# LifeWave X39 Pilot Demonstrates Light Triggered Changes

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# Abstract

**Purpose -** To determine if metabolic and physiological changes were produced by participants wearing the LifeWave non-transdermal X39 phototherapy patch.

*Materials* - LifeWave X39 non-transdermal patches, Sabre Sciences urine and saliva test kits. Amino Acid panel was performed using a TSQ Quantum Triple Quadrupole LC-MS/MS Mass Spectrometer. Adrenal stress panel, cortisol and DHEAS, from saliva were performed using a Sciex 4000 Triple Quadrupole LC-MS/MS Mass Spectrometer and Dynex DSX 4-plate automated ELISA processing system respectively. Physiology testing was done using a Thought Technology Biograph Infinity Physiology Suite with Cardio Pro software. Blood pressure was taken with a Model #BPAG1-20CVS monitor. Questionnaires included: Marlow-Crowne, Arizona Integrative Outcome Scale for Vitality, Pittsburg Sleep Quality Index, McGill Pain Scale, WAIS III- memory subtest, Profile of Mood States.

**Method** - Fifteen subjects of convenience age 40-65 were recruited through flyers and email. Subjects were consented and baseline measures were taken. Patches were worn 8-12 hours each day placed at either the GV14 or CV6 acupuncture points as selected by a computerized algorithm and selected for ease and clarity of patch placement for participants. All measures were taken at all test points: Baseline/day1, day 2 and day 7. Participants were asked to apply patch one hour prior to testing on day 2 and 7.

Cortisol levels were obtained from 8am, 12pm, 4pm, 8pm and 12am time collection saliva Samples were purified with Phenomenex Strata polymeric reversed phase columns and analyzed on Sciex 4000 Triple Quadrupole LC-MS/MS Mass Spectrometer using Phenomenex Kinetex C8 3x100mm, 2.6um, 100A column with linear gradient.

Dehydroepiandrosterone sulfate (DHEAS) levels were obtained at 8am, 8pm and 12am time collections using Pantex salivary cortisol ELISA kit on Dynex DSX 4-plate automated ELISA processing system.

Urine samples were taken by 10am at each test point. WAIS III data was taken immediately, at 10 minutes and at 20 minutes. A full 10 number sequence was used which was randomly generated for each subject at each data point. Amino acid analysis was obtained on TSQ Quantum Triple Quadrupole LC-MS/MS Mass Spectrometer using Phenomenex EzFaast amino acid kit.

**Results -** Significant changes were seen in most essential and nonessential amino acids over the period of one week. These changes include:

- 1. Changes of inflammatory markers and their metabolites
- 2. Improvement in sleep quality
- 3. Reduction in blood pressure
- 4. Improvement in short term memory
- 5. Improvement in reported feelings of vitality

**Conclusion** - While this is a small sample of convenience the positive results in this study suggest that further investigation needs to be done. Both the effect that appears to produce a number of significant amino acid changes in a short period of time and the improvement in short term memory which are particularly relevant to an ageing population and should be explored.

Keywords: Photobiology, Phototherapy, Meridian

# Introduction

The X39 patch uses phototherapy to stimulate the tripeptides GHK and GHK- Cu (Connor et al, May 2020). This tripeptide (glycine, histidine, and lysine) was first isolated in 1973 by Dr. Loren Pickart. This is important as the "copper tripeptide-1 belongs to a group of emergency response molecules which are released during injury and come to the body's aid..." (DeHaven, C., 2014) It is naturally released by the body when there is any type of injury to tissue. For example: the "copper tripeptide-1 has been suggested to have a potential therapeutic role in age-related neurodegeneration and cognitive decline. It improves axon survival and maintenance of nerves." (DeHaven, C., 2014)

This pilot study explores the metabolic and physiologic results of wearing the LifeWave nontransdermal patch over the period of one week. Measures were taken at baseline, 24 hours and at 7 days of wearing the patch. A sample of convenience of 15 subjects made up of both men and women aged 40-65 were selected to participate in this study. Study approval from National Foundation for Energy Healing number NFFEH 8-28-18-07.

# Non-transdermal Patch

The LifeWave patch is non-transdermal. This means that instead of having chemicals imbedded in the patch and intended to be absorbed, nothing contained within the sealed patch enters the body. Instead, this allows the light to flow through the patch without any changes to the contents of the patch throughout the time the patch is worn.

