over 7 days, in Quality of Sleep at significant levels within 24 hours and a self reported increase in vitality at near significant levels in 7 days as measured by the Arizona Integrative Outcomes scale.*

Our findings suggest this patch is not only stimulating the biosynthesis of copper-peptide production, but also increases neurotransmitters production and improves metabolism. Additional studies could address underlying mechanisms of action to the phototherapy process and longer periods of study might be explored for additional potential physical changes and longevity of the demonstrated changes.

Keywords: *Photobiology, Photobiomodulation, Amino Acid, Metabolism*

INTRODUCTION

The Lifewave X39 non-transdermal patch focuses on stimulating the copper tripeptide GHK- Cu [1,2,3]. "Copper tripeptide-1(GHK-Cu) is a small protein composed of the three amino acids glycine, histidine, and lysine combined in a specific geometric configuration with the physiologically beneficial mineral (copper)" [4]. The goal of this research was to determine X-39 patch impact in stimulation of Copper peptide biosynthesis and bio-available amino acid levels, neurotransmitters production, memory, sleep quality, vitality, muscle

relaxation and blood pressure. This tripeptide was first isolated from human plasma albumin in 1973 by Dr. Loren Pickart. Pickart, noticed differences in the levels of fibrinogen based on age. He additionally noticed that these differences stopped when the older liver cells were incubated in blood from younger individuals. "In 1977, David Schlesinger of the Harvard University Chemistry Department confirmed that the growth modulating peptide isolated by Pickart was a glycyl-L-histidyl-L-lysine peptide"[5]. It is interesting to note that this peptide has also been found in saliva, urine, and collagen. Additional research has established the strong affinity the GHK peptide has for copper, and exists in two forms, as this was not covered in the initial experiment. These two forms are GHK and GHK-Cu. It is also important to mention that none of the research around GHK has ever found it to cause an issue [4].

Research has identified that the peptide is used to signal the beginning of the natural repair process. This benefit has specifically been documented through research for post-laser or surgical wounds, ischemic, burns, skin or hair transplants, and diabetic ulcers. "Diabetic wounds healed three times faster in the presence of Copper tripeptide-1. Time to re-epithelialization is shortened" [4]. The tripeptide has also been demonstrated to improve tissue remodeling. "It increases keratinocyte proliferation and normal collagen synthesis, improves skin thickness, skin elasticity and firmness, improves wrinkles, photodamage and uneven pigmentation, improves skin clarity, and tightens protective barrier proteins" [4]. This has an impact on both scars and other effects of damage to the skin, and natural aging processes. The effects of tissue remodeling also appear to have an impact on cancerous cells. "The fact that GHK was able to suppress 70% of genes involved in the development of an aggressive metastatic form of colon cancer indicates that GHK is capable of the regulation of various biochemical pathways on a gene level and it seems to be resetting the gene activity back to health, which leads to the improvement of tissue repair" [6].

GHK-Cu also has a demonstrated impact on other organs in the body after they have been damaged. "A collaborative study conducted by scientists from Boston University, University of Groningen, University of British Columbia, and University of Pennsylvania established that the GHK peptide reverses the gene expression signature of COPD, which is manifested by emphysema, inflammation, lung tissue destruction, and significant reduction of lung capacity" [6]. It is also important to note that "…the level of GHK is about 200 ng/mL(10−7M) at age 20 and it declines to 80 ng/mL by age 60" [6]. This likely explains the increasing effects of aging. It would also suggest that increased levels over time of GHK-Cu would have a positive effect on both life expectancy and aging.

While phototherapy has been defined as "the use of ultraviolet (UV) light for its healing effects" [7], the LifeWave patches have been specifically developed to reflect light in the infrared and visible light bands back onto the skin where the patch has been placed. Supported by normal electro-dermal skin conductance [8,9], the human body gives off a number of materials biochemically including particulate release, gas emission, ultraviolet, infrared, near infrared, and visible spectrum light. This then stimulates the area of skin, producing improved physiological effects. Variations on phototherapy have been used for at least 100 years. In that time there has been little evidence of negative side effects. This suggests that this is a relatively untapped option for healing with relatively few risks.

