Report for Human Clinical Pilot Study
Lifewave Glutathione Patch

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Abstract: A pilot human clinical study examined the efficacy of the LifeWave Glutathione Patch to increase blood glutathione on nine healthy subjects. This study also measured enzymes of the glutathione pathway (Glutathione S-transferase and Reductase) to determine possible mechanisms of action of the patch. To determine whether the LifeWave patches improve detoxification, urine mercury was measured. Results indicate that Life Wave Patches significantly increased blood glutathione and had no overall effect on GSH enzymes. Urine mercury was elevated in some of the subjects.

Introduction

Glutathione (GSH) is a tripeptide composed of the amino acids glutamic acid, cysteine and glycine. It is the major antioxidant in humans and is found in every cell. GSH neutralizes free radicals and reactive oxygen compounds and maintains exogenous antioxidants such as vitamins C and E in their reduced (active) forms. The highest concentration of GSH is found in the liver, where it is important in detoxification (e.g. mercury).

Glutathione is also required to maintain the normal function of the immune system. It is known to play a critical role in the multiplication of lymphocytes, which occurs in the development of an effective immune response. Animal and laboratory studies have demonstrated that GSH has the potential to fight almost any disease, particularly those associated with aging, since free radical damage is the cause of many of the diseases of old age.

Since GSH is not well absorbed into the body when taken by mouth, precursors of GSH are a form of supplementation used to increase GSH. LifeWave has developed a novel way to increase GSH, the non-transdermal GSH Patch. The LifeWave Patches send information signals into the body after being placed on acupuncture points. A previous study demonstrated that LifeWave Patches increased blood GSH an average of three times the baseline values.

The purpose of this trial was to determine if LifeWave GSH Patches increase blood reduced GSH, enzymes involved in the GSH pathway and urine mercury levels over a four-day period after the patches were applied. In an effort to elucidate the mechanism of action of the GSH Patches, the following enzymes were measured: GSH S-transferase levels and GSH reductase.

Glutathione S-transferases (GSTs) are a group of enzymes important in the detoxification of many xenobiotics in mammals. The enzymes protect cells against toxin by conjugating the thiol (sulfhydryl) group of the glutathione to the xenobiotics, and thereby defend cells against the mutagenic, carcinogenic, and toxic effects of toxins. GST activity is present in

plants, insects, yeast, bacteria, and most mammalian tissues especially in the liver, which plays a key role in detoxification.

Glutathione reductase is a ubiquitous enzyme that catalyzes the reduction of oxidized GSH (GSSG) to GSH. Glutathione reductase is essential for the GSH redox cycle that maintains adequate levels of reduced cellular GSH, which serves as an antioxidant reacting with free radicals and organic peroxides.

Results of this study demonstrate that blood GSH levels increased in subjects. There was no detectable change in the GST or GSH reductase enzymes. Urine mercury was increased

Methods

Nine healthy individuals (5 male and 4 female) ranging from 18-65 years of age with no history of disease, pregnancy, drug or alcohol use, or on any medications were subjects in this pilot study. The study was open label, meaning that subjects were told about what the patches do and that the endpoints measured were glutathione and related enzymes in blood. The patches were given to the subjects in unlabeled packaging and they were not told whether the substances are expected to increase, decrease, or not change, to avoid subject psychological influence on the outcome. Samples were labeled with a code to prevent bias by laboratory technicians and the assays are objective tests that cannot be manipulated.

Measurements of whole blood glutathione, glutathione enzyme activities and urine mercury levels were taken at Labcorp, Boulder, CO. These measurements were taken at days 1, 4 and 7 to establish an accurate baseline. Following baseline measurements, the patches were applied by the subject approximately 2" below the navel and replaced every 24 hours (alternating with the sternum as a placement site). Subsequent identical measurements were repeated on days 9, 11, 14 and 16. Urine samples for mercury determination were collected on days 4, 8 (baseline), 14 and 16. Samples were frozen until assayed.

