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Report for:

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Report 206-001. Clinical study on the X39 patch.

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Report 171-003. Clinical study on the X39 patch.

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Report 171-003. Clinical study on the X39 patch.

1 Executive Summary

Lifewave is a leader in the phototherapy section of the wellness industry. Lifewave's lead product is the X39 patch, which emits infrared light when applied to the skin.

The goal for this clinical proof-of-concept study was to document acute and long-term effects of wearing the X39 patch, where the acute effects were compared to changes when wearing a placebo patch, using a randomized double-blind design. This data is important to verify mitochondrial and inflammation-related effects.

Study

Twelve healthy adults were enrolled and attended two clinic visits for the acute phase of the trial. On these clinic visits, baseline blood draws were immediately followed by the application of either the X39 patch or the placebo patch in randomized order. Additional blood draws were performed after 1 and 2 hours. Then the patches were removed, and the X39 patch applied, for the beginning of the long-term phase. Participants were instructed to apply a new patch each morning and remove it before bed. Additional clinic visits happened on Day 1, Week 1, and Week 4 of using the X39 patch.

Results and conclusions

Wearing the X39 patch was associated with rapid and sustained increase in mitochondrial volume, indicating mitochondrial biogenesis. Wearing the X39 patch was associated with increased mitochondrial resilience under oxidative and inflammatory stress. This was initially associated with a decrease in mitochondrial membrane potential (1-2 hours), but already at Day 1 the mitochondrial membrane potential showed an increase. Please also refer to Figure 40 on page 98.

The use of the X39 patch showed an orchestrated wave of events, starting with mitochondrial biogenesis, and continuing with

increased mitochondrial membrane potential, mitochondrial resilience against oxidative and inflammatory stress, increased anti-inflammatory cytokines, reduced pro-inflammatory biomarkers, and a subjective increase in energy. Please also refer to Figure 41 on page 99.

Further work

The most imminent further work would involve testing of the culture supernatants from the ex vivo immune challenges, that remain banked frozen.

Further work may also involve testing additional markers on serum samples that remain banked frozen.

Additional clinical trials may address rapid effects on brain waves, and long-term recovery in chronic health situations (fatigue, pain).

Manuscript writing may be discussed.

Report 171-003. Clinical study on X39.

2 Purpose

The goal for this clinical proof-of-concept study was to document acute and long-term effects of wearing the X39 patch, where the acute effects were compared to changes when wearing a placebo patch, using a randomized double-blind design. This data is important to verify mitochondrial and inflammation-related effects.

3 Background

Lifewave's lead product is the X39 patch, which emits infrared light when applied to the skin. Lifewave has performed many studies and various types of testing over the years to document effects of the patch on biological functions, bioelectrical measurements, and physical and mental well-being. One of the core findings was that wearing the X39 patch increased the blood levels of GHK copper peptide,[i] which is known to have rejuvenating properties.[ii] [iii] [iv]

Photobiology involves photons of light that are absorbed by a chromophore, which is a molecule that transduces the photon energy into measurable biological effects. Chromophores include heme, myoglobin, and a variety of enzymes such as cytochromes. One of these molecules is Cytochrome C oxidase, responsible for the final step in the mitochondrial energy production, driven by the mitochondrial membrane potential (electrical charge).

Photobiomodulation therapy, also discussed in the literature as **low-level laser therapy (LLLT),** is widely used in complementary medicine, but has not yet become an accepted mainstream medical therapy. Photobiomodulation has been studied for over 120 years, but very few devices have received regulatory approval, and some have been banned. The fundamental principles of photobiomodulation are very promising, but inconsistent parameters in published clinical studies have diluted the impact of the studies on current medical practices. Some studies used mainstream outcome measures, but combined with sub-optimal or misunderstood light sources, and other studies have used devices that work well but where the studies focused on outcome measures of limited acceptance in mainstream medicine. This has contributed to confusion regarding the scientific and medical value.[v] It has been suggested by researchers in the field that a possible reason for the lack of regulatory acceptance of this type of method may be due to lingering uncertainty about the **mechanisms of action** at the **molecular and cellular levels**.[vi]

For Lifewave's device X39, there is a need for clinical documentation of both acute and long-term effects, combined with in vitro testing at the cellular level. There is a need for **bridging** language, from marketing claims typical for the natural products industry with mainstream scientific terms acceptable to regulatory agencies. There is also an interest in weaving in other contributing findings from bioenergetic methods and to help bridge mainstream and less-accepted scientific methods.

In parallel to the clinical study, we planned in vitro work on the biological effects of light emitted from the patches on well-characterized cellular reactions, to help form a framework for presenting at least some underlying mechanisms of action in a language acceptable to regulatory agencies.

Lifewave's X39 patch is designed to work on the whole body, tapping into subtle energy fields, such as meridians, where research still meets skepticism from mainstream science. There is a need to bridge this with methods that are widely acceptable in today's scientific and medical fields, and to make data visible in peer-reviewed, PubMed-visible publications.

While energy fields and bioenergetic shifts are likely part of the initiating effects of the X39 patch, there are also likely to be measurable cellular effects, starting in the skin where the patch is attached, and cascading, in part, via the immune system, designed as a rapid communication system throughout the body. This area of science is termed photoimmunology, with skin-residing immune cells as initiators, including mast cells and Langerhans' cells. While a lot of photoimmunology has been done to document effects of UV light on immune status, there are also important findings documented with the use of far-red light (670 nm).[vii]

This clinical proof-of-concept study aimed at documenting **acute** and **long-term** effects of using the X39 patch through evaluation of changes to mitochondrial resilience to stress, pro- and anti-inflammatory cytokines and restorative growth factors, and general wellness.

4 Study Design

4.1 Clinical Study Design Combining Acute and Long-Term Changes

For this clinical trial, participants were tested following an established study design, including a **randomized, double-blinded, placebo-controlled, cross-over study design for testing acute changes,** followed by an **open-label phase to monitor long-term changes.** [viii] [ix] [x] [xi] [xii] [xiii]

Below is a simplified diagram illustrating the involvement of each participant.

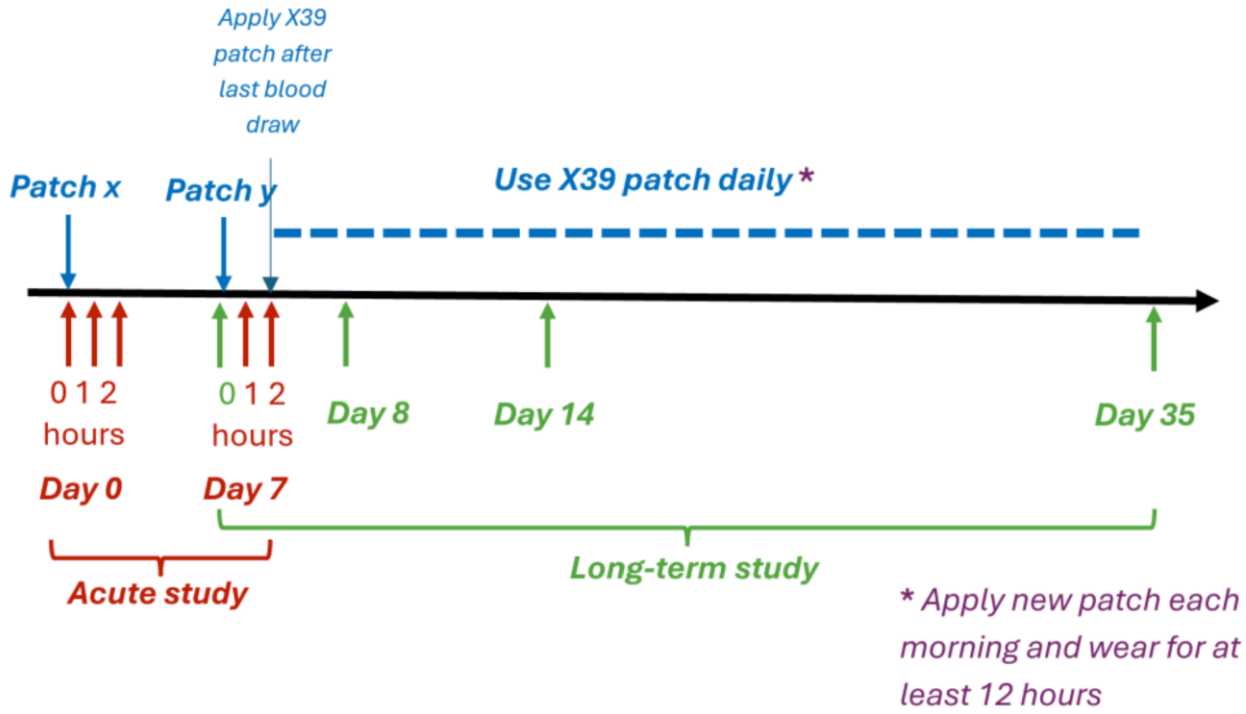


Figure 1. Diagram showing the timing and study procedures of the combined acute and long-term study here. On the report graphs, the time points for the acute effects are named "Baseline", "1 hour", and "2 hours". For the long-term effects, the time points are named "Baseline", "Day 1", "Week 1", and "Week 4".