# Phototherapy

Phototherapy uses light to promote changes in the body. The patch uses infrared, light, near infrared light, and visible spectrum light passing through specially processed crystals in solution to send out specific wave lengths that facilitate those changes. The LifeWave patches help to stimulate the body by using that light to create physiological effects. In the past 100 years, in repeated trials of various phototherapy products, there has been only rare reported incidents of negative side effects.

#### Meridian Implications and Patch placement

LifeWave patches are placed on specific meridian points to maximize effectiveness. The theory of balancing the body based on the Chinese meridian system is over 3000 years old. Current information now maps the meridian system to parts of the lymphatic system. The concept of the release of "Qi" on an area of the lymphatic system is consistent with the evidence that the body has a variety of electrical-dermal potentials across its surface (Becker & Selden, 1985, Flick, 2004).

There are two options which were selected for patch placement. The first GV14 effects Yang meridians of the body, which helps prevent illness. It also clears heat, which would include inflammation and redness, which is a common sign of tissue damage. (Deadman, P., 2001) The second option CV-6, supports the Kidneys and harmonizes blood. (Deadman, P., 2001) Computer randomization was used with half the participants using the CV6 point and half using the GV14 point.

# Metabolic test Panel

It is well known fact that this tripeptide glycine-histidine-lysine (GHK) has the ability to bind on the cell surface and transmit copper in and out of cells, which may help copper enzyme activities, including phenol oxidase, alanine synthase, tyrosinase, and cytochrome oxidase.

The purpose of this study is to follow the production of tripeptide glycine-histidine-lysine

its degradation into glycine, histidine and lysine amino acids, the relation to glutathione (GHK), production, and their metabolic effect.

We have selected the Sabre Sciences HPA4 metabolic panel, **which** involves urine and saliva collection. The urine testing evaluates all 22 natural amino acids, in addition their metabolites including major inflammatory markers such as glutathione, cysteine to cystine ratio, homocysteine and homocystine ratio.

This test also determines level of neurotransmitters and their intermediates including 5-HTP, Serotonin, Dopamine, Epinephrine, Norepinephrine, GABA, Glutamic acid, Histamine, L-DOPA, Normetanephrine, Metanephrine, and 3-Methoxytyramine. The salivary testing evaluates adrenal hormones such as cortisol and DHEAS.

Metabolic testing consisted of one 10am urine taken at baseline/day one, day two and day seven. Saliva Testing consisted of six swabs taken in one day at baseline/day one, day two and seven. Subjects self-administered the swab when in the laboratory under the supervision of Dr. Connor. The swab was then placed back in the shipping container and labeled with the subject number. Samples were kept in the freezer at -20F and were shipped to the Sabre Sciences laboratory in Carlsbad, CA on a daily basis.

# **Physiology Suite**

In this study the initial baseline readings were taken, and then the X39 patch was applied. The participant was asked to wear the patch 8-12 hours each day. The participant removed the patch at night and a fresh patch was applied each morning prior to 8 am. The patch was worn for a minimum of 1 hour before the additional data measures were taken. Patches were worn for a total of 7 days. Data taking with the patch applied was done on day one, day two and day seven.

All study participants had the following physiological testing done at base line, day 2 (24 hours) and 7 days: Six minute recordings of EKG, pulse, respiration, heart rate variability (HRV), temp, blood volume pulse, galvanic skin response, and 2 EMG (muscle) leads (one on each shoulder area). At baseline testing, participants were checked for any allergic reactions to the adhesive used on patches. No participants in this study had a reaction to the patch adhesive.

#### Questionnaires

A series of questionnaires were administered to support the metabolic findings.

- The Marlowe Crowne is a 13 Item true/false short form that is a measure of subjects' tendencies to give answers that they may perceive are desired by the interviewer and/or reflect perceived positive social norms. This variable can produce inaccurate or misleading findings unless properly controlled for in statistical analysis. Estimated completion time 2 minutes. This instrument was normed by Reynolds in 1982.