Glutathione Assay

The spectrophotometric/microplate reader assay method for glutathione (GSH) involves oxidation of GSH by the sulfhydryl reagent 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5'-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm. The glutathione disulfide (GSSG) formed can be recycled to GSH by glutathione reductase in the presence of NADPH. Production of TNB over a 5 minute period is proportional to GSH concentration.

Kits of GSH determination were originally obtained from Sigma-Aldrich though significant modification was needed for reliable performance. The most notable modification was substitution of metaphophoric acid (MPA) for 5-sulfosalicylic acid (SSA) for deproteinization of the blood samples. MPA was the reagent used in the original development of the recycling assay, but some researchers substituted SSA because it was better for some auxiliary assays and good for tissues. Other researchers

have found positive interference and erratic results from SSA with blood samples, thought to be a SSA-heme complex. We also found the MPA to give more consistent analyses and adopted its use.

Glutathione S-Transferase Assay
The assay utilizes 1-Chloro-2,4-dinitrobenzene (CDNB), which is suitable for the broadest range of GST isozymes. Upon conjugation of the thiol group of GSH to the CDNB substrate, there is an increase in the absorbance at 340 nm. The assay is intended for the measurement of the total GST activity in cell and bacterial lysates, tissue homogenates, and plasma and erythrocytes lysates.

Glutathione Reductase Assay
The assay uses spectrophotometric determination of GSH reductase activity either by following the decrease in absorbance caused by the oxidation of NADPH at 340 nm (UV assay) or the increase in absorption caused by the reduction of dithiobis (2-nitrobenzoic acid) (DTNB) at 412 nm (colorimetric assay).
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Mercury Analysis
Analysis was performed by a NELAC-accredited modification of EPA Method 245.7, which describes a flow-injection system for cold-vapor atomic fluorescence detection of mercury in water samples. The Hg-Thiourea complex Liquid Chromatography/Cold-Vapor Atomic Fluorescence system (Hg-Tu/IC-CVAFS) incorporates on-line preconcentration and LC separation for speciation of monomethyl and inorganic mercury forms in prepared sample solutions. Extensive validation of this patented system can be found on www.quicksilverscientific.com.

Statistics
Descriptive statistics were generated to summarize the data. Glutathione, GSH Reductase, GSH S-Transferase, Urinary MeHg and Urinary Hg levels were summarized in terms of number of observations, means and standard deviations. Baseline values were computed as the averages across day 1 to day 7 measurements (day 8 for the urinary measurements). Absolute and percentage changes between baseline assessments were computed and evaluated using a paired-t-test. Urinary MeHg levels below the detection level were censored. Histograms were used to verify the normality assumption. The data analysis was performed using SAS® version 9.2 software (SAS Corp., Cary, NC) by Jens Eickhoff, PhD, Associate Professor at Colorado University, Dept. of Statistics and Clinical Studies.

Results
Figure 1 shows overall profile plots for all study outcomes from day 1-16 and as seen, changes were observed in blood GSH and urine mercury. There was a trend towards an increase in GSH. No significant changes in blood GSH Reductase, S-Transferase and no overall changes in urine mercury or methyl mercury were observed. However, there was some variability in response to urine mercury both in baseline and post patch placement measures. Extra data points are included because some subjects gave extra urine.

Figures 2-7 show plots for each subject of blood GSH, GSH Reductase, S-Transferase, urine mercury and methyl mercury. As seen in Figure 2 and Table 1, there was a trend toward an increase in blood GSH. When comparing averaged baseline to each day after patch application, all of the measurements after patch placement were higher than baseline and were as high as 154% (Table 2A). There was some variablity in baseline GSH measures, both between and within subjects (Figure 2).