The test parameters evaluated do not necessarily stay constant, even over a few hours, since they are related to people's metabolism, individual circadian rhythms, and other normal physiological parameters. Therefore, studies of this nature must include a placebo test day, allowing *within-subject* analysis of changes between the test days for each person. This strengthens the data analysis from this type of pilot study.

4.2 Outcome Measures

Primary outcome measure: **Mitochondrial resilience to stress.** Support of cellular energy production.

Secondary outcome measure: **Communication in the body.** Changes to serum cytokine levels, including pro- and anti-inflammatory cytokines, and regenerative growth factors.

Tertiary outcome measure: **Immune cell alertness and priming.** Cell cultures were performed, material was banked frozen. Screening for cytokine and growth factor changes can be done later **(separate budget)**, without repeating the clinical study.

Additional outcome measures: **C-reactive protein, acute effects on energy/focus (questionnaire), long-term effects on Wellness (questionnaire).**

5 Study Population

5.1 Study Participants

Twelve healthy people of any gender were enrolled after IRB-approved, written informed consent. The inclusion/exclusion profile for a study of this nature is not trivial, and each potential study participant was carefully evaluated prior to enrollment. To minimize anticipatory stress and apprehension during initial clinic visits for the study, each study participant must either have participated in previous studies at our facility or must attend a visit where we go through the study procedures, prior to a clinical study day.

5.2 Inclusion Criteria

- Healthy adults;
- Age 40 – 75 years (inclusive);
- BMI between 18.0 and 34.9 (inclusive);
- Veins easy to see in one or both arms (to allow for the multiple blood draws);
- Willing to comply with study procedures, including:
    - Maintaining a consistent diet and lifestyle routine throughout the study,
    - Consistent habit of bland breakfasts on days of clinic visits,
    - Abstaining from exercising and nutritional supplements on the morning of a study visit,

- Abstaining from use of coffee, tea, and soft drinks for at least one hour prior to a clinic visit;
- Abstaining from music, candy, gum, computer/cell phone use (airplane mode is allowed), during clinic visits.

5.3 Exclusion Criteria

- Cancer during past 12 months;
- Chemotherapy during past 12 months;
- Currently taking prescription weight loss drugs (such as semaglutide);
- Currently taking cholesterol-lowering medication (for example: statins);
- Currently experiencing intense stressful events/life changes;
- Currently in intensive athletic training (such as marathon runners);
- Currently taking anxiolytic, hypnotic, or anti-depressant prescription medication;
- Immunization during past 6 months;
- An unusual sleep routine (examples: working graveyard shift, irregular routine with frequent late nights, studying, partying);
- Unwilling to maintain a constant intake of supplements over the duration of the study;
- Anxiety about having blood drawn;
- Pregnant, nursing, or trying to become pregnant;
- Known allergies related to adhesive materials.

Other considerations:

- Other prescription medications were evaluated on a case-by-case basis.
- If taking blood pressure medications, we reviewed in more detail. One participant was taking blood pressure medication (calcium channel blocker) at a low maintenance dose of 10 mg/day.
- If taking over-the-counter anti-inflammatory medications on a daily basis, we worked with the participant on timing. For example, not taking Tylenol 8 hours prior to a clinic visit is acceptable for this study design.

- If using cannabis products, we reviewed the doses and timing with the participant before deciding if the person should be enrolled into the study. For example, people using low-to-moderate doses at bedtime, but not using cannabis at any other time of the day, are acceptable for this study design.

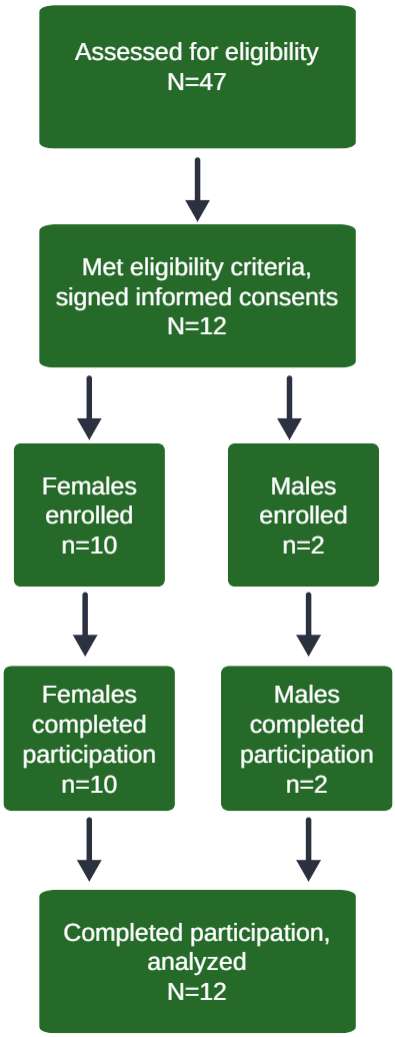


Figure 2. CONSORT flow chart.

5.4 Demographics

Table 1. List of study participants

Participant Number	BMI	Age	Randomization	Gender
P001	22.59	56	A	Female
P002	31.35	61.8	B	Female
P003	29.23	71.8	A	Female
P004	25.6	57.1	B	Female
P005	24.96	73.2	B	Male
P006	24.78	60.6	A	Female
P007	25.68	72.9	A	Male
P008	22.27	57.9	B	Female
P009	19.8	62.8	A	Female
P010	28.83	71.4	B	Female
P011	28.06	44.6	A	Female
P012	22.14	65.2	B	Female

Table 2. . Demographics of the study population.

	N	Age average[a]	Age range	BMI average[a]	BMI range
Females	10	60.9 ± 7.9	44.6 - 71.8	25.5 ± 3.8	19.8 - 31.4
Males	2	73.1 ± 0.2	72.9 - 73.2	25.3 ± 0.5	25 - 25.7

[a] *The average ± standard deviation is shown.*

5.5 Test Products: X39 and Placebo Patches

Test patches were provided by the study sponsor. On each clinic day, immediately after the baseline blood draw, participants used a single patch of either the active test product or a placebo. The patch was applied by clinic staff on the upper back, near the neckline. The participant was instructed to keep the patch on during the clinic visit and keep it on for 12 hours. Participants were encouraged to consume water throughout the clinic visit.

At the end of the second visit, participants were provided with X39 patches to use on a daily basis for the following 28 days. Participants were asked to wear the patch for 12 hours a day.

5.6 Adverse Events

Adverse Event (AE): An adverse event covers any health-related event that is unusual to a person, affects daily living, and occurs during a study. AEs are recorded with the date the problem started, whether study product was discontinued, whether medical advice or treatment was sought, and date resolved. All records are electronically linked between the study, the study participant, and the informed consent form. Adverse events that are judged possibly study- or product-related would be sent to the study sponsor as soon as possible. There were no such adverse events in this trial.

There was one health-related incident reported during this study:

1. **Participant P011:** 44-year-old female in good health. Participant said she had an allergic reaction, from either being exposed to lily flowers or possibly mold that was discovered growing in her heating/ventilation system during her last week of study participation. She does not know if it was a combination of the two that caused the reaction or if it was one or the other isolated. She went to the emergency room and received a 4 mg dexamethasone shot on Day 27 and Day 28 and was fully recovered by her last clinic visit on Day 35.

Note: This incident was not a study- or product-related adverse event.

5.7 Randomization

There were two phases in this study:

1. Double-blinded, placebo-controlled, cross-over phase for testing acute effects of wearing the X39 patch;
2. Open-label phase for documenting long-term changes with daily use of the X39 patch.

For the double-blinded, acute phase, we had even distribution of the number of participants who used the X39 patch on the first clinic day, to the number of participants who used the placebo patch on the first clinic day.

- The first woman enrolled into the study was assigned to the “11” patch, and remaining women alternated between the “22” patch and the “11” patch.
- The first man enrolled into the study was assigned to the “22” patch, and remaining men alternated between the “11” patch and the “22” patch.

- After all analysis and auditing was completed, unblinding revealed that “11” was placebo and “22” was the X39 patch.