Materials and Methods

This study was done as a whole systems research randomized controlled double blind trial of 50 participants. Human studies ethics approval for this research was given NFFEH 03-31-19-02. Participants were recruited using flyers, emails and radio announcements. A sample of convenience drawn from those who responded and met inclusion/exclusion criterion of men and women age 40-81 with no major mental health issues who were then randomized into control and active groups via computer and to patch placements (GV14, CV6). Patches used in the trial were mailed prepackaged as groups A and B and the blind was not broken until after the statistical analysis was done. Participants were scheduled for the same time each day for each of the data points (day1/baseline, day2/24 hours, day 7). Participants were consented, and data was taken in the following order: questionnaires, urine sample, physiology measures. On day one the participant was given 9 days supply of the patches for use in the study. A one month supply of the active patch was given at the end of the week's participation and $20 toward the participants time and gasoline.

The trial was conducted at the Arizona School of Acupuncture and Oriental Medicine clinic in Tucson, AZ.

Non-transdermal Patch and Patch Placement

All X39 patches are sealed so that none of the substances in the patch actually penetrate the skin. This allows for consistent patch promotion of the light flow during the period of patch application. There is sufficient evidence of electrical-dermal response in orthopedic research to use previously measured acupuncture points as "strategic conductors of electromagnetic signals" [10]. Two points were selected for this research so the process of patch placement could be standardized across the participant population. Earlier studies of this patch selected acupuncture/acupressure points designated GV14 and CV6 [2, 3] and based on pilot results, the same patch placement was selected for consistency. Participants were randomized by computer into groups and the point usage was selected based on the randomization.

Metabolic Suite

Sabre Sciences Inc. conducted the urine analysis using their HPA1 metabolic panel using LC-MS/MS tandem mass spectrometry. The metabolic panel measures excreditory level of amino acids and their metabolites, including catecholaminergic, serotonergic, glutamatergic pathways and metabolites, and trans-sulfuration and histidine pathways. Three urine data points were taken on each subject before 10am each of baseline/day1, at 24 hours/day 2 and on day 7. Samples were frozen and then shipped to the Sabre Science laboratory in Carlsbad, CA each day for analysis.

Physiology Suite

The Thought Technology Biography Infinity physiology suite was used for six minute measures of heart rate variability (HRV), galvanic skin response (GSR), EMG, EKG, blood volume pulse (BVP), temperature and respiration at each data point.

Questionnaires

Questionnaires and memory test were administered at each data point with the exception of the Marlow-Crowne which was administered day one and day seven.

Marlowe Crowne

The Marlowe Crowne is a 13 Item true/false short likert scale that measures political correctness. This instrument was selected to confirm accuracy of the data. The instrument takes about 2 minutes to do for most participants. (Norming: [11].)

Pittsburg Sleep Quality Index

Normed by Cole et al (2006) this scale is a mix of quantitative questions and five likert scale questions 0-3 addressing the participants quality of sleep.

Arizona Integrative Outcome Scale, Visual Analogue Scale (AIOS-VAS) for Vitality

The AIOS- VAS (Normed: [12]) rates subject's "overall sense of well-being and vitality." It uses a 100mm one-line visual analogue scale on which the participant notes their sense of well being with an x on the line.

WAS III memory sub-test

A subsection of the WAS III was administered which looks at the level of short, mid and longer term memory. The test was administered concurrent to the filling out of other questionnaires. Ten numbers which were taken from a randomized list were repeated without inflection three times to each participant. Participants were asked to immediately repeat the numbers back at the end of the third repetition, then again asked to repeat the numbers back at 10 minutes and at twenty minutes. Count was taken of the number of correct digits in the order originally given. Count was stopped when an error in the order was made.

Global Mood Scale

Denollet normed the Global Mood Scale in 1993. It includes a twenty item, five factor likert scale that assesses participants current mood and a one item, ten factor likert scale assessing well being at the current moment. It was selected both as a redundant measure comparison to the AIOS-VAS and an overall assessment of mood.