Figure 1. Means and standard errors of study outcomes for day 1-16,

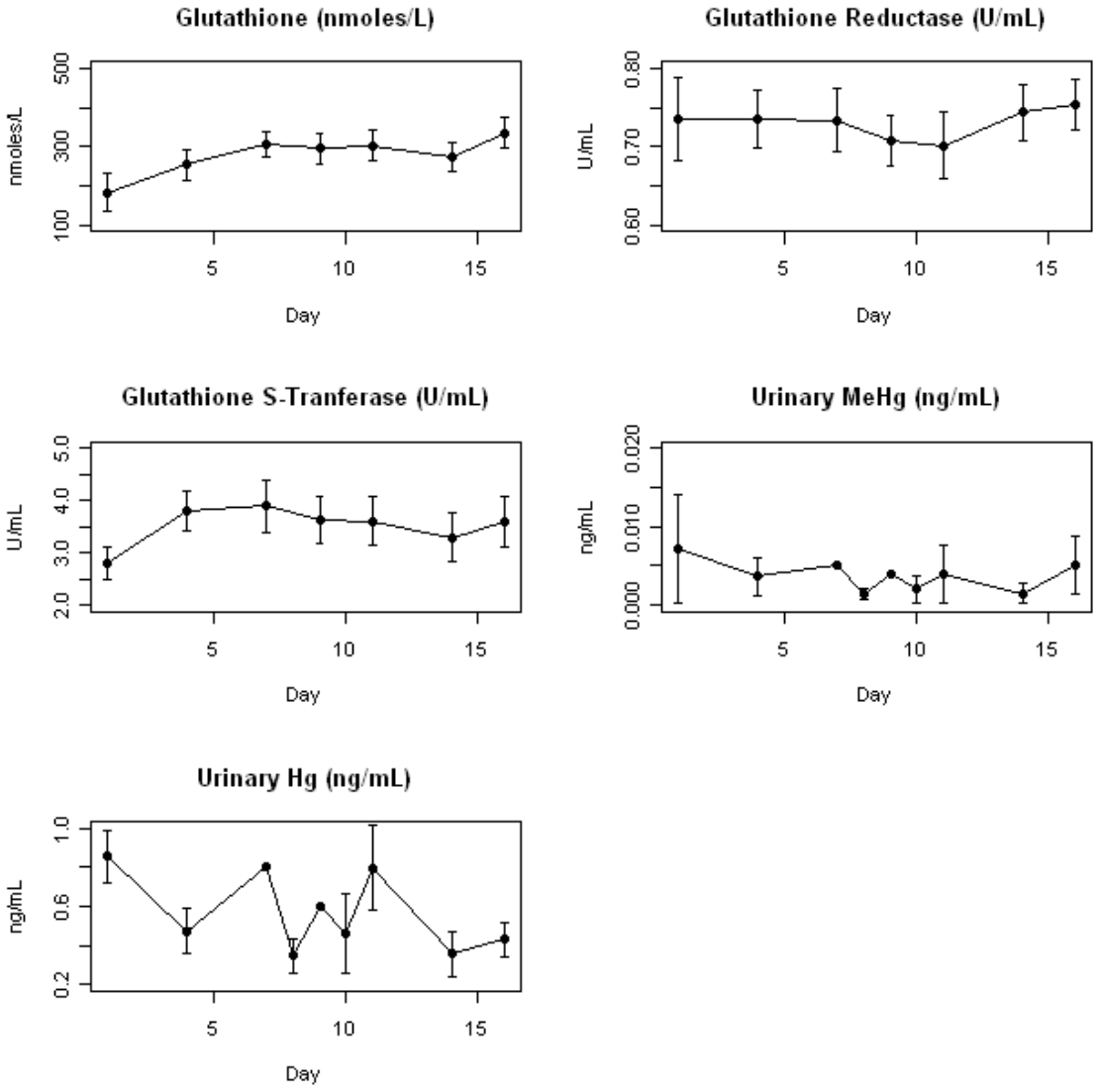


Figure 2. Profile plot for subjects 1 -9 for Glutathione.