For the open-label phase, participants each received a sleeve containing thirty X39 patches. The open-label phase of the study started at the end of the second clinic day of the acute phase:

- The blinded patch that a participant had been using for 2 hours in the clinic was removed.
- The sleeve with the participant’s number code was opened, and the clinic staff instructed the participant on where and how to apply the X39 patch on the back of the neck.
- Participants were instructed to remove the patch before bedtime and apply a new patch the next morning.
- Participants were instructed to return the sleeve with unused patches at the last clinic visit. The count was used to estimate compliance.

5.8 Compliance

For the double-blinded, placebo-controlled, cross-over phase for testing acute effects of wearing the X39 patch, there was 100% compliance. The placebo versus X39 patches were applied by clinic staff after the baseline blood draws.

For the open-label phase, the X39 patches were applied by the participants. Compliance ranged from 81-100%, with an average of 92% across all participants.

5.9 Study Environment

The study of acute changes to levels, activation status, and functionality of immune cells is not trivial. All test parameters undergo circadian changes, and are negatively affected by stress, adrenaline, lack of sleep, and recent illness. The study participants were instructed to call and reschedule a certain clinic day if they felt that any of these things were reasons to do so.

Also, the study environment is kept controlled for stressors. Cell phones are turned off at entry to clinic. Clinic phones and door chimes are off. Sensory input such as music, coffee/food smells, noises, etc., were eliminated or kept to an absolute minimum. In order to keep

volunteers in a state of mind we refer to as 'perpetually bored', but not falling asleep, they are offered a choice of light reading or crossword puzzles.

Upon arrival on the morning of each clinic day, participants rested quietly for 1 hour prior to baseline blood draw. This resting period is crucial to gain representative baseline data. During this time, questionnaires were completed to monitor previous meals, snacks, exercise, stressors, and recent sickness. After the baseline blood draw, a patch was applied. Two more blood samples were drawn at 1 and 2 hours after applying the patch.

5.10 Study Procedures

5.11 Table Over Study Procedures

				Acute module (randomized)						Long-term module		
				Clinic visit A (Placebo)			Clinic visit B (X39)					
		Pre-screen	Screen	Base line	1 hour	2 hours	Base line[1]	1 hour	2 hours	Day 1	Week 1	Week 4
	PHYSICAL											
1	Medical history	X										
2	Health status interview	X										
3	Current medications	X	X	X			X			X	X	X
4	Current supplements	X	X	X			X			X	X	X
5	Informed consent		X									
6	Height		X									
7	Weight (BMI)		X	X			X			X	X	X
8	Blood pressure		X	X			X			X	X	X
	BLOOD TESTS											
9	Mitochondrial resilience[2]			X	X	X	X	X	X	X	X	X
10	Bodily Communication: Cytokines and growth factors[3]			X	X	X	X	X	X	X	X	X
11	Ex vivo immune cell challenges[4]			X	X	X	X	X	X	X	X	X
12	C-reactive protein[5]			X	X	X	X	X	X	X	X	X
13	Serum banking[6]			X	X	X	X	X	X	X	X	X
	QUESTIONNAIRES											
14	Brief questionnaire on energy/focus (acute phase)[7]			X	X	X	X	X	X			
15	Wellness questionnaire (long-term phase)[8]			X			X			X	X	X
16	Compliance			X			X			X	X	X
17	Adverse events			X			X			X	X	X

Please see footnotes on the following page.

Footnotes to table:

[1]This blood draw served as baseline for both the long-term study and the second day of the acute study.

[2]White blood cells were purified from fresh blood samples. The cells were cultured in the absence of stress, and in the presence of a) oxidative stress, b) inflammatory stress. Flow cytometry analysis was performed on the same day as the blood was drawn, and analysis was performed on x) mitochondrial volume per cell, and y) mitochondrial membrane potential, on 1) lymphocytes, 2) monocytes, and 3) granulocytes (neutrophils), simultaneously within the same samples.

[3] Serum cytokine profile includes 27 pro- and anti-inflammatory cytokines, anti-viral peptides, and regenerative growth factors: IL-1beta, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, basic FGF, G-CSF, GM-CSF, IFN-gamma, IP-10, MCP-1 (MCAF), MIP-1alpha, MIP-1beta, PDGF-BB, RANTES, TNF-alpha, and VEGF.

*[4]The ex vivo immune challenge involves an inflammatory bacterial challenge and a viral mimetic (Poly I:C). Cultures are performed on the PBMC cells, and 24-hour supernatants frozen. This allows cytokine profiling to be done at a later time (**separate budget**) without repeating the clinical study.*

[5]Blood samples were used for testing for C-reactive protein using a high-sensitivity ELISA method.

[6]Banking of serum in multiple small portions will allow for subsequent tests to be added on separate budgets, without repeating the clinical study.

[7]As part of the acute phase of the study, a brief 5-question questionnaire was asked immediately prior to each hourly blood draw.

[8]Thirty-point questionnaire on physical, mental, emotional, energy, pain, sleep, and general health.

5.11.1 Explanation of the Clinical Study Procedures

In a clinical trial to monitor rapid changes to bodily communication (cytokines) and cellular metabolic state, we expected a cascade of events, starting by light-activation of cells in the skin, systemic changes to cytokine levels, followed by changes to various metabolic markers. Blood samples offer a convenient window into the events happening after a patch is applied.

5.11.2 Blood Draws

For each blood draw, we obtained 1 heparinized vial and 1 serum separator vial, for a total of 14 mL blood per blood draw.

- The serum separator vials were allowed to sit for 30-60 minutes at room temperature to facilitate complete coagulation, and the vials were centrifuged. The serum was harvested and aliquoted before freezing at -80°C.
    - **Cytokines and growth factors:** One aliquot of serum was tested for a broad panel of pro- and anti-inflammatory cytokines and restorative growth factors as part of this study protocol.
    - **C-reactive protein:** Serum was tested using commercial high-sensitivity ELISA kits.
    - Extra aliquots remain stored frozen, and this allows for additional tests to be added at a later time (separate budget). This may include exosome cytokines, neuroendocrine markers, antioxidant status, and other tests.
    - Remaining frozen serum aliquots will be stored frozen at -80°C for up to 3 months after the clinical study report is received by the study sponsor, to allow sponsor to make that decision.

- The heparinized blood was used for the testing described below:
    - **Ex vivo testing of mitochondrial resilience when blood cells were under stress:** White blood cells were purified from each sample and exposed to oxidative and inflammatory stress outside the body, to see whether the cells in the blood circulation changed how robustly their mitochondria were able to maintain functionality after using the patch.
    - **Immune cell priming: Ex vivo challenging of immune cells with microbial antigens:** A bacterial antigen (LPS) and a viral mimetic antigen (Poly I:C). After 24 hours of culture, we banked the culture supernatants at -80°C for cytokine testing (**future testing, separate budget**).

5.12 Data Analysis

During a study of this design, we are tapping into the circadian cycle of each study participant. From chronobiological studies it is known that immune cells traffic and circulate following a personal circadian clock. Not only do the numbers of immune cell types vary during day and night, but their epigenetic programming and gene expression, cytokine production, and alertness vary during the circadian cycle.[14] This circadian cycle is disrupted in people working night shifts or otherwise sleeping during the day and staying awake until morning. Therefore, this study excluded people with such an irregular sleep cycle. In the figure below, is an example of published data on circadian rhythms of monocytes and T lymphocytes (from: Cuesta et al., 2016). The blue boxes show the time of the day when this clinical study was conducted.

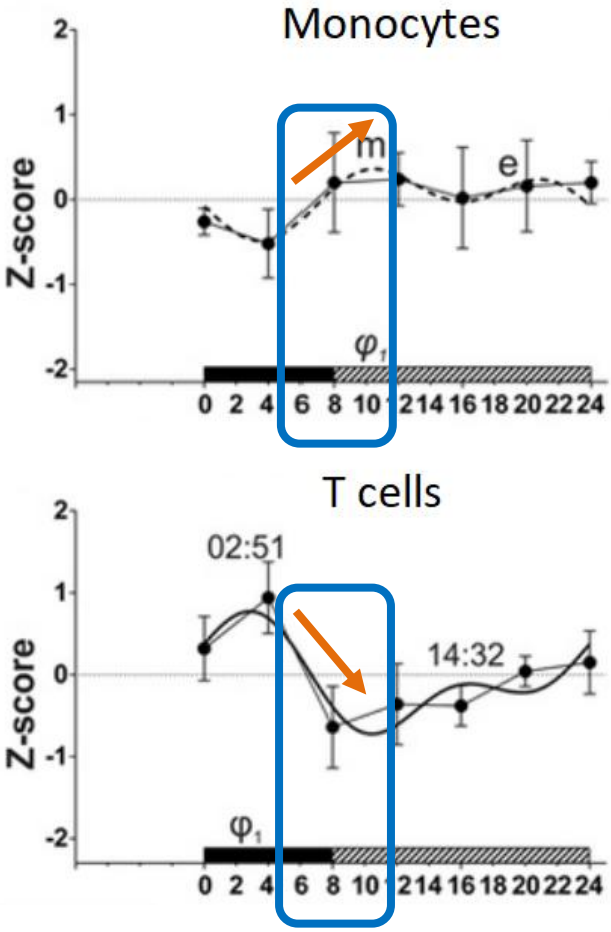


*Figure 3. Circadian rhythms of monocyte and T lymphocyte blood counts (from: Cuesta et al., 2016). Cells from 9 participants with a normal day/night cycle were counted by flow cytometry. Group harmonic regression is given in z-scores for monocytes and T cells. The two **blue boxes** show the time of the day where this clinical study was conducted. The general trend of changes in cell numbers in the blood is indicated by the **orange arrows.***

5.1 Statistical indicators

Overview tables:

The P-values in the overview tables are provided with color-coding, where the more intense a green color the higher the level of statistical significance the results have. In addition, P-values between 0.10 and 0.25 are coded in blue, meaning that they did not reach statistical significance, but in a small study like this, the p-value still deserves attention.

