

# Bone and Muscle Support in Ageing Women with Life Wave X49 TM Patch

Connor MH<sup>1,2\*</sup>, Connor CA<sup>2,3</sup>, Horzempa D<sup>2,4</sup>, Yue D<sup>5</sup>, Eickhoff J<sup>6</sup>, Perry M<sup>2</sup> and Young D<sup>2</sup>

<sup>1</sup>Director of Research, Akamai University, Hilo HI, USA

<sup>2</sup>Earth songs Holistic Consulting, Tucson, AZ, USA

<sup>3</sup>Assistant Professor, CAM, Akamai University, Hilo, HI, USA

<sup>4</sup>Mind-Body MD, Tucson, AZ, USA

<sup>5</sup>AxisPharm Laboratory, San Diego, CA, USA

<sup>6</sup>Senior Scientist, University of Wisconsin Madison, Madison, WI, USA

**\*Corresponding author:** Melinda H Connor, Director of Research, Akamai University, Hilo Hawaii and Founder, Earthsongs Holistic Consulting 31907 South Davis Ranch Rd. Marana, AZ 85658, USA



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## ABSTRACT

**Purpose:** To determine if the Life Wave X49TM patch supports bone and muscle health in women ages 40-80.

**Materials and Method:** Urine test kit, lavender top blood tubes, BD Vacutainer with Pre-attached holder; cryo tubes, racking, freezer, gloves, band aids, wipes, masks, UVC sterilizing wands, sterile eye droppers, sterile cotton balls, tourniquets, dry ice, shipping containers. Measures were taken at baseline, day 2, 7, 30 and 60 days of wearing the patch. A sample of 24 subjects made up of women aged 40-80 with the goal of 20 subjects completing the study, was selected to participate in this study. Participants used the X49 TM patch at the GB34 point and X39 TM patch at the CV6 point or GV14 point. Acupuncture points were used for ease of correct patch placement. Food diaries were maintained throughout the study by participants. Participants were asked to have a minimum of 6oz of Leucine based foods each day. Food diaries were reviewed on a weekly basis to confirm participant adherence. Metabolic testing (amino acid panel) consisted of one 10am urine taken at baseline/day one, day two, day 7, 30 and 60. Samples were kept in the freezer at -20F and were shipped with ice by UPS to the Sabre Science lab in Carlsbad, CA. Two lavender top tubes were drawn from each participant at each data point. Plasma was separated, placed in cryo tubes and flash frozen. Samples were kept in the freezer at -20F and at study end were shipped to Axis Pharm in San Diego for analysis of both AHK and NTx.

**Results:** Significant decreases from baseline were observed for AHK-Cu. The percentages of subjects with a >30% decrease in creatine levels from baseline (NTx response) to the post intervention time points were significant. Secondary to NTx, we also saw a significant decrease in Hydroxyproline at the post intervention assessment time points. The combination of these three points in this early data suggests that X49 TM has the potential to decrease the breakdown of bones during the cycle of bone repair. In addition, we saw 14 amino acids change production levels at significance over the 60 days. The amino acids which changed were also spread between the catecholamine, serotonergic, glutaminergic, transulfuration, and histidine pathways, giving a very broad impact.

**Conclusion:** This study explores changes in AHK-Cu peptide production and changes in NTx production to see if the Life Wave X49 TM patch supports improved bone density. There was a significant change in both AHK-Cu and NTx. Study data is sufficiently significant to warrant further research.

## Introduction

Osteoporosis has become a «major public health problem» [1]. As the average population age is increasing it is beginning to affect a greater proportion of the population [1]. Unfortunately, at this point in time the medications available to treat osteoporosis also have some unfortunate side effects [2]. These side effects are preventing some individuals from seeking treatment [3]. L-Alanine-L-Histidyl-L-Lysine Copper (AHK) has a documented impact on a variety of areas in the body, specifically skin, hair, and bone [4-6]. Since AHK has a documented impact on bone it was decided to test if the X49 TM patches would have an effect on bone density, starting with healthy women in the age range at risk to develop osteoporosis. It was also decided to test if the increase in GHK and GHK-Cu from the Life Wave X39 patch would impact the production of AHK or the effect on bone density, since the patches are likely to be used in combination. A dexascan was determined to be impractical due to scheduling and frequency of testing needed, so an alternative method to test for changes in bone density was chosen with serum NTx. AHK, amino acids, and hormones were also checked to develop as clear an understanding of the effects.