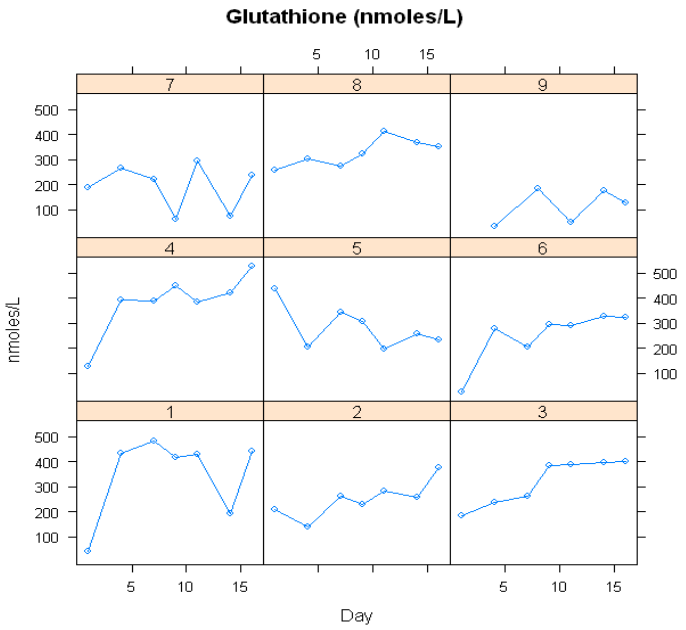


Figure 3. Profile plot for patient 1-9 for Glutathione Reductase

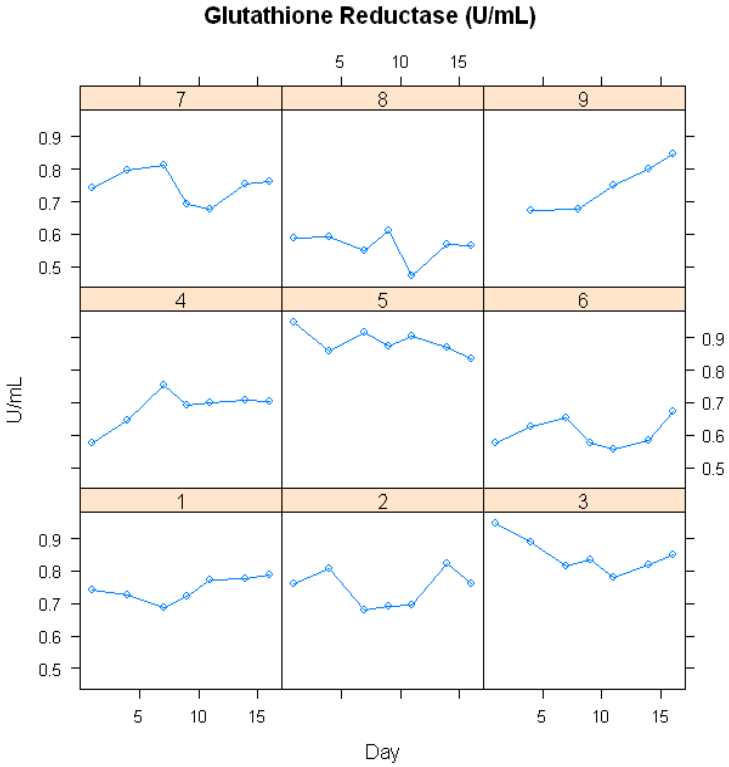
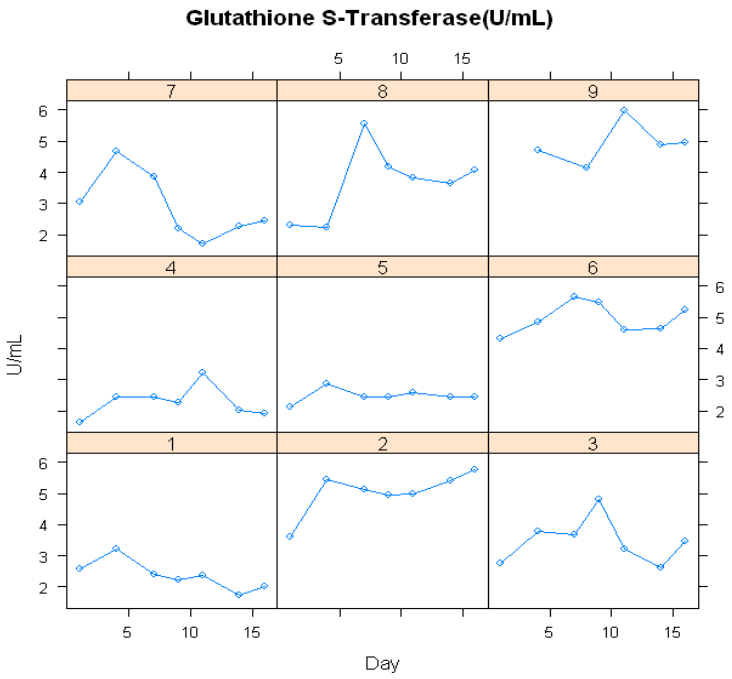


Figure 4. Profile plot for patient 1-9 for Glutathione S-Tranferase.