P<0.01	0.0001	indicating a high level of statistical significance
P<0.05		indicating statistical significance
P<0.1	0.0510	indicating a statistical trend
P<0.25		indicating worthy of attention

6 Results

6.1 Mitochondrial Resilience Under *Ex Vivo* Stress

Mitochondria are intracellular organelles, responsible for producing cellular energy. They are present in all living cells, except specialized cells such as mature mammalian red blood cells.

The testing of mitochondrial function is a relative measure, with a high degree of day-to-day variability. Therefore, **the most meaningful measure in a clinical study** is to **compare the functionality of resting mitochondria to those of mitochondria under oxidative stress**. This provides a relative measure of **mitochondrial resilience** towards stress, which can be compared between test-days for a given person. The method followed our previously published protocol.[15]

The testing was performed on white blood cells (leukocytes) from peripheral blood drawn from study participants at baseline and at 1 and 2 hours after applying the X39 patch or a placebo patch, as well as after 1, 7, and 28 days of wearing the X39 patch.

Cells were tested under three separate conditions:

- Normal culture conditions;
- Oxidative stress culture conditions where cells are exposed to hydrogen peroxide;
- Inflammatory culture conditions where cells are exposed to bacterial lipopolysaccharide.

Following the cultures, cells were stained with fluorescent probes:

1. The Mitotracker dye stains mitochondria in proportion to mitochondrial volume per cell.
2. The JC-1 dye provides fluorescence signals dependent on mitochondrial membrane potential.

6.1.1 Mitochondrial Volume Per Cell

The mitochondria are highly dynamic and can divide into multiple separate organelles or fuse into a continuous system of tubes, depending on cellular demands. Therefore, the overall volume of mitochondria inside each cell is a more meaningful measure than numbers of mitochondria per cell. Here we present this as mitochondrial volume per cell.

The Mitotracker dye stains mitochondria in proportion to mitochondrial volume per cell. Below is a diagram showing green-labeled mitochondria, as they were measured by flow cytometry.

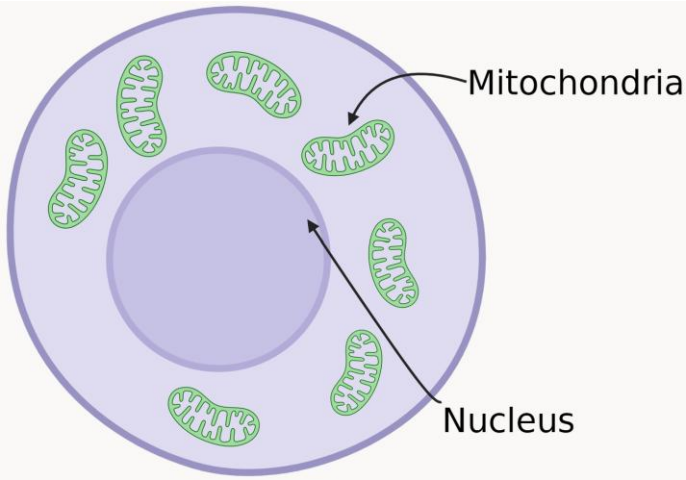


Figure 4. Mitochondria are subcellular organelles inside almost all living cells and are responsible for the majority of cellular energy production. The mitochondria are dynamic and can change shape, as well as undergo fusion and fission. Therefore, we don't count the number of mitochondria, we evaluate the overall mitochondrial volume per cell. Mitochondria qre labeled with a green fluorescent dye, and the relative amount of fluorescence per cell is used to monitor changes to mitochondrial volume per cell.

The Attune® acoustic aligning flow cytometer tracks cell numbers per volume unit and provides fluorescence intensity measurements per cell. The flow cytometry displays the cells based on

size and granularity, which allows analysis of lymphocytes, monocytes, and PMN cells within each sample. This is important, since these cell types have different activity levels, both under normal and under stressed conditions.

Below is a diagram from the flow cytometry analysis, showing the scatter plot for particle size (FSC-A) and granularity (SSC-A), and the electronic gates for the three cell types. Below the scatter plot is shown an example of the evaluation of fluorescence intensity for each cell type.

Blood cell populations:

- ***Lymphocytes*** are a type of white blood cell that are round in shape with a round nucleus that occupies the majority of the cell. Three types of cells belong to the lymphocyte class. These are natural killer cells, T lymphocytes and B lymphocytes. Most blood lymphocytes are in a resting state, and only after activation will enter highly active metabolic states.
- ***Monocytes*** are the largest type of white blood cell. They are present in the blood circulation, and after leaving the blood and migrating into tissue they become macrophages and serve a crucial link between the innate and adaptive immune responses. Monocytes are active scavengers and respond immediately to inflammation and oxidative stress.
- ***Neutrophils*** are also known as polymorphonuclear ***(PMN) cells*** because they contain a nucleus whose shape (morph) is irregular and contains many (poly) lobes. They are the most abundant type of white blood cell and are the first line of defense for bacterial infection. Neutrophils engulf and kill bacteria by releasing enzymes stored in intracellular granules. Neutrophils are also involved in inflammation, including migrating to sites of tissue injury in response to chemical signals. PMN cells are active scavengers and respond immediately to inflammation and oxidative stress.