## Background

Bones go through a cycle of repair to both microdamage and old bone «through sequential osteoclastic resorption and osteoblastic bone formation» [7]. NTx is released during «proteolytic cleavage of bone collagen by osteoclasts» [8]. This means that NTx is released during the osteoclastic resorption phase of bone repair. It has specifically been found to correlate with changes in the rate of bone loss [9]. Most importantly, a decrease in NTx levels has been shown to correlate to a decrease in osteoclast activity, and thus a decrease in bone breakdown [10] which means there is less bone for the osteoblastic bone formation to replace. NTx in urine has been used to index changes in bone density since at least 1994 [11]. Unfortunately, its utility is dependent on dilution levels, which are matched using creatinine. Fortunately, NTx is also found in blood [8], and has been shown to reflect changes in bone density equally well [12].

Amino Acids are used to create proteins and peptides in the human body. Some amino acids can be produced out of smaller component pieces, but nine of them must be obtained through diet [13]. Two of these amino acids which cannot be created by the body are essential amino acids and are utilized in AHK. Because these amino acids can be used to create a broad variety of important components, they also have a broad variety of functions. L-Alanine-L-Histidyl-L-Lysine Copper (AHK) is a copper polypeptide with a variety of different functions. It «increases dermal cell proliferation and viability while increasing the deposition of collagen to renew the extracellular matrix» [4]. It also «promotes the growth of human hair follicles, as is caused by stimulation of the proliferation and the

preclusion of the apoptosis of dermal papilla cells» [5]. AHK linked to Vitamin C has also been shown to have «an enhancing effect on osteoblast proliferation and differentiation through activation of Smad1/5/8 and MAPK ERK1/2 and p38 signaling and without significant cytotoxicity» [6]. These functions make a fair amount of sense given the individual actions and effects of the amino acid components.

Alanine has been shown to «reduce lactate concentrations during exercise and thus can improve exercise performance in endurance athletes» [14]. Alanine also has a stabilizing impact on glucose levels in the human body [15]. Histidine is used as a «component of solutions used for organ preservation and myocardial protection in cardiac surgery» [16]. It also seems to have an impact on «neurological disorders, atopic dermatitis, metabolic syndrome, diabetes, uraemic anaemia, ulcers, inflammatory bowel diseases, malignancies, and muscle performance during strenuous exercise» [16], though unfortunately more research is needed in those areas to clarify the impact. Lysine is «crucial for collagen fibre crosslinking» [17]. All of these areas are likely to have an impact on bone development. The Life Wave X39 TM patch has previously been shown to improve the production of both GHK and GHK-Cu [18-21]. GHK is a related peptide to AHK, and also has a number of positive health impacts, including signaling the start of the natural repair process and improved tissue remodeling [22]. The increase in keratinocyte proliferation and normal collagen synthesis [22] were specifically thought to have a potential impact on the improvement in bone density. Given this possibility the decision was made to test both the X49 patch alone and in combination with X39.

## Phototherapy

Phototherapy has shown benefits for a variety of skin diseases [23], foot ulcer healing, specifically with diabetes [24], and even a first line therapy for mycosis fungoides [25] and has been used in various forms for over 100 years. Few negative side effects have been found so phototherapy has been found to be a relatively safe process. The Life Wave X49 TM patch uses phototherapy to stimulate production of specific frequencies which support the positive balance of the body and changes in production of specific chemicals. Merriam-Webster Dictionary defines «Phototherapy» as «light therapy» «It is the use of light in specific wavelengths that vary based on the intended effect to stimulate a specific physiological change.» [26]. Research has identified that peptides are used to support the natural repair process and demonstrated to improve tissue remodeling [22].