As seen in Figures 3 and 4, the individual enzyme responses in most of the subjects were not significantly changed.

Figure 5 shows individual subject responses for urinary methyl mercury. Subject 1 had a significant increase at day 16, the last day of the experiment. Subject number 7 showed a significant increase at day 11, 3 days following the first GSH Patch application. In most of the subjects, there were no observable changes in urinary methyl mercury.

Figure 6 shows individual responses for urine mercury. As seen, subjects number 3, 7 and 8 showed a significant increase at day 11. Subject 3 had no change in urinary methyl mercury and subject 7 had an increase in urinary methyl mercury at day 11. The increases in subject number 7's urinary mercury correlated with the urinary methyl mercury increase for this subject.

Overall, there were some increases in both urinary methyl mercury and mercury in some of the subjects, which did not correlate with increases in GSH in those particular subjects. Interestingly, there were no changes in either mercury measurement for subjects 1 and 4, who showed the largest increase in GSH.

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Figure 5. Profile plot for patient 1-9 for Urinary MeHg.

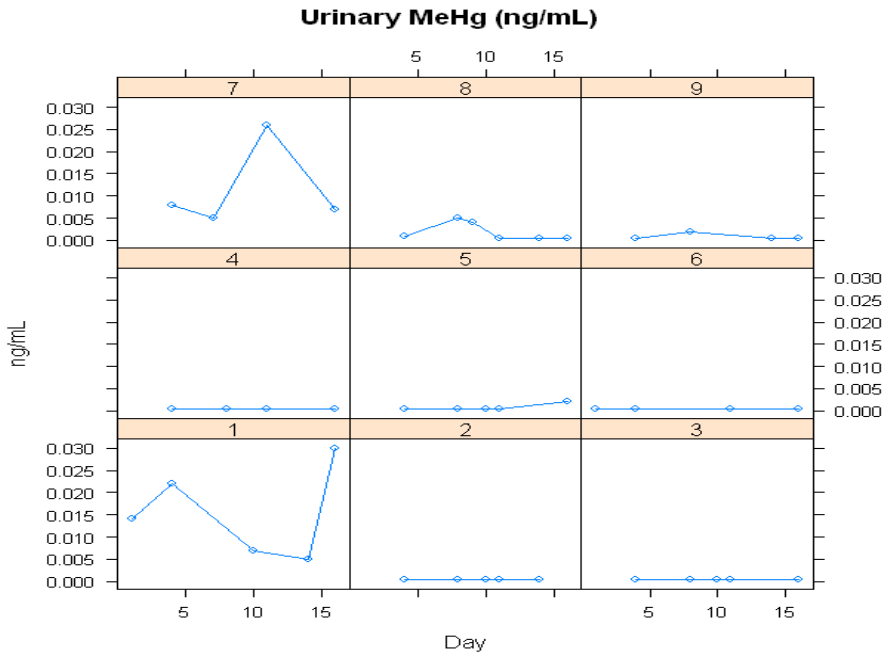

Figure 6. Profile plot for patient 1-9 for Urinary Hg.