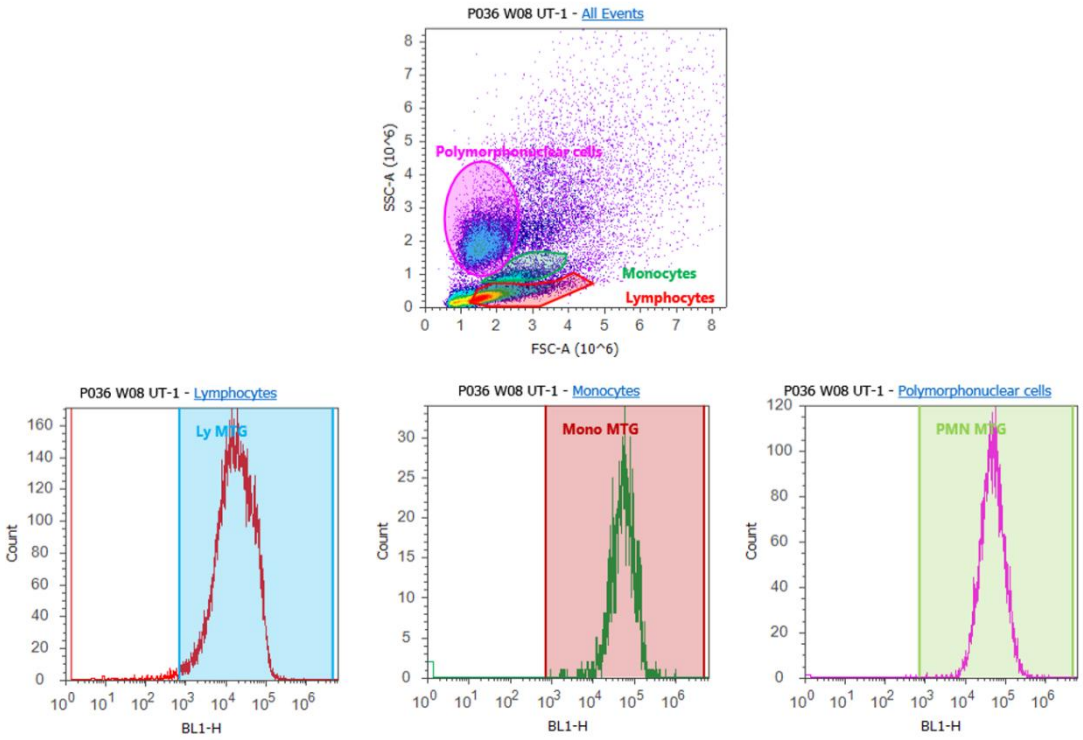


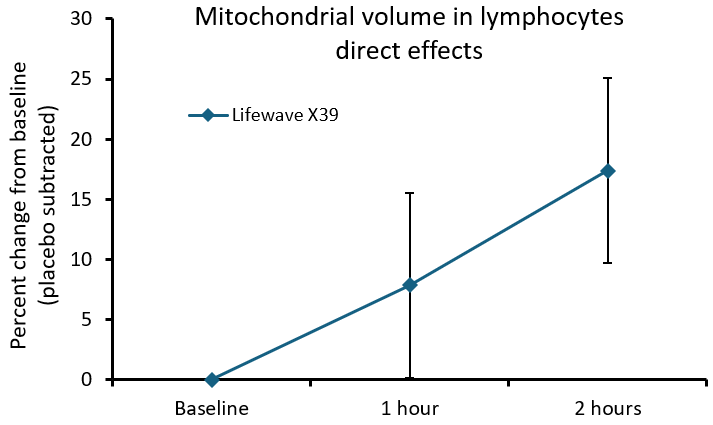
Figure 5. Flow cytometry analysis of mitochondrial volume per cell. Electronic gates are set to define lymphocytes (Ly), monocytes (Mono), and polymorphonuclear (PMN) cells based on their forward scatter (size) and side scatter (granularity) properties. The green fluorescence intensity (BL1-H) is measured for each cell type.

6.1.2 Mitochondrial biogenesis when wearing the X39 patch

Synopsis:

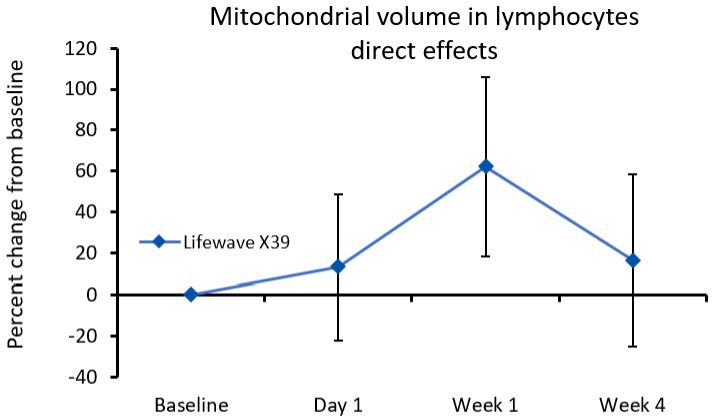
Wearing the X39 patch triggered mitochondrial biogenesis in lymphocytes, monocytes, and neutrophils.

- **Acute changes:** The increased mitochondrial volume per cell reached a high level of significance at 2 hours for both lymphocytes and monocytes.
- **Long-term changes:** The increased mitochondrial volume per cell reached statistical significance for lymphocytes and monocytes at Week 1, and a high level of significance for neutrophils at Week 1. For all three cell types, there was a return towards baseline levels at Week 4. This may be related to increased energy expenditure and a long-term adaptation to the effects of wearing the X39 patch.



Statistical Comparison

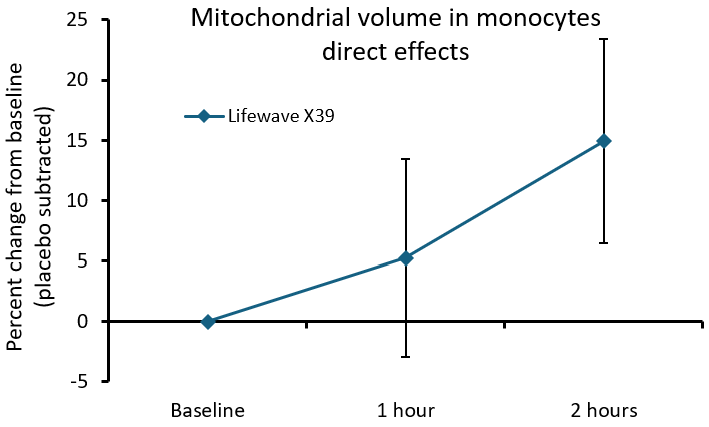
Baseline to		(*)	**
1 hour to			*



Statistical Comparison

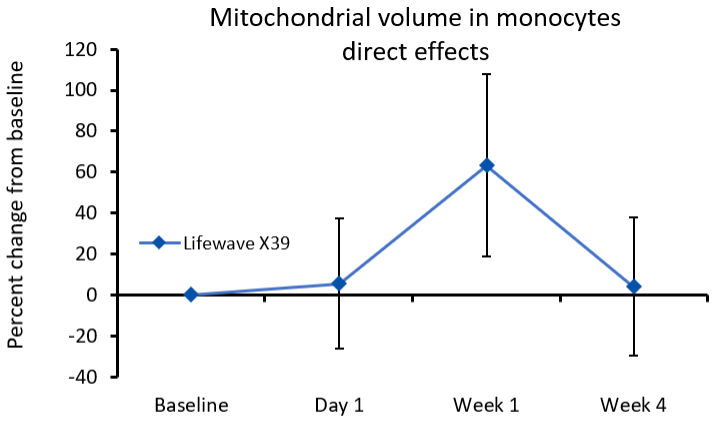
Baseline to			*	
Day 1 to			**	
Week 1 to				**

Figure 6. ***Top****: Acute changes to mitochondrial volume in lymphocytes in blood circulation when wearing the Lifewave X39.* ***Bottom****: The change in mitochondrial volume in lymphocytes in blood circulation when wearing the X39 patch over 4 weeks.*



Statistical Comparison

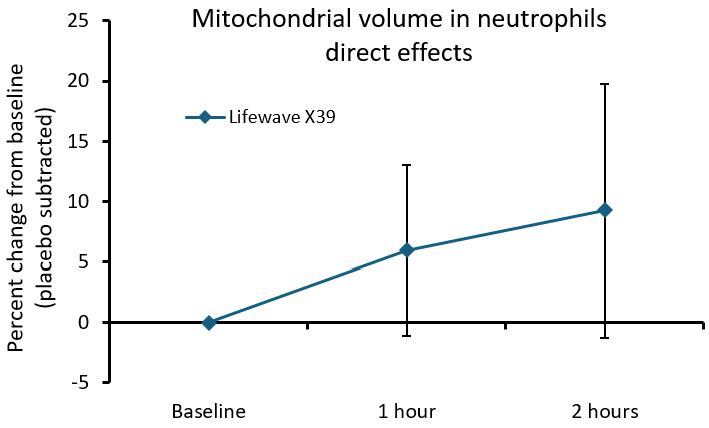
Baseline to			**
1 hour to			*



Statistical Comparison

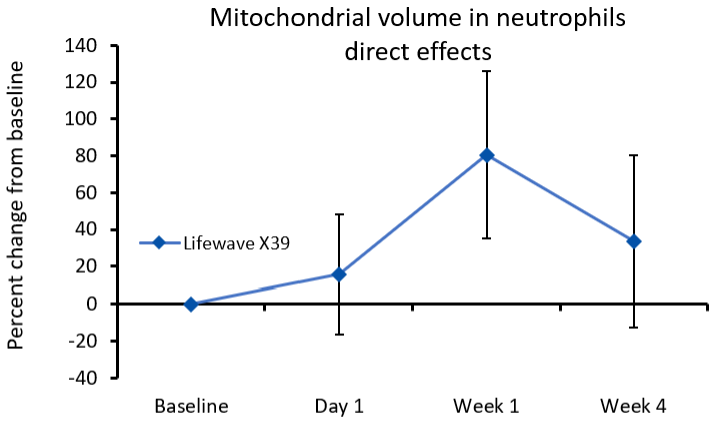
Baseline to			*	
Day 1 to			**	
Week 1 to				**

Figure 7. ***Top****: Acute changes to mitochondrial volume in monocytes in blood circulation when wearing the Lifewave X39 patch.* ***Bottom****: The change in mitochondrial volume in monocytes in blood circulation when wearing the X39 patch over 4 weeks.*



Statistical Comparison

Baseline to			
1 hour to			



Statistical Comparison

Baseline to			**	
Day 1 to			**	
Week 1 to				**

Figure 8. ***Top****: Acute changes to mitochondrial volume in neutrophils in blood circulation when wearing the Lifewave X39 patch.* ***Bottom****: The change in mitochondrial volume in neutrophils in blood circulation when wearing the X39 patch over 4 weeks.*

6.1.3 Mitochondrial volume under oxidative stress

Synopsis:

Mitochondria are vulnerable to oxidative stress and can respond to such stress by increasing the mitochondrial volume per cell; a process called mitochondrial biogenesis. We calculated this by comparing the mitochondrial volume per cell in unstressed cell cultures to the mitochondrial volume in H_2O_2-mediated oxidatively stressed cultures.