- The McGill Pain Scale is comprised of three sections regarding a subject's pain. Section number one asks, "What Does Your Pain Feel Like?" and then lists 20 descriptive categories that are numerically rated as 1 – 5. Section number two asks, "How Does Your Pain Change with Time?" The first question asks the subject to describe the pattern of their pain; and this is followed by 22 descriptive terms regarding their effects on increasing or decreasing pain. The third section reflects upon the strength of the pain and has six questions that give a context and comparison to the current pain compared to other types of pain suffered in the past. Again this section is ranked numerically 1-5, with one being mild and five being excruciating. (Melzack, R., 1975)

- The Pittsburg Sleep Quality Index is comprised of a mix of quantitative questions and five Likert scale questions 0-3. It explores the type and quality of sleep experienced by an individual

over the past month. It was normed by Cole et al. (2006). (normed is correctly used, can't get spellcheck to accept it)

- The Arizona Integrative Outcome Scale, Visual Analogue Scale (AIOS-VAS) for Vitality rates subject's "overall sense of well-being and vitality" over the past 24 hours, using a 100mm one-line visual analogue scale. This instrument was normed by Bell in 2004.

- The Profile of Mood States (POMS) is a 37-item 5 factor scale which is a basic measure of affective state in several dimensions including vigor, mood, anxiety and depression. 8 minutes maximum completion time. It was normed by McNair et al., 1971/1981; Shacham, 1983.

#### - The WAS III

A subsection of the WAS III, the memory subtest was used to determine level of short, mid and long-term memory and if there is an improvement in memory from baseline to 7 days.

# **Participants**

A sample of convenience of individuals who responded to locally placed flyers consisted of 15 individuals. There were five men and 10 women in the study which had a mean age of  $61.9 \pm 9.3$  years.

# **Statistical Analysis**

Data from questionnaires were collected on standard answer sheets and scored. Then the data points were entered into an excel spread sheet for statistical evaluation. Standardized tests were scored and reviewed for data trends and significant results. All questionnaires parameters were summarized in terms of means and standard deviation, stratified by assessment time point. Changes between assessment time points were evaluated using a paired t-test or nonparametric Wilcoxon Signed Rank test. All physiology parameters were summarized in terms of means and standard deviation, stratified across the 6 study epochs. Changes from pre- to post-patch administration were evaluated using a paired t-test. Normal probability plots were examined to verify the distribution assumptions. All reported P-values are two-sided and P<0.05 was used to define statistical significance. All metabolic parameters were summarized in terms of means and standard deviation, stratified by assessment time point. Changes from day 1 (pre-patch) to day 2, day 2 to day 7, and day 1 to day 7 were evaluated using a paired t-test or nonparametric Wilcoxon Signed Rank test. Cortisol levels were obtained at 8am, 12pm, 4pm, 8pm and 12am.

DHEAS levels were measured from 8am, 8pm and 12am collection times. The area under the curve (AUC) for Cortisol and DHEAS levels over the data collection periods were calculated using the trapezoid rule. AUC levels were summarized in terms of means and standard deviations, stratified by assessment time point. Changes between assessment time points were evaluated using a paired t-test or Wilcoxon signed rank test.

# Results

Significant results of the X39 patch testing are as follows:

	N (%)
Gender	
Female	10 (67%)
Male	5 (33%)
Age (yrs), means ± SD	61.9 ± 9.3

# Table 1: Demographics (N=15)

# **Questionnaire Outcomes**

- Marlowe-Crowne
- AIOS-VAS
- WAIS III
- Profile of Mood States (POMS)
- Modified Pittsburg Sleep Quality Index
- McGill Pain Instrument

# Table 2: Summary of Marlowe-Crowne instrument scores, stratified by day

Day	Ν	Mean	SD
1	15	16.0	2.1
7	15	15.5	2.4

# Table 3: Changes in Marlowe-Crowne instrument scores from day 1 to day 7

	Mean Change	SD	p-value
Change from day 1 to day 7	-0.47	0.2	0.4614

The Marlowe-Crowne looks at social desirability. It is used to measure the reliability of the data. One common confounding variable in research is research subjects telling the researchers the answers they think the researchers want. The middle range that is being shown here suggests that this was not an issue with this particular group. It is also interesting that this group had a non-significant negative mean change, indicating that the responders became more honest.