Statistical Analysis

All data results were entered into spread sheets and they were then analyzed for significant results. Questionnaires were analyzed for mean and standard deviation and stratified by assessment time point. Paired t-test or nonparametric Wilcoxon Signed Rank tests were then done. Physiology measures were summarized stratified across the 6 study epochs looking at means and standard deviation. Pre - post changes were evaluated using a paired t-test. Distribution assumptions were then verified

using normal probability plots. The values for p are two-sided and statistical significance was defined as $p<0.05$. The metabolic analysis included stratification by assessment point and summary means and standard deviation. Analysis was done including changes from baseline to day 2, day 2 to day 7 and day 1 to day 7. These were evaluated using Paired t-test or nonparametric Wilcoxon Signed Rank tests.

Results and Discussion

This was a double blind randomized controlled trial with a sample of convenience and which had a seven day intervention period. While this was a sample of convenience participants were recruited through five different methods including: radio announcements, email, posting in cafe's and at university center's, prior study participant announcement lists and through area community groups. So while this could be considered a study weakness, the range of recruiting methods insured a diversity of population who decided to participate. There were 26 individuals in the Active group and 24 in the Control group, with an overall mean age of 63 and a range of 40-81 years. The Active groups mean age matched the overall mean, while the Control group was slightly lower at 62 years. Despite the limitations of smaller participant sample size (N=50) in this study, there were several interesting significant changes, as well as some changes of near significance within the study test results. There were two adverse events, where two individuals in the study had headaches while using the patches. These headaches took place during monsoon season, and both were individuals who had a previous history of barometric pressure triggered migraine headaches. The IRB reviewed the cases and determined that they were both consistent with the individuals past history and had nothing to do with the product. Two individuals were also dropped from the study when they did not complete the study visits.

The specific impact of research location should also be recognized. Aside from the barometric pressure effects of monsoon season, this study largely took place over the summer in Arizona. This meant that participant hydration was a constant issue, especially with the necessity of a urine sample. As such, it was determined that water had to be available and was offered to each participant at the beginning of each study appointment. Included are the significant and some near significance findings for the LifeWave non-transdermal X39 phototherapy patch.

The urine analysis data showed an increase in amino acids: Creatinine, Normetanephrine, methionine, homocystine, isoleucine, glutamine, cysteine, 5-hydroxytrytophane, β-aminobutyric-acid. These are important findings as branched chain amino acids like isoleucine, which is constantly oxidized in muscle, methionine and homocystine, are major ingredients of trans-sulfuration pathway. This pathway is responsible for methyl donor S-adenosyl-methionine (SAMe) production. SAMe is readily donates methyl groups to other substances, enabling cardiovascular, neurological, reproductive and detoxifying systems. 5-hydroxytrytophane is major building block of important neurological monoamine neurotransmitter, serotonin, which later methylates into melatonin, and is a main hormone which regulates sleep-wake cycle. Normetanephrine is a norephinephrine break down product in dopamine pathway and is major hormone of neuromodulatory system.

Table 1: *Comparisons of amino acid concentrations between Control group vs. Active group at day baseline (1), day 2 (2) and day 7 (7) All amino acids concentration results were summarized in terms of means and standard deviations (SD), stratified by group (Control vs. Active) and assessment time point (1, 2, 7). Absolute changes from baseline (1) to 2 and baseline (1) to 7 were evaluated using a paired t-test or nonparametric Wilcoxon signed rank test. All reported p-values are two-sided.*

Parameter	Unit	Day	Control group Mean (SD)	Active group Mean (SD)	p-value
Creatinine	mg/ml	1	147.7 (69.7)	88.2 (52.7)	0.002
Creatinine	mg/ml	2	159.5 (67.9)	82.7 (50.6)	0.000
Creatinine	mg/ml	7	147 (77)	104 (62.2)	0.040
Normetanephrine	mg/g-c	2	66.5 (32.4)	93.1 (49.7)	0.036
Normetanephrine	mg/g-c	7	68.9 (37)	97.7 (52)	0.036
Methionine	mmol/g	1	7.4 (3.6)	10.3 (6.4)	0.066

Table 2: *Comparisons of absolute changes in amino acid concentrations from baseline (1) to day 2 and baseline (1) to day 7 between Control group vs. Active group*