Both in the acute and the long-term phases of this study, oxidative stress triggered increases to the average mitochondrial volume per cell, as the cells reacted to this stress.

In blood samples taken from the participants as they were using the X39 patch, the mitochondrial biogenesis was more robust. This was seen for all three cell types: Lymphocytes, monocytes, and neutrophils.

- The increased mitochondrial volume seen under oxidative stress, in blood samples drawn when wearing the X39 patch, was statistically significant when compared to blood samples drawn when wearing the placebo patch, for:
    - Lymphocytes (1 hour)
    - Monocytes (1 hour)
    - Neutrophils (1 and 2 hours)

- The long-term effects for the different cell types:
    - Lymphocytes: The increase reached statistical significance on Day 1 and a high level of statistical significance at Week 4 after wearing the X39 patch.
    - Monocytes: The increase reached a high level of statistical significance on Day 1 and Week 4 after wearing the X39 patch.
    - Neutrophils: The increase reached a statistical trend at Week 4 after wearing the X39 patch.

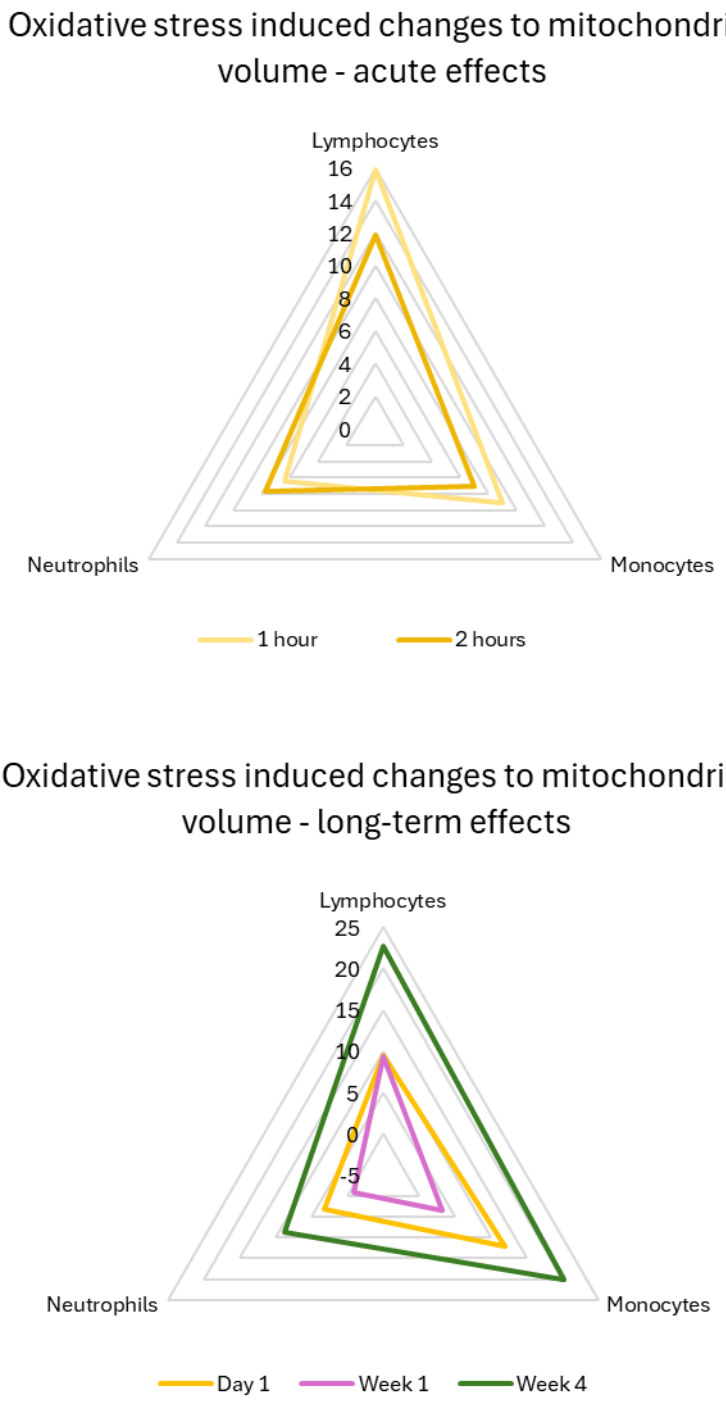


Figure 9. Oxidative stress-induced changes to mitochondrial volume after wearing the X39 patch. ***Top****: Acute effects to the oxidative stress-induced change, compared to wearing the placebo patch.* ***Bottom****: Long-term changes when compared to baseline.*

Table 3. Mitochondrial volume under oxidative stress, acute effects - levels of significance.

	N	Baseline average [a]	P-value [b]	One-hour average [c]	Change [d]	P-value [e]	P-value [f]	P-value [g]	Two-hour average [h]	Change [i]	P-value [j]	P-value [k]	P-value [l]
Lymphocytes													
Placebo	12	77.4 ± 10.9		83.3 ± 10.4	Increase	0.1889			84.2 ± 7.4	Increase	0.1559		
Lifewave X39	12	68.3 ± 11.8	0.1237	90 ± 12.6	Increase	0.0001	0.1677	0.0232	86.9 ± 8.7	Increase	0.0003	0.5214	0.0749
Monocytes													
Placebo	12	23.6 ± 4.9		20.4 ± 4.3	Decrease	0.2450			19.2 ± 2.8	Decrease	0.1270		
Lifewave X39	12	18.7 ± 3.8	0.0897	24.5 ± 7.3	Increase	0.1307	0.3047	0.0338	21.3 ± 3.4	Increase	0.2335	0.3883	0.0603
Neutrophils													
Placebo	12	9.8 ± 5.4		8.2 ± 2.5	Decrease	0.6265			7.7 ± 3.1	Decrease	0.5413		
Lifewave X39	12	3.4 ± 1.9	0.1074	8.3 ± 1.8	Increase	0.0089	0.8206	0.0248	9.1 ± 2.1	Increase	0.0030	0.4030	0.0196

[a] Average ± standard error of the mean at baseline
[b] Significance to placebo at baseline
[c] Average ± standard error of the mean at 1 hour
[d] Change from baseline at 1 hour
[e] Significance of change from baseline to 1 hour
[f] Significance to placebo at 1 hour
[g] Significance to placebo, percent change at 1 hour
[h] Average ± standard error of the mean at 2 hours
[i] Change from baseline at 2 hours
[j] Significance of change from baseline to 2 hours
[k] Significance to placebo at 2 hours
[l] Significance to placebo, percent change at 2 hours

Table 4. Mitochondrial volume under oxidative stress, placebo subtracted, acute effects - levels of significance.

	N	Baseline average [a]	One-hour average [b]	P-value [c]	Two-hour average [d]	P-value [e]	P-value [f]
Lymphocytes							
Lifewave X39	12	0 ± 0	15.9 ± 10.8	0.0201	11.9 ± 10.4	0.0678	0.4200
Monocytes							
Lifewave X39	12	0 ± 0	9 ± 6.2	0.0240	7.1 ± 5.8	0.0621	0.7977
Neutrophils							
Lifewave X39	12	0 ± 0	6.4 ± 4.5	0.0242	7.8 ± 5.3	0.0194	0.2793

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at 1 hour

[c] Significance of difference in percent change from baseline to 1 hour

[d] Average ± standard error of the mean at 2 hours

[e] Significance of difference in percent change from baseline to 2 hours

[f] Significance of difference in percent change from 1 hour to 2 hours

Table 5. Mitochondrial volume under oxidative stress, long-term effects - levels of significance.