Day	Ν	Mean	SD
1/Baseline	15	62.7	22.8
2	14	71.1	27.3
6	15	78.0	23.1

# Table 4: Summary of AIOS-VAS instrument scores, stratified by assessment time point

Table 5: Changes in AIOS-VAS instrument scores from Consent to 1.2,Consent to day 2, and Consent to day 7 assessments

	Mean Change	SD	p-value
Change from 1.1 to 2	7.6	15.3	0.0877
Change from 1.1 to 7	15.3	20.6	0.0151

The AIOS-VAS looks at the overall wellness of an individual. In this case there was a clear, though not quite significant positive shift established by the second day of testing. It had increased to significance by day 7 showing clear overall improvement in the feelings of vitality and wellness.

Time	Outcome	Ν	Mean	SD
Day 1	# Short	15	6.2	1.9
	# Mid	15	5.3	2.3
	# Long	15	5.1	2.3
Day 7	# Short	15	7.3	2.3
	# Mid	15	6.1	2.9
	# Long	15	6.1	3.1

# Table 6: Summary of WAISIII instrument scores, stratified by assessment time point

Table7: Changes in WAISIII instrument memory test scores from day 1 to day 7

	Memory Test	Mean Change	SD	p-value
Change from Day 1 to Day 7	#Short/Immediate	1.1	2.4	0.0872
	# Mid/ 10 min	0.8	2.9	0.3008
	# Long/ 20 min	1.1	3.2	0.2170

Memory is a common issue for people above age 45. The WAIS III is a well-established intelligence test which includes a standard memory test. There was a clear trend, though not significant, improvement in short term memory by day 7. It is likely that this would get more significant with a larger group of people and a longer intervention period. It is also interesting that there was improvement in both mid and long-term memory as well, though to a much lower degree. Further research should be done in this specific area as a non-invasive support to memory as one ages has the potential to be of benefit to many individuals.

# Table 8: Summary of modified PSQI instrument total scores,stratified by assessment time point

Time	Ν	Mean	SD
Day 1	11	6.7	2.8
Day 2	11	5.2	2.7
Day 7	7	4.6	2.9

# Table 9: Changes in modified PSQI instrument scoresfrom day 1 to day 2 and from day 1 to day 7

	Mean Change	SD	p-value
Change from Day 1 to Day 2	-1.0	1.3	0.0676
Change from Day 1 to Day 7	-3.0	2.9	0.0522

The Pittsburg Sleep Quality Index was used to look at sleep, which is also a common issue once people get past 45. This questionnaire showed an immediate strong shift the first night,

though it was not quite significant. The shift by day 7 was significant. This is particularly important as sleep strongly effects everything else related to health and wellbeing.

There were no significant changes in the Profile of Mood States and the McGill Pain scores from the baseline (pre-patch) assessment to the close of the study.

## **Metabolic Outcomes**

Marker	Change	Mean Change	SD	p-value
Alanine	Day 1 to Day 2	-20.17	36.89	0.0526
Cystine	Day 2 to Day 7	-16.07	23.86	0.0206
Epinephrine	Day 1 to Day 2	-2.09	3.08	0.0197
Epinephrine	Day 2 to Day 7	1.59	2.94	0.0552
Glutamate	Day 2 to Day 7	-3.82	6.73	0.0453
Glutamate	Day 1 to Day 7	-5.82	10.37	0.0475
Glycine	Day 1 to Day 2	-72.54	117.73	0.0317
HCys2	Day 1 to Day 2	0.35	0.55	0.0296
Histamine	Day 1 to Day 2	-46.32	75.35	0.0320
Histamine	Day 1 to Day 7	-46.64	49.35	0.0026
Leucine	Day 1 to Day 2	-4.84	7.84	0.0313
Normetanephrine	Day 2 to Day 7	-13.06	23.32	0.0479
PEA Phenylethylamine	Day 1 to Day 7	-0.59	1.12	0.0589
Phenylalanine	Day 2 to Day 7	6.33	10.94	0.0418
Tryptophan	Day 2 to Day 7	-10.81	18.55	0.0406
Alpha-aminobutyric acid	Day 1 to Day 7	-8.90	13.79	0.0256
Alpha-aminobutyric acid	Day 2 to Day 7	-5.29	7.79	0.0198

Table 10: Change from day 1 (pre-patch) to day 2, day 2 to day 7,and day 1 (pre-patch) to day 7

Amino acids and neurotransmitters play a critical role in the health and wellbeing of individuals. If an individual's amino acid and neurotransmitter production pathways are imbalanced, the individual cannot maintain body health for long.