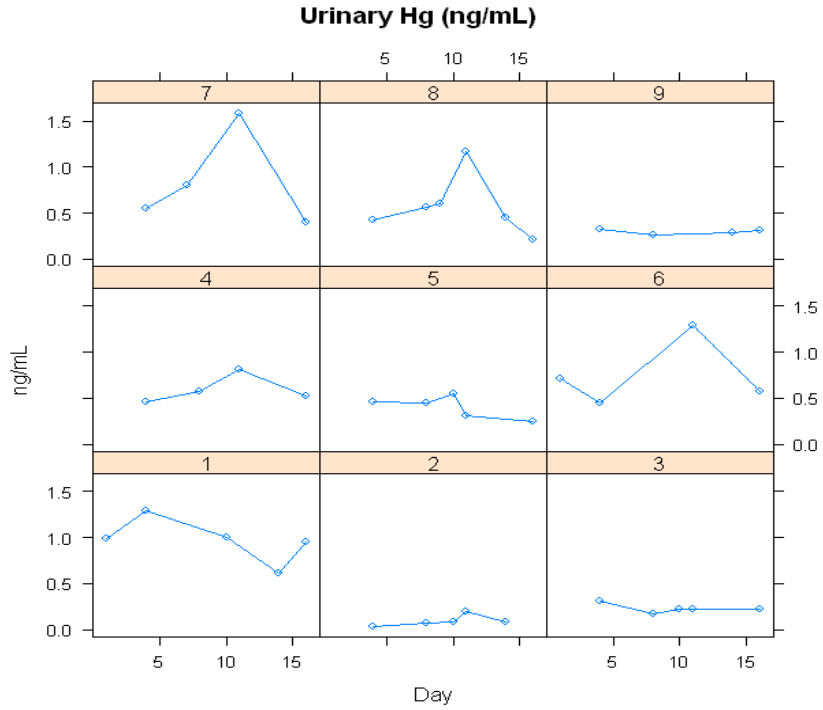

Table 1 shows averaged measures of GSH, GSH reductase, GSH S-transferase, urinary MeHg and urinary HgII levels for each measurement time point (day 1-16). As seen, there was variability in baseline GSH measures, both between and within subjects. However, there was less variability in GSH after patch application, all values were above 248 nmoles/L.

Table 1 Means and standard deviations of Glutathione, Glutathione Reductase, Glutahione S-Transferase, Urinary MeHg and Urinary HgII levels for each measurement time point (day 1- day 16).

	Glutathione (nmoles/L)		
Day	N	Mean	SD
1	8	184.000	130.724
4	9	253.556	121.880
7	8	305.750	94.252
Baseline	9	248.000	122.534
9	9	295.444	121.399
11	9	303.444	121.431
14	9	274.556	114.157
16	9	335.556	121.488
	Glutathione Reductase (U/mL)		
	N	Mean	Standard Deviation
1	8	0.735	0.153
4	9	0.735	0.108
7	8	0.734	0.115
Baseline	9	0.735	0.120
9	9	0.708	0.095
11	9	0.701	0.128
14	9	0.744	0.106
16	9	0.754	0.095
	Glutahione S-Transferase (U/mL)		
	N	Mean	Standard Deviation
1	8	2.794	0.861
4	9	3.802	1.165
7	8	3.895	1.415
Baseline	9	3.509	1.223
9	9	3.630	1.349
11	9	3.606	1.370
14	9	3.297	1.371
16	9	3.586	1.475

	Urinary Hg (ng/mL)		
	N	Mean	Standard Deviation
1	2	0.855	0.193
4	9	0.475	0.340
8	7	0.412	0.258
Baseline	9	0.493	0.313
9	1	0.602	.
10	4	0.461	0.409
11	7	0.797	0.567
14	4	0.357	0.226
16	8	0.431	0.251
	Urinary MeHg (ng/mL)		
	N	Mean	Standard Deviation
1	2	0.007	0.010
4	9	0.004	0.007
8	7	0.002	0.002
Baseline	9	0.003	0.006
9	1	0.004	NA
10	4	0.002	0.003
11	7	0.004	0.010
14	4	0.002	0.002
16	8	0.005	0.010

Table 2A shows the comparison between the averaged baseline measurements and each measurement after patch application. Day 9 showed the highest GSH increase (154.7%) and day 14 showed a 149% increase.

Since there was variability within subjects for baseline measurements, comparisons were made between each baseline measurement and each post-patch application time point (Tables 2B-D). As seen in Figure 2B, the increase in GSH ranged from 434.15-481.15% when comparing the lowest baseline (day 1) measurement to each time point after patch application.