			Control group		Active group		
Parameter	**Unit**	**Day**	**Mean (SD)**	**p-value[1]**	**Mean (SD)**	**p-value[1]**	**p-value[2]**
Epinephrine	mg/g-c	2	-1.8 (4.1)	0.054	0.9 (5.9)	0.464	0.083
Normetanephrine	mg/g-c	2	-8.2 (32.7)	0.255	9.9 (29.3)	0.097	0.049
Homocystine	mmol/g	2	0.1 (0.5)	0.234	0.4 (0.7)	0.010	0.180
β-aminobutyric-acid	mmol/g	2	5.5 (40.2)	0.524	14.9 (33.4)	0.032	0.384
Isoleucine	mmol/g	2	0.7 (4.9)	0.495	2.2 (5.1)	0.037	0.310
Glutamine	mmol/g	2	0.9 (75.9)	0.956	33.4 (83)	0.051	0.166
5-hydroxytryptophane	mg/g-c	7	-0.3 (24)	0.954	11.3 (28.3)	0.058	0.140
Cysteine	mmol/g	7	0.4 (33.2)	0.953	10.1 (24.2)	0.047	0.255

[1]:p-pvalue for evaluating changes from baseline to 2 and baseline to 7 within each arm

[2]:p-value for comparing changes from baseline to 2 and 7 between arms

All questionnaire scores were summarized in terms of means and standard deviations (SD), stratified by group (Control vs. Active) and assessment time point (1, 2, 7). Absolute changes from baseline (1) to day 2 and baseline (1) to day 7 were evaluated using a paired t-test or nonparametric Wilcoxon signed rank test. All reported p-values are two-sided.

Questionnaire outcomes:

- WAIS III (three subscales #Short, # Mid, #Long)
- Global Mood Scale (two subscale: Positive Affect (PA), Negative Affect (NA))
- Pittsburgh Sleep Quality Index (total score – higher score indicates worse sleep quality)
- Marlow-Crowne (total score)
- AIOS-VAS (total score)

Within the questionnaire results the shifts in sleep were particularly interesting, as they were significant in both groups, in opposite directions. The Active group had a significant improvement in sleep, and the control group had a significant decline in sleep. Vitality also showed a particularly interesting shift, with significant improvement within 24 hours which dropped slightly at day 7. The Global mood scale showed a change in negative affect in both groups. Additionally the Marlow-Crowne showed no significance so that responses on the questionnaires were not as a result of the desire to provide socially correct or desired answers.

Table 3: *Significant and almost significant WAIS III scores between baseline (1) and day 7 (7)*

Subscale	Day	Control group Mean (SD)	Active group Mean (SD)	p-value
#Short	7	9 (1.3)	6.5 (2.6)	<0.001
#Mid	7	7.3 (2.3)	6 (2.7)	0.098

Table 4: *Comparisons of significant absolute changes in GMS subscale scores (PA-Positive Affect, NA-Negative Affect) from baseline (1) to day 2 and baseline (1) to day 7 between Control group vs. Active group*

		Control group		Active group		
Subscale	**Day**	**Mean (SD)**	**p-value[1]**	**Mean (SD)**	**p-value[1]**	**p-value[2]**
GMS-NA	2	-3 (4.7)	0.008	-2.2 (5.3)	0.067	0.576

[1]:p-pvalue for evaluating changes from baseline to 2 and baseline to 7 within each arm

[2]:p-value for comparing changes from baseline to 2 and 7 between arms

Table 5: *Comparisons of significant absolute changes in PSQI total scores from baseline (1) to day 2 and baseline (1) to day 7 between Control group vs. Active group*

		Control group		Active group		
Total Score	**Day**	**Mean (SD)**	**p-value[1]**	**Mean (SD)**	**p-value[1]**	**p-value[2]**
PSQI Total Score	2	-1.4 (2.3)	0.049	0.2 (1.6)	0.646	0.042

[1]:p-pvalue for evaluating changes from baseline to 2 and baseline to 7 within each arm

[2]:p-value for comparing changes from baseline to 2 and 7 between arms

Table 6: *Comparisons of Marlow-Crowne scores between Control group vs. Active group at time baseline (1) and day 7 (7)*