The number of statistically significant changes demonstrated in this study shows the powerful impact which can be created by the use of phototherapy products and the clear positive changes produced by the application of this specific non-transdermal product X-39. Key findings are in changes of many amino acids levels, including glutamate, histamine, glycine, and indicates distinct reduction of inflammation produced by the patch.

Of note the amino acids cysteine (cystine, an oxidized form of cysteine) glycine and glutamate, which are building blocks of glutathione, and which showed a drop at level of significance indicates biosynthesis glutathione in a trans-sulfuration pathway. Glutathione is

- one of major antioxidant naturally produced in liver
- facilitates metabolism of xenobiotics
- decreases toxins by conjugation and excretion
- prevents cellular damages by scavenging reactive oxygen species,
- free radicals, and heavy metals
- acts as neurotransmitter by modulating the NMDA receptors
- induces GABA release, which is a major inhibitor of neurotransmitters

The changes in all aromatic amino acid levels show improved metabolic activity on dopamine and serotonin pathways as well, and increase in level of epinephrine. More importantly, changes in normetanephrine level gives clear indication of liver function change, specifically monoamine oxidase (MOA) and catechol-O-methyltransferase (COMT) enzyme activity changes. Therefore these enzymes are responsible for regulation of neurotransmitters. Tryptophan is a major building block of serotonin, which is mood booster and healthy sleep producer in dim light.

Further changes in branched chain amino acids, along with alanine level change, indicate glycine-histidine-lysine tripeptide (GHK) synthesis stimulation and positive impact on copper containing enzyme activities, including alanine synthase and tyrosinase, cytochrome oxidase.

# **Physiology Outcomes**

# *Table 11: Change from pre-patch to last-patch (day 7) of HF, LF/HF NN50, PNN50, Power, RMSSD, and VLF, stratified by Epoch (1-6) for Blood Volume Pulse (BVP)*

Source	Outcome	Epoch	Mean Change	SD	p-value
BVP	HF	5	-1085.13	2038.55	0.05830
BVP	NN50	1	-3.13	5.34	0.03950
BVP	NN50	3	-1.73	2.89	0.03580
BVP	NN50	5	-2.73	3.03	0.00360
BVP	PNN50	1	-0.05	0.08	0.03820
BVP	PNN50	3	-0.03	0.04	0.04290
BVP	PNN50	5	-0.04	0.04	0.00360
BVP	RMSSD	5	-21.13	36.49	0.04160
BVP	SDNN	5	-19.42	27.08	0.01480
BVP	VLF	5	-382.47	426.65	0.00370

Source	Outcome	Epoch	Mean Change	SD	p-value
BVPHR	Average	2	4.51	7.83	0.0426
BVPHRMaxMin	Average	5	-2.23	3.13	0.0153
BVPHRMaxMin	Mode	1	-1.18	2.17	0.0533

Table 12: Change from pre-patch to last-patch (day 7) of BVP-HR, for Average, Mode, and Area, stratified by Epoch (1-6)

Table 13: Change from pre-patch to last-patch (day 7) of BVP-HR and EMG for Average,
Mode, and Area, across all 6 Epochs

Source	Outcome	Mean Change	SD	p-value
BVPHR	Average	2.54	8.31	0.0047
BVPHR	Mode	2.31	9.60	0.0249
BVPHRMaxMin	Average	-1.67	5.50	0.0049
EMG	Average	-39.44	124.62	0.0035
EMG	Mode	-38.45	128.72	0.0057
EMG	Area	-2366.63	7477.30	0.0035

Reduction in blood pressure and improved muscle relaxation are consistent changes which are present in the physiology data.

#### Discussion

It is important to recognize that this was both a sample of convenience and a short-term pilot study with a small sample size. However, there are several areas that showed significant change, as well as a few that showed a clear trend towards change, though not quite to the level of significance. It should also be noted that the shift in sleep is especially interesting, given that the research subjects' sleep was being interrupted every four hours for the saliva testing on the days those questionnaires were administered. Therefore, the results may have been stronger without this confounding variable.

The lack of shift in POM, which looks at overall mood, is also interesting. The patch itself was not expected to shift the research subjects' mood, but there was a possibility of this being a secondary effect. Improved sleep and energy often also improve people's mood.