## Non-Transdermal Patch

All Life Wave X49 TM patches are sealed so that none of the substances in the acrylic sleeve of the patch actually penetrate the

skin. Patches are designed to reflect wavelengths of light in the infrared, near infrared, and visible light bands. The patches act as a transducer and transmitter, like a router on a computer network, or one of the old crystal radio sets. This allows for patch promotion of the electrodermal skin response. Electrodermal activity (EDA) is the property of the human body that causes continuous variation in the electrical characteristics of the skin. The patches receive signals from the body, strengthen them, and sends them back. This promotes specific frequencies to support specific body processes. The patches use the same adhesives as band-aids. This limits the level of irritation caused by the adhesive which might be developed through consistent daily use of the patch.

## Purpose

To determine if the LifeWave X49 TM patch supports bone and muscle health in women ages 40-80.

## Materials

Urine test kit, lavender top blood tubes, BD Vacutainer with Pre-attached holder, cryo tubes, racking, freezer, gloves sterile, band aids, hand sanitizer, clorox wipes, masks, UVC sterilizing wands, sterile eye droppers, sterile cotton balls, tourniquets, dry ice, shipping containers.

## Method

This study sought to explore the metabolic implications of wearing the Life Wave X49 TM patch vs. the X49 TM in combination with X39 TM patch over the period of eight weeks. Measures were taken at baseline, 24 hours, at 7 days, and 30 days and 60 days of wearing the patch. A random sample of 24 subjects made up of women aged 40-80 with the goal of 20 subjects completing the study, were selected to participate in this study. Once 20 subjects completed the study stopped recruiting and consenting. This study focused on the metabolic and physiologic impact of patch usage, with the X49 TM participants using the GB34 point and X39 TM using the CV6 point or GV14 point as the person prefers. This study explored changes in AHK peptide production, changes in NTx production and improved bone density.

## Recruitment

Three methods of recruitment were used for this study. Individuals who had previously expressed interest in participating in research studies were sent IRB approved emails with descriptions of the study including the inclusion/exclusion criteria. There was a phone number to call if they were interested in participating. IRB approved flyers were also distributed to a variety of local business and community boards. These also included a phone number to call if the individual was interested in participating. The final method was word of mouth. People who had already learned about the study told other people and provided them with the phone number to call. Once the individual called the phone number the

study personnel who answered went over the study description, as well as the inclusion/exclusion criteria. They also answered any questions the individual had. If the individual was still interested and matched the inclusion/exclusion criteria a time was set for consenting.

## Consenting

All consenting was done in person. The individual being consented was handed two copies of the IRB approved consent form, two copies of the IRB approved HIPAA, as well as a demographics form. The consent form was gone over in detail, including the study description and inclusion/exclusion criteria, and any questions were answered. The individual was also informed that they could leave the study at any point in time and for any reason and requested to ask any questions at any point in time. The contact numbers stated on the consent for was pointed out, including the phone number for the IRB contact person in case they had any concerns. If they were still interested in participating, they signed all of the forms, as did a representative of the study team. The participating individual kept a copy of the forms, as did the study team.

## Questionnaires

Food diaries were maintained throughout the study by participants. Participants were asked to have a minimum of 6oz of Leucine based foods each day. Food diaries were reviewed on a weekly basis to confirm participant adherence.

## Metabolic Analysis One

AxisPharm laboratory in San Diego, CA did blood analysis for both AHK and NTx. Two lavender top tubes were drawn from each participant at each data point. The blood samples went through centrifuge processing into plasma. Plasma was separated and placed in cryo tubes and flash frozen. Samples were kept in the freezer at -20F and shipped with ice by UPS to Axis Pharm in San Diego for analysis.

## Metabolic Analysis Two

Sabre Sciences laboratory did a metabolic analysis (amino acid panel). Metabolic testing consisted of one 10am urine taken at baseline/day one, day two, day 7, 30 and 60. Samples were kept in the freezer at -20F and shipped with ice by UPS to the Sabre Science lab in Carlesbad, CA.