Table 2A-D. Change from Baseline Analysis. Absolute and percentage changes between baseline assessments were computed and evaluated using a paired-t-test. Baseline values were calculated as the average across the day 1 – day 7 measurements (day 8 for the urinary measures).

Table 2A: Means and standard deviations for absolute and percentage changes from baseline (day 1- day 7) for Glutathione

Glutathione (nmoles/L)				
Absolute Changes (nmoles/L) from Baseline (Day 1 – Day 7)				
Day	N	Mean	Standard Deviation	p-value
9	9	63.37	105.68	0.10972
11	9	71.37	87.18	0.03957*
14	9	42.48	125.92	0.34113
16	9	103.48	97.71	0.01305*
Percentage Changes from Baseline (Day 1 – Day 7)				
9	9	71.18%	154.71%	0.20484
11	9	37.10%	33.30%	0.01019*
14	9	63.72%	149.11%	0.23571
16	9	72.51%	89.25%	0.04072

* indicates a statistically significant change

Table 2B. Means and standard deviations for absolute and percentage changes from Day 1 for Glutathione

Glutathione (nmoles/L)				
Absolute Changes (nmoles/L) from Day 1				
Day	N	Mean	Standard Deviation	p-value
4	8	97.13	200.38	0.21272
7	8	121.75	167.62	0.07901
9	8	125.00	197.00	0.11577
11	8	151.13	187.26	0.05642
14	8	102.75	177.95	0.14646
16	8	177.63	201.72	0.04156*
Percentage Changes from Day 1				
4	8	270.93%	446.54%	0.12985
7	8	257.07%	403.05%	0.11422
9	8	287.20%	454.23%	0.11686
11	8	302.51%	443.27%	0.09489
14	8	239.72%	434.15%	0.16233
16	8	337.41%	481.15%	0.08773

* indicates a statistically significant change

Table 2C. Means and standard deviations for absolute and percentage changes from Day 4 for Glutathione

Glutathione (nmoles/L)				
Absolute Changes (nmoles/L) from Day 4				
Day	N	Mean	Standard Deviation	p-value
7	8	24.63	76.85	0.39492
9	9	41.89	108.39	0.27974
11	9	49.89	66.25	0.05381
14	9	21.00	141.69	0.66837
16	9	82.00	83.81	0.01885*
Percentage Changes from Day 4				
7	8	16.19%	41.43%	0.3057
9	9	66.03%	156.27%	0.2406
11	9	29.61%	37.64%	0.0460
14	9	59.51%	150.10%	0.2684
16	9	66.57%	98.34%	0.0767

* indicates a statistically significant change

Table 2D. Means and standard deviations for absolute and percentage changes from Day 7 for Glutathione

Glutathione (nmoles/L)				
Absolute Changes (nmoles/L) from Day 7				
Day	N	Mean	Standard Deviation	p-value
9	8	3.25	93.97	0.92482
11	8	29.38	97.18	0.42085
14	8	-19.00	148.10	0.72742
16	8	55.86	93.35	0.13428
Percentage Changes from Day 7				
9	8	1.94%	38.23%	0.8899
11	8	15.62%	33.22%	0.2254
14	8	-0.21%	47.75%	0.9903
16	8	22.90%	31.5%	0.0806

*indicates a statistically significant change.

For individual GSH responses, individuals with a lower baseline measure exhibited higher increases in GSH. Therefore, subjects were separated into 2 groups, low and high baseline and the baseline measures were averaged for these 2 groups and comparisons were made between low and high baselines and time points following GSH patch application (Table 3). When these comparisons were made, the percentage increase for the low GSH was as high as 207.71 % whereas the highest percentage increase for the high GSH baseline group was only 42.87%.

Table 3. Comparison of changes from baseline in Glutathione values for days 9 – 16 between patients with low glutathione baseline values (<228nmoles/L) and patients with high glutathione baseline values (≥228nmoles/L). 228 nmoles/L is the Median baseline value of Glutathione for the 9 patients in this study.