There are several further key pieces of information in this data. The first is the improvement in blood pressure. Further exploration of this parameter should be a priority, as phototherapy is a non-invasive process, in contrast with the challenging side-effects caused by most blood pressure medications, in a more vulnerable population of those who are aging. Reduction in blood pressure would correlate with the aromatic amino acid findings which also would support a GHK tripeptide increase and GHK copper binding peptide increase. This peptide development does not only have antioxidant effect, it helps iron transmission while regulating cardiovascular homeostasis. Further exploration and direct confirmation of tripeptide production should be done.

The second is the impact of the over all improvement in amino acid production, stimulation of enzyme activities, metabolism and overall bodies detoxifying ability. While amino acid production and metabolism change on a daily basis and are strongly impacted by which foods we eat and by our daily activities, the data taken over several days gives a snapshot of change. Longer studies looking at stability of change should be considered. Key findings in the amino acid production are increasing amino acids bioavailability, which are necessary building blocks of protein and energy. The is an interactive process in which the body is engages where the GHK tripeptide stimulates some amino acids production and metabolism, and the result is that some of these amino acids act as inducers for biosynthesis of natural detoxifying agents like glutathione. Glutathione is one of the major detoxifying agents our body produces and has inhibiting effect on amyloid peptide oligomerization, which is well known cause of Alzheimer's disease. Glutathione is one of the liver's main helpers along with cytochrome P450, by conjugating toxins, and allowing free radicals to be excreted from the body.

The third interesting finding is that flexibility in gastrointestinal imbalance is restored. As one ages there is often less flexibility in all the body systems. Flexibility is key in human survival. We must be able to adapt, moment to moment, in order to survive. This patch triggers change in the gut and may be determined in longitudinal studies to be restoring fitness with the body's adaptability to change.

The fourth interesting finding is in the changes to non-essential amino acid levels. Only significant findings were listed in the tables. This indicates it is not only helping the gut microbiome restore fitness and adaptability to change. It is reducing over all gut Inflammation, as seen in the changes in cystine and glutathione production.

The fifth finding is the improved short-term memory within a week, which almost reached significance (0.08). We have noted some aromatic amino acid level increase. These are the major building blocks for dopamine, norepinephrine, epinephrine, and serotonin. Numerous neurological disorders, Parkinson's disease, attention deficit hyperactivity disorder (ADHD), Alzheimer's disease are associated with dopamine and serotonin systems imbalances. By stimulating amino acid production, the potential exists to delay the natural aging process, improve the memory, and protect overall neuronal health. Further exploration in this area should be done.

Longer studies, with larger numbers of subjects to explore the impact of this phototherapy product should be considered.

#### Summary

This study provides information on the experience of participants' metabolic and physiological effects as a result of wearing the LifeWave X39 patch for 12 hours per day for a period of one week. Study scope included questionnaires which were administered concurrently, covering a range of previously reported anecdotal experiences with the X39 patches. Questionnaire data demonstrated improvements in quality of sleep, memory, feelings of vitality, reduction of pain and inflammation, and an overall sense of well-being.

The X39 patch showed clear, significant improvements in metabolic, blood pressure, memory, sleep and other factors over the course of one week. These areas warrant study over longer periods of time in future studies so that a better understanding of the comprehensive nature and effects of the phototherapy produced by this patch may be demonstrated.

The data results of improvement in blood pressure, 17 statistically significant amino acid changes over the 7 days, significant improvement in anti-inflammatory response, improvement in sleep levels, reduction in blood pressure, improvement in short term memory, improvement in reported feelings of vitality all suggest that further research with a larger sample size and over a longer period of time is warranted to confirm this data and the previously reported anecdotal responses in the testing of earlier subjects.

Since this study was done on an aging population it is also important to note that the patch appears to support shifts toward fitness of the gut system and improved adaptability to change. The human body must live in such a way that it can adapt and respond to change moment to moment in order to survive. One of the first areas of the body which shows a reduction in responsiveness to environmental change as we age, is the gut. While this study covered only a one-week period, the effect in the data over the sampling period showed an improved response promoting body fitness and adaptability to change on each subject. If one can change in healthy ways in response to the environment, one can survive longer. Combined with the anti-inflammatory response shown in this data, there is a potential for a positive impact on longevity. Further longitudinal studies need to be undertaken to determine if these effects are maintained or improved over time.

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