## Statistical Analysis

NTx - Creatinine levels and changes in creatinine levels were not normally distributed and therefore summarized in terms of medians and ranges. Changes from baseline (day 1) to day 2, day 7, day 14, day 30 and day 60 were analyzed using a nonparametric Wilcoxon Signed Rank test. Creatine responses were defined as a decrease of at least 30% from baseline. The frequencies and percentages of creatine responses were summarized in tabular

format. Creatine response rates were reported along with the corresponding two-sided 95% confidence intervals (CI) which were constructed using the Wilson score method. AHK outcomes were normally distributed and summarized in terms of means and standard deviations and changes from baseline were evaluated using a paired t-test. AHK and NTx outcomes were checked for differences between the single patch group and the double patch group. Amino Acid outcome parameters were summarized in terms of means, standard deviations, and medians. Changes between

consecutive time points (day 1 vs. day 2, day 2 vs. day 7, day 7 vs. day 14 etc.) were evaluated using a nonparametric Wilcoxon signed rank test (Table 1). Absolute changes from baseline (day 1) to day 2, day 7, day 14, day 30 and day 60 were also summarized in terms of means, standard deviations, medians, and interquartile ranges. All reported P-values are two-sided and P<0.05 was used to define statistical significance. Statistical analyses were conducted using R software, version 4.1.0.

**Table 1:** NTX response (>30% decrease in creatine levels from baseline).

Day	N	Number of Responses	Response Rate (95% CI)
2	18	5	28% (12-51%)
7	18	8	44% (25-66%)
14	18	7	39% (20-61%)
30	18	8	44% (25-66%)
60	17	7	41% (22-64%)

## Results

### NTx (Tables 1 & 2)

Because this study was only 60 days, and the bone development cycle occurs over 150 days we did not see significance across all parameters. We did see in all data points (2-6) the necessary 30% decrease in NTx-creatinine levels showing that osteoclastic resorption was taking place. This test should be repeated in a

way that covers all the cycles involved and specific analysis on a subject-by-subject basis should be done to confirm what % of participants see calcium re-absorption into the bone and changes in the osteoclastic resorption phase. A significant decrease in AHK-Cu concentration (-12.65 ng/ml, p=0.0489) was observed from baseline to Day 30, indicating that AHK-Cu was being used to build bone, muscle, tendons, skin, and hair.

**Table 2:** NTx: Creatinine levels at each assessment time point.

Day	N	Median	Range
1 (baseline)	18	2.45	0.03-24.88
2	18	4.78	0.03-12.74
7	18	2.58	0.03-22.34
14	18	2.73	0.03-36.77
30	18	2.45	0.00-34.90
60	17	2.7	0.32-75.44

### AHK-Cu

(Tables 3 & 4).

**Table 3:** Changes in AHK-Cu concentration from baseline to day 30.

AHK Outcome	Day	Mean	SD	p-value
AHK-Cu Peak Area	30	-4626	9252	0.0489
Amount of AHK-CU injected (ng)	30	-0.19	0.38	0.0488
Total AHK-Cu in Sample (ng)	30	-18.97	37.95	0.0489
AHK-Cu Concentration in Sample (ng/ml)	30	-12.65	25.3	0.0489

**Table 4:** AHK: AHK outcomes at each assessment time point.

Outcome Parameter	Day	N	Mean	SD
AHK-Cu Peak Area	1 (baseline)	18	11708	8104
	2	18	11669	13313
	7	18	9731	5507
	14	18	10564	6526
	30	18	7082	4441
	60	17	7737	5417
Total AHK-Cu in Sample (ng)	1 (baseline)	18	48.02	33.24
	2	18	47.86	54.61
	7	18	39.91	22.59
	14	18	43.33	26.77
	30	18	29.05	18.22
	60	17	31.74	22.22
AHK-Cu Concentration in Sample (ng/ml)	1 (baseline)	18	32.01	22.16
	2	18	31.91	36.4
	7	18	26.61	15.06
	14	18	35.02	36.59
	30	18	19.36	12.14
	60	17	26.85	33.17

**Specific Amino Acids of Significance**

(Tables 5 -7).