Total Score	Day	Control group Mean (SD)	Active group Mean (SD)	p-value
Marlow-Crowne	1	15.8 (6.6)	15.9 (5.4)	0.920
Marlow-Crowne	7	14.9 (6.3)	14.7 (6.7)	0.887

Table 7: *Comparisons of AIOS-VAS scores between Control group vs. Active group at time baseline (1), day 2 (2) and day 7 (7)*

Total Score	Day	Control group Mean (SD)	Active group Mean (SD)	p-value
AIOS-VAS	1	73.2 (11.3)	58.6 (24.4)	0.039
AIOS-VAS	7	76.4 (14.5)	64.5 (24.3)	0.086

Physiological measures showed significant normalization in blood pressure in VLF $p<0.02$ and near significance in LF $p<0.09$ and power $p<0.06$. Average respiration became deeper by day 7 at $p<0.04$ and neck and shoulder muscles showed improved relaxation effects by day 7 at $p<0.08$.

Table8: *Summary of significant and near significance physiological parameters, stratified by time (day) and group. Since the physiology parameters were non-normally distributed, all parameters were summarized in terms of medians and interquartile ranges (IQR). Changes from run 1 (baseline) to run 3 were evaluated using a nonparametric Wilcoxon signed rank test. Comparisons between groups were conducted using a nonparametric Wilcoxon rank sum test. All reported p-values are two-sided and P<0.05 was used to define statistical significance.*

			Control group	Active group	
Source	Parameter	Day	Median (IQ1)	Median (IQ1)	p-value[2]
B:BVP	VLF	7	217.9 (65.4-431.4)	57.4 (18.7-147.2)	0.0262
B:BVP	LF	7	194.5 (89.1-1122.4)	99.1 (45.5-167.5)	0.0976
B:BVP	Power	7	503.9 (295.5-1651.5)	238.1 (132.3-665.1)	0.0677
Resp.Rate	Average†	7	14.6 (13.7-14.9)	13.6 (11.9-14.2)	0.0490
C:EMG	Average†	7	15.1 (10-40.4)	7.8 (4.9-25.8)	0.0866

[1]: IQR: Interquartile range (25^{th} to 75^{th} percentile)

[2]: P-value for comparison between Control group vs. Active group

Average†: Average across Epoch 1-6

A comparison of this studies results with the pilot study showed some interesting differences. The first was the change in the level of significance in memory improvement. While the pilot study showed a near significant improvement in short-term memory, the double blind showed very clear significance, $p<0.001$, as well as near significance in mid-term memory. It would be very interesting to see if this improvement continued to strengthen with a longer intervention period or a population with a mean age of 70+.

Of note, was the difference in which amino acids were significant between the pilot and the current study. The amino acids which has significance were further down the catecholaminergic, serotonergic, and glutamatergic pathways that the significant findings of the pilot study. This may be due to the difference in median age, with the double blind having an older median population or may be due to utilization by the body when higher concentrations of the amino acids were available for use. It should also be noted that while we saw more prevalent amino acid changes tied to antioxidant events in the pilot study, the data from the double blind shows an overall rebalancing of the gut and overall improvement in gut performance.

It would be interesting to determine how long and at what continuing rate changes to gut performance, physiological and overall wellness support would be produced by wearing the LifeWave X-39 patch. Longer periods of study such as six to twelve weeks including comprehensive blood and metabolism testing might be considered in the future.

Conclusion

The results of the double blind randomized controlled trial of 50 subjects on the LifeWave non-transdermal X-39 phototherapy patch worn for 8-12 hours per day for seven days produced an increase in 8 amino acids at 10 significant levels over the 3 time periods. There was additional increase in 3 amino acids at near significance. These specific changes served to rebalance the gut toward a positive homeostatic balance. There was an increase in short term memory as measured by the WAIS III memory test at significant levels over 7 days. There was an improvement in Quality of Sleep at significant levels within 24 hours and a self reported increase in vitality at near significant levels in 7 days as measured by the Arizona Integrative Outcomes scale. Given the mean age of the population at 63 years this is a substantial improvement in overall quality of life. Additional studies could address underlying mechanisms of action to the phototherapy process and longer periods of study might be explored for additional potential physical changes.

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