	Low Glutathione Baseline <228 nmoles/L (=Median)			High Glutathione Baseline ≥228 nmoles/L (=Median)			
Day	N	Mean	Standard Deviation	N	Mean	Standard Deviation	P-value
Absolute Changes (from baseline)							
9	5	60.333	135.29	4	67.1667	73.078	0.93064
11	5	89.733	54.878	4	48.4167	122.427	0.51688
14	5	74.733	133.909	4	2.1667	120.311	0.42687
16	5	121.133	69.039	4	81.4167	133.925	0.57992
Percentage Changes (from baseline)							
9	5	110.33%	207.711%	4	22.2447%	23.5329%	0.43272
11	5	52.881%	18.79%	4	17.376%	39.4027%	0.11533
14	5	112.772%	191.175%	4	2.4093%	39.1648%	0.2993
16	5	108.494%	104.456%	4	27.5393%	42.8692%	0.19302

Discussion

Results of this pilot study demonstrate that the LifeWave GSH Patch increases blood GSH significantly in several of the subjects. Although there was variability in baseline GSH measurements, all of the averaged measurements after patch placement for each time point were above 264.6%, and more importantly, above the average baseline measurement benefit of LifeWave GSH patches. Furthermore, when comparing the lowest baseline value to post-patch values, the increase was as high as 454%.

The GSH increases are substantial in subjects with a lower GSH baseline value, indicating that LifeWave GSH patches are more beneficial for individuals that are deficient in GSH. Another potential benefit for the LifeWave GSH Patches is that they do not overstimulate the GSH system, which could potentially cause harm.

There were no appreciable changes in either GSH Reductase, or GSH S-Transferase, suggesting that these enzymes are not involved in the actions of LifeWave GSH Patches.

There were some spikes in urine mercury levels in some of the subjects, indicating that a consequence of increased GSH levels is an enhanced detoxification. However, the changes in GSH and mercury were not tightly correlated, which does not demonstrate conclusively that LifeWave GSH Patch induced increases in GSH are related to changes in mercury. Further characterization of the amount of GSH needed to change mercury levels and the timing of increases in GSH and mercury are needed. However, it appeasrs that the LifeWave Patches are altering mercury levels, which likely means they are contributing to detoxification of heavy metals., which would be an important benefit of LifeWave GSH Patches. Further investigation is need to determine if LifeWave Patches alter mercury levels and to characterize the effects on GSH production.

References

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Shade, Christopher W. and Hudson, Robert J.M., 2005 Determination of MeHg in environmental sample matrices using Hg-Thiourea Complex Ion Chromatography with on-line cold vapor generation and atomic fluorescence spectrometric detection (HgTU/IC-CVAFS), Environmental Science & Technology, 39, 4974-4982

Research Team

Research was conducted by Lisa Tully, PhD, Andrew Lange, ND and Christopher Shade, PhD

Dr. Lisa Tully received her PhD in Pharmacology and Toxicology from the Indiana University School of Medicine. Dr. Tully has several publications in peer-reviewed medical journals and has presented her research at international scientific conferences. Following her postdoctoral fellowship, Dr. Tully shifted from academic medical research to pursuits in integrative medicine and has attended many international medical conferences over the past decade, evaluating low cost and effective health care. Dr. Tully is currently on the Scientific Advisory Board of several companies and non-profit organizations and is founder of the Energy Medicine Research Institute, whose mission is to assess the efficacy of vibrational medicine technologies and therapies.

Andrew Lange N.D. is a Naturopathic Physician. He served as Chair of the Department of Homeopathic Medicine and Supervising Clinical Physician at Bastyr University in Seattle. He has taught internationally, serving on the faculty of the College of Homeopathy in London. He has consulted in clinics of natural therapeutics in Europe and India. Dr. Lange is the author of Getting to the Root: Treating the Deepest Source of

Disease and a contributing author to A Textbook of Natural Medicine by Pizzorno and Murray.

Dr. Christopher Shade is founder and president of Quicksilver Scientific LLC. He obtained a PhD in environmental sciences specializing in mercury biogeochemistry and analytical chemistry. He founded his company based on patented analytical technology he developed for mercury speciation analysis. Since founding the company he has focused on biochemical aspects of and diagnostic measurements for mercury toxicity and the development of detoxification products and strategies.