**Table 5:** Absolute decreases from baseline (Day 1).

Parameter	Day	N	Mean	SD	Median	Lower 25ht Percentile	Upper 75th Percentile	p-value1
DA	7	17	-14.9	27.9	-11.4	-23	-1.6	0.0395
Hist	30	16	-2.1	5.4	-3.9	-6	0.5	0.0739
LDOPA	2	17	-4.4	8	-1.2	-10.3	2.8	0.0963
LDOPA	30	16	-4.7	8.7	-2.8	-10.5	1	0.0676
Cystathionine	2	17	-1.8	2.8	-1.8	-3.6	-0.7	0.0224
HYP	7	17	-2.9	2.3	-2.9	-4.8	-1.4	0.0003
HYP	14	17	-2.1	3	-2.5	-4.6	0.5	0.0202
HYP	30	16	-2.2	2.8	-2.6	-4	0.1	0.0092
HYP	60	14	-2.5	3	-1.8	-5.4	-0.3	0.0061
Sar	60	14	-2	3.5	-1.4	-3.4	0.7	0.05
Leu	60	14	-1.8	3.2	-2	-5.1	0.7	0.0676
His	2	17	-29.1	75.8	-7.7	-23.8	1	0.0569
Ser	60	14	-10.8	20.5	-5.9	-13.4	-0.2	0.0756
Ala	2	17	-4.2	33.3	-0.7	-6.3	1.3	0.7467
Ala	7	17	-2.2	27.2	2.3	-10.9	8.5	1
Ala	14	16	-1.3	29.1	3.2	-2.5	6.1	0.3484
Cys	60	14	-7.4	12.3	-3.6	-7.8	0	0.0068

**Table 6:** Absolute increases from baseline (Day 1).

Param	Day	N	Mean	SD	Median	Lower 25th Percentile	Upper 75th Percentile	p-value1
GSH	7	17	0.2	0.5	0.3	0	0.5	0.0627
Gln	7	17	36.9	48.9	22.8	6.6	59.6	0.004
Gly	2	17	39.9	101	13.6	0.4	24.5	0.0038
Asn	14	16	21.3	38.8	11.4	-4.1	42.5	0.0654
Ala	30	15	0.8	27	1.2	-4.8	15.9	0.4543
Ala	60	14	3.4	21.2	2.1	-5.4	9.5	0.6257

**Table 7:** Summary statistics of outcome parameters, stratified by time point.

Parameter	Day	N	Mean	SD	Median	p-value1	p-value2	p-value3	p-value4	p-value5
5-HT	1	17	109.7	24.7	110	0.6441	0.3061	0.0695	0.025	0.9999
5-HT	2	17	107	25.2	102.4					
5-HT	7	17	101.8	24.1	101.7					
5-HT	14	17	115.9	24.4	121.3					
5-HT	30	16	101.8	28.6	103.3					
5-HT	60	14	103	26.8	105.8					
NE	1	17	37.6	9.3	37.3	0.4307	0.0448	0.174	0.5966	0.5416
NE	2	17	35.4	8.5	36.2					
NE	7	17	39.7	9	38					
NE	14	17	35.8	8.5	35.9					
NE	30	16	34.9	8.6	36.6					
NE	60	14	32.8	8.9	32.5					
ME	1	17	27.3	6.9	27.9	0.4874	0.3348	0.0459	0.0021	0.6698
ME	2	17	26.3	6.7	26.5					
ME	7	17	25.2	8	22.1					
ME	14	17	29.1	6	29.4					
ME	30	16	25.1	7.3	25.1					
ME	60	14	24.5	8.1	25.3					
HCys2	1	17	1.4	0.6	1.2	0.1328	0.9522	0.5896	0.6278	0.0308
HCys2	2	17	1.6	0.6	1.3					
HCys2	7	17	1.5	0.7	1.3					
HCys2	14	17	1.3	0.5	1.3					
HCys2	30	16	1.3	0.5	1.1					
HCys2	60	14	1.1	0.4	1.1					
Hcys/HCys2	1	17	0.9	0.3	0.9	0.1626	0.5871	0.4304	0.5336	0.0513
Hcys/HCys2	2	17	0.8	0.3	0.7					
Hcys/HCys2	7	17	0.8	0.2	0.8					
Hcys/HCys2	14	17	0.9	0.4	0.8					
Hcys/HCys2	30	16	0.9	0.3	0.9					
Hcys/HCys2	60	14	1	0.4	1.1					
Cystathionine	1	17	14.9	6.8	13.9	0.0224	0.4874	0.48	0.6412	0.8198
Cystathionine	2	17	13.1	6.6	11.4					
Cystathionine	7	17	14.9	7.2	13.9					
Cystathionine	14	17	13.3	6.7	13.9					
Cystathionine	30	16	12.4	6.8	12.6					
Cystathionine	60	14	14.7	8.4	14.4					