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one Average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
Lymphocytes											
Lifewave X39	12	72.7 ± 10.9	82.2 ± 6.9	Increase	0.0269	82 ± 7.7	Increase	0.2004	95.4 ± 9.2	Increase	0.0018
Monocytes											
Lifewave X39	12	23.3 ± 5	35.3 ± 6.7	Increase	0.0000	26.6 ± 3.6	Increase	0.1722	43.6 ± 6.5	Increase	0.0000
Neutrophils											
Lifewave X39	12	8.4 ± 5.2	11.6 ± 3.6	Increase	0.3126	7.6 ± 2.7	Decrease	0.8022	17.1 ± 4.6	Increase	0.0907

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at day 1

[c] Change from baseline at day 1

[d] Significance of difference in percent change from baseline to day 1

[e] Average ± standard error of the mean at week 1

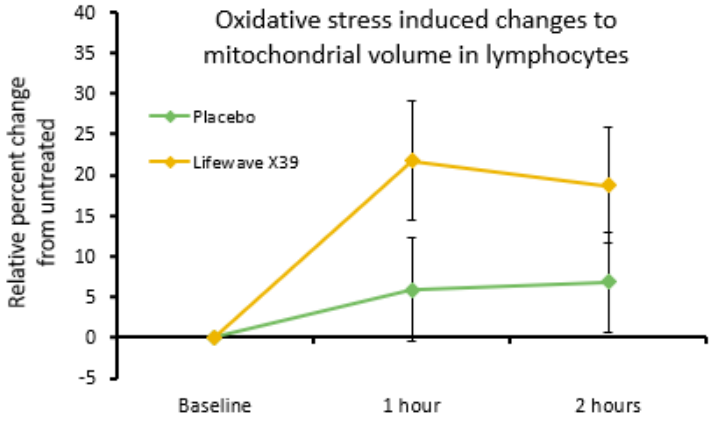
[f] Change from baseline at week 1

[g] Significance of difference in percent change from baseline to week 1

[h] Average ± standard error of the mean at week 4

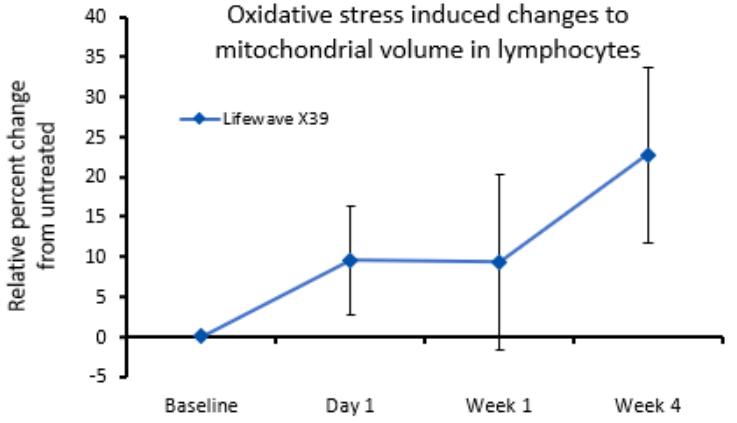
[i] Change from baseline at week 4

[j] Significance of difference in percent change from baseline to week 4



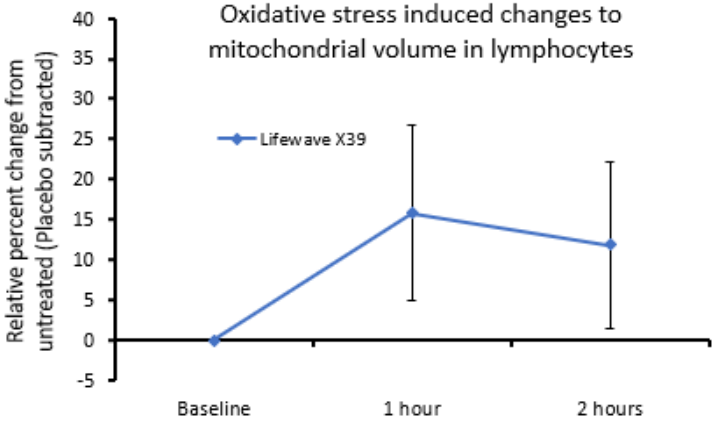
Statistical Comparison

Lifewave X39 - Placebo		*	(*)



Statistical Comparison

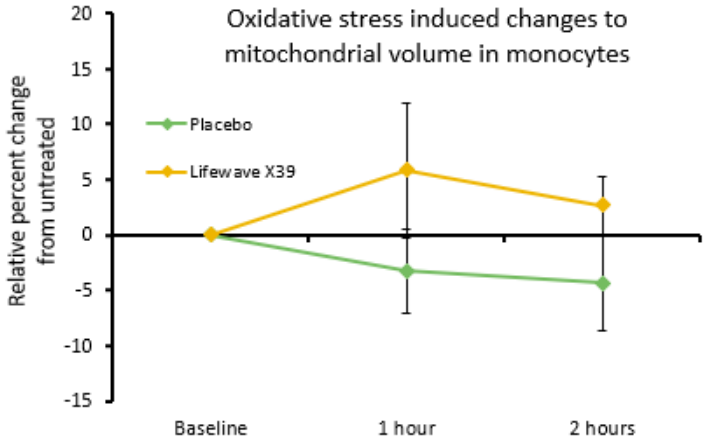
Baseline to		*		**
Day 1 to				*
Week 1 to				(*)



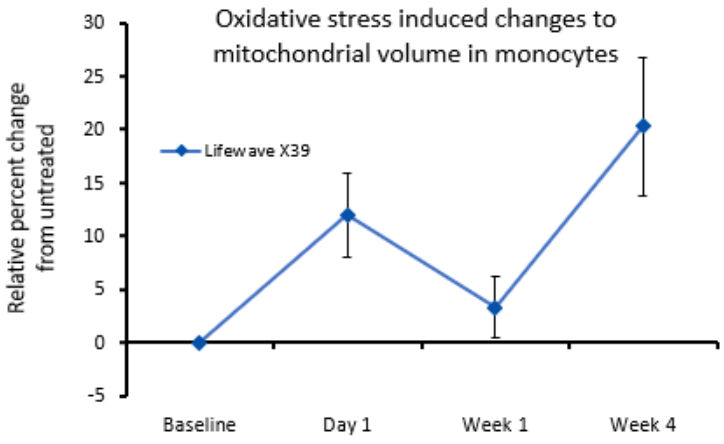
Statistical Comparison

Baseline to		*	(*)
1 hour to			

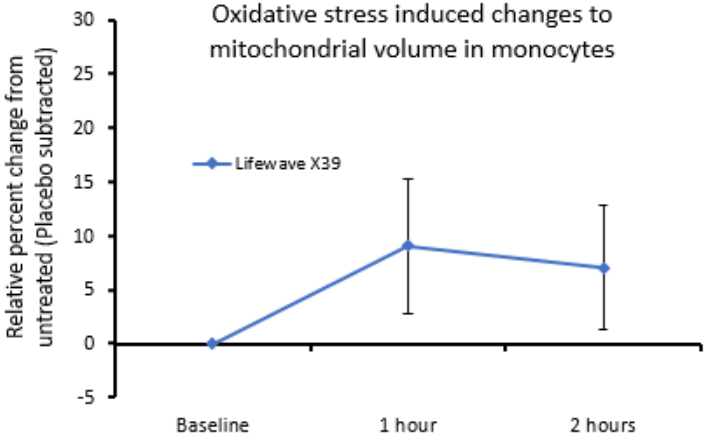
Figure 10. ***Top left****: Acute changes to mitochondrial volume in lymphocytes in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to oxidative stress ex vivo.* ***Bottom left****: The change in mitochondrial volume in lymphocytes in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in oxidative stress-induced changes to mitochondrial volume in lymphocytes in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*



Statistical Comparison			
Lifewave X39 - Placebo		*	(*)