AAA	1	17	13.4	9	11.4	0.2744	0.2293	0.4657	0.0258	0.4917
AAA	2	17	15.2	12.1	12.1					
AAA	7	17	14.3	12.4	9.6					
AAA	14	17	14.2	11.1	7.9					
AAA	30	16	12.3	10.7	6.6					
AAA	60	14	10.9	10.6	5.6					
ABA	1	17	7.5	3.1	7.1	0.9357	0.4515	0.091	0.4716	0.4854
ABA	2	17	7.4	2.2	6.9					
ABA	7	17	7.1	2.6	6.3					
ABA	14	17	8.2	2.9	6.9					
ABA	30	16	7.8	3.2	6.5					
ABA	60	14	7.3	2.6	7.6					
HYP	1	17	14	2.8	15.3	0.1231	0.0348	0.2633	0.98	0.7728
HYP	2	17	12.9	3.2	13.7					
HYP	7	17	11.2	2.8	11.3					
HYP	14	17	12	3.1	11.6					
HYP	30	16	11.7	3.8	12					
HYP	60	14	11.4	3.8	10.5					
GSH	1	17	1.4	0.5	1.3	0.4262	0.0857	0.9222	0.2217	0.5085
GSH	2	17	1.4	0.4	1.3					
GSH	7	17	1.6	0.5	1.5					
GSH	14	17	1.6	0.5	1.8					
GSH	30	16	1.4	0.4	1.4					
GSH	60	14	1.4	0.5	1.3					
Lys	1	17	26.2	23.5	16.4	0.2633	0.0032	0.1973	0.9341	0.946
Lys	2	17	20.2	12.7	15.9					
Lys	7	17	30	23.3	15.2					
Lys	14	16	25.4	30.5	15.2					
Lys	30	15	18.5	8.9	16					
Lys	60	14	16.8	12.3	12.8					
Gln	1	17	169.2	70.9	193.7	0.6356	0.0004	0.1754	0.5336	0.2163
Gln	2	17	178.8	87	197					
Gln	7	17	206	82.8	231					
Gln	14	16	188.4	89.2	194.5					
Gln	30	15	190.3	96.2	194.7					
Gln	60	14	165	96.7	154.4					
His	1	17	102.8	88.6	69.8	0.0569	0.0013	0.91	0.1354	0.4548
His	2	17	73.7	53.2	58.9					
His	7	17	93.8	65.7	73.8					
His	14	16	93.7	68.4	75.9					
His	30	15	125.3	108.2	80.2					
His	60	14	87.9	84.8	57.1					
Trp	1	17	14.8	14.2	13.6	0.6526	0.2078	0.1826	0.0859	0.6909
Trp	2	17	13.7	13	16.1					
Trp	7	17	17.5	17.9	19.2					
Trp	14	16	11.3	9.6	11.7					
Trp	30	15	15	16.4	12.5					
Trp	60	14	10.1	10.5	8.5					

Note:

p-value<sup>1</sup>: p-value for comparing Day 1 vs. Day 2

p-value<sup>2</sup>: p-value for comparing Day 2 vs. Day 7

p-value<sup>3</sup>: p-value for comparing Day 7 vs. Day 14

p-value<sup>4</sup>: p-value for comparing Day 14 vs. Day 30

p-value<sup>5</sup>: p-value for comparing Day 30 vs. Day 60

## Food Diaries

Food diary summaries showed that most vegetarians and vegans involved in the study failed to eat sufficient foods which contained lysine.

## Discussion

This data demonstrates that the LifeWave X49 TM patch triggers the use of more AHK-Cu. AHK-Cu is involved in the creation of bone, tendon, skin, hair etc. X49 TM does effect calcium re-absorption in a cycle 30-40 days as shown by the NTx and Hydroxyproline data. The change in NTx, as a measure of bone change, while not statistically significant across all participants at all data points, is still an exciting finding. It is also supported by the significant decrease in Hydroxyproline at 5 datapoints. The decrease in osteoclast activity means that less of the bone is being broken down, [8] which means there is less bone for the osteoblastic bone formation to replace. We can see that clearly at data point 5 (30 days). The standard cycle of bone formation of 150 days is longer than the measurement period of this study. As a result, not every participant showed a change in re-absorption. Additional studies of longer duration are suggested so that the effects of the X49 TM patch, on the full cycle of the bone repair process, can be documented. Most medications for bone support have significant side effects. We had no side effects reported. In addition, we had two individuals in the study who had standard bone density scans during the study. Both individuals showed a slowing of bone loss and an increase in bone density in areas that had been previously compromised. This is a wellness product and not a medical product, however, consideration should be given to this patch which could potentially be used to mitigate early bone loss.

There were some surprises in the data. X49 TM triggered support of the dopamine pathway. It was significant on day 2 and day 30. X49 TM triggered support for 5HT in the catecholamine pathway supporting serotonin production. Women in the study got less depressed and probably slept better. This is a very interesting finding as many women at the peri-menopausal and menopausal stage suffer from chronic imbalances between serotonin and dopamine and can have fairly significant mood swings. It is also logical that the trans-sulferation pathway is stimulated and X49 TM triggers more glutathione production. In addition, we saw 14 amino

acids change production levels at significance over the 60 days. In general, we would expect to see an occasional spike in one or two amino acids but like the X39 TM product we again saw significance in a much larger than expected number. The amino acids which changed were also spread between the catecholamine, serotonergic, glutaminergic, transulferation, and histidine pathways, giving them a very broad impact. In addition to the amino acid changes we saw a significant increase in 2-amino adipic acid, which is produced by lysine degradation. This lends support to the theory that amino acids were being used. AHK and NTx outcomes were checked for differences between the single patch group and the double patch group. No significant differences were found.

What was expected and was not seen was an individual increase of Alanine as a direct amino acid measured by urine, but the amino acid data instead showed that Leucine went to almost significance at 0.06 on day 60 and it is converted into Alanine. That may be because we were not checking blood and AHK-Cu was being used and was not available free in the urine. It is also possible checks were not made for a long enough period given the 150-day bone replacement cycle. It was clear that AHK-Cu dropped significantly at 30 days which is a result if the tri-peptide was being used more effectively. Histidine had significance on day 2 at 0.05 and almost significance on day 30 at 0.07. This means that much of the expected pathway information was demonstrated. It should be noted that this was an open label study. Subsequent trials should repeat this research both as a double-blind and with a 150 day plus duration. This would allow a complete bone re-absorption and formation cycle to be monitored. Additional follow up studies could include lysine supplements, as it was found that multiple participants were not consuming a minimum daily requirement in their diet.

## Conclusion

There was a significant change in both AHK-Cu and NTx. A decrease in NTx levels has been shown to correlate to a decrease in osteoclast activity, and thus a decrease in bone breakdown [10] which means there is less bone for the osteoblastic bone formation to replace. Secondary to NTx, we also saw a significant decrease in Hydroxyproline at 5 datapoints. «Hydroxyproline is mostly used as a diagnostic marker of bone turnover» [27]. The combination of these three points strongly suggests that X49 TM decreases the



breakdown of bones during the cycle of bone repair. In addition, we saw 14 amino acids change production levels at significance over the 60 days. The amino acids which changed were also spread between the chatecholamine, seratinergic, glutaminergic, transulferation, and histidine pathways, giving them a very broad impact. Study data is sufficiently significant to warrant further research.

## Declarations

Human Studies Ethics Board approval study number is NFFE01-11-21-1.

## Consent for Publication

Consent to publication was obtained from all participants included in the study.

## Availability of Data and Materials

The datasets used and/or analyzed during the current study are available to appropriately qualified individuals with an approved request.

## Competing Interests

All study team members were paid by Earthsongs Holistic Consulting as consultants to the study. Analysis of blood samples was done at the independent laboratory Axis Pharm in San Diego, CA. Analysis of urine samples was done at the independent laboratory Sabre Sciences Laboratory in Carlesbad, CA. Statistical Analysis was done independently by Dr. Eickhoff, University of Wisconsin -Madison.

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## Authors' Contributions

Each author have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; has approved the submitted version (and version substantially edited by journal staff that involves the author's contribution to the study); agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature. All authors have read and agreed to the published version of the manuscript.

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