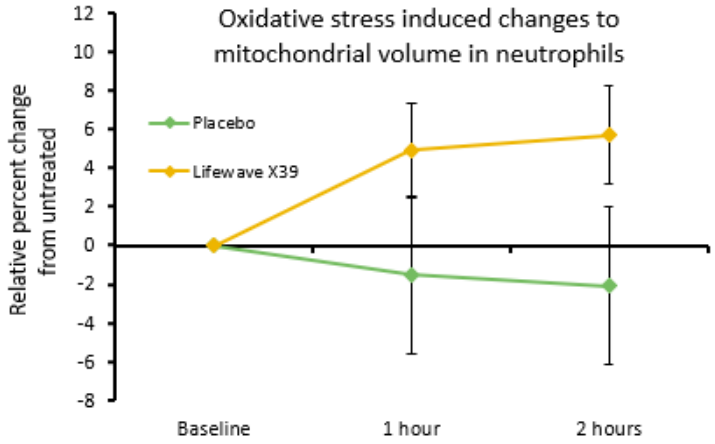


Statistical Comparison				
Baseline to		**		**
Day 1 to			**	*
Week 1 to				**

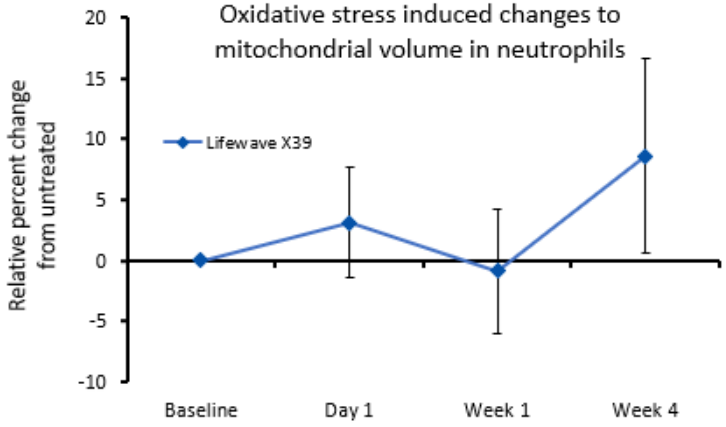


Statistical Comparison			
Baseline to		*	(*)
1 hour to			

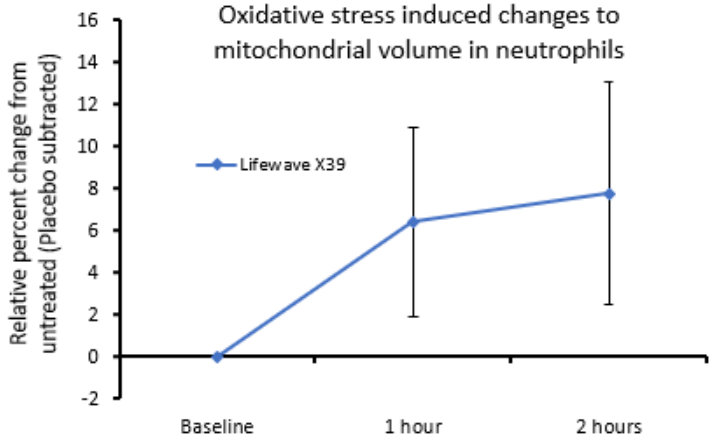
Figure 11. ***Top left****: Acute changes to mitochondrial volume in monocytes in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to oxidative stress ex vivo.* ***Bottom left****: The change in mitochondrial volume in monocytes in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in oxidative stress-induced changes to mitochondrial volume in monocytes in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*



Statistical Comparison			
Lifewave X39 - Placebo		*	*



Statistical Comparison				
Baseline to				(*)
Day 1 to			*	
Week 1 to				*



Statistical Comparison			
Baseline to		*	*
1 hour to			

Figure 12. ***Top left****: Acute changes to mitochondrial volume in neutrophils in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to oxidative stress ex vivo.* ***Bottom left****: The change in mitochondrial volume in neutrophils in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in oxidative stress-induced changes to mitochondrial volume in neutrophils in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*

6.1.4 Mitochondrial volume under inflammatory stress

Synopsis

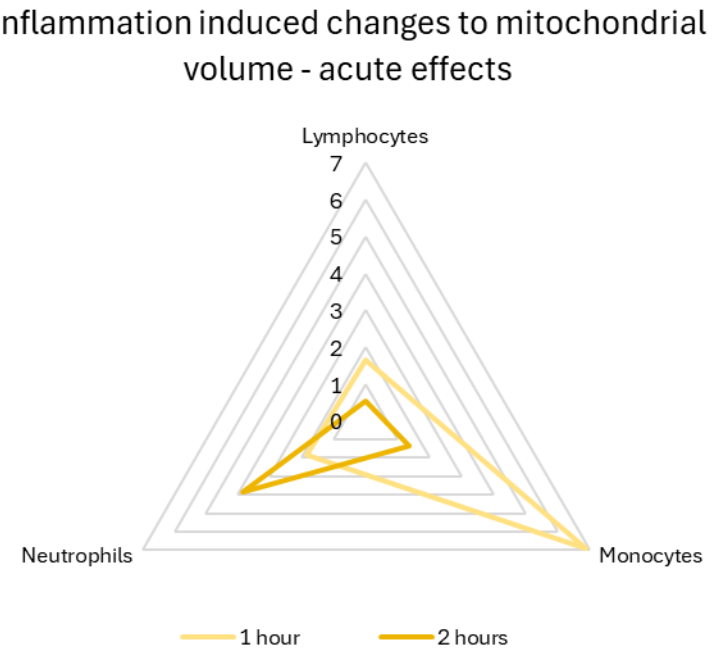
Mitochondria are sensitive to inflammatory stress and can respond to such stress by increasing the mitochondrial volume per cell; a process called mitochondrial biogenesis. We calculated this by comparing the mitochondrial volume per cell in unstressed cell cultures to the mitochondrial volume in cell cultures inflamed with the bacterial toxin LPS.

Both in the acute and the long-term phases of this study, the inflammatory stress triggered increases to the average mitochondrial volume per cell, as the cells reacted to this stress.

In blood samples taken from the participants as they were using the X39 patch, the mitochondrial biogenesis was more robust.

- The increased mitochondrial volume seen under inflammatory stress, in blood samples drawn when wearing the X39 patch, was notably higher when compared to blood samples drawn when wearing the placebo patch, for:
    - Monocytes (statistical trend at 1 hour);
    - Neutrophils (statistical trend at 2 hours).

- The long-term effects for the different cell types:
    - Lymphocytes: The increase reached a high level of statistical significance on Day 1, Week 1, and Week 4 after wearing the X39 patch.
    - Monocytes: The increase reached a high level of statistical significance on Day 1, Week 1, and Week 4 after wearing the X39 patch.
    - Neutrophils: The increase reached a high level of statistical significance on Day 1, Week 1, and Week 4 after wearing the X39 patch.



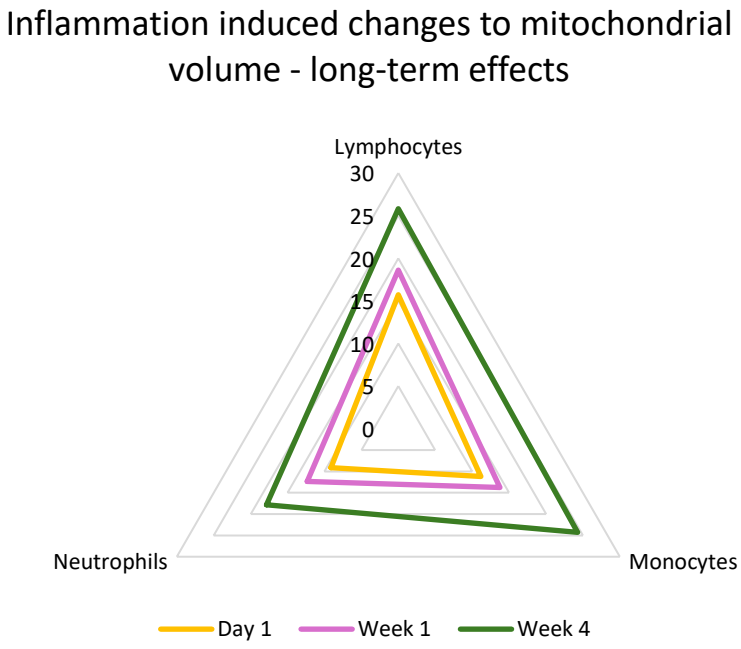


Figure 13. Inflammation-induced changes to mitochondrial volume after wearing the X39 patch. ***Top****: Acute effects to the inflammation-induced change, compared to wearing the placebo patch.* ***Bottom****: Long-term changes when compared to baseline.*

Table 6. Mitochondrial volume under inflammatory stress, acute effects - levels of significance.

	N	Baseline average [a]	P-value [b]	One-hour average [c]	Change [d]	P-value [e]	P-value [f]	P-value [g]	Two-hour average [h]	Change [i]	P-value [j]	P-value [k]	P-value [l]
Lymphocytes													
Placebo	12	2.4 ± 2.4		1.5 ± 2.3	Decrease	0.5924			4.3 ± 2.2	Increase	0.3173		
Lifewave X39	12	0.1 ± 4.1	0.4689	0.8 ± 3.5	Increase	0.7633	0.8186	0.5501	2.5 ± 3.5	Increase	0.4411	0.5255	0.8663
Monocytes													
Placebo	12	1.5 ± 2.4		-0.5 ± 2.1	Decrease	0.2942			1.9 ± 2.8	Increase	0.8810		
Lifewave X39	12	-2.6 ± 4.5	0.2436	2.3 ± 5.6	Increase	0.1647	0.4964	0.0335	-0.9 ± 3.1	Increase	0.5921	0.3467	0.6416
Neutrophils													
Placebo	12	-2.9 ± 2.4		-2.9 ± 2	Increase	0.9760			-2.3 ± 1.8	Increase	0.6761		
Lifewave X39	12	-6.2 ± 3	0.2229	-4.3 ± 3.3	Increase	0.2553	0.6003	0.2799	-1.7 ± 3	Increase	0.0282	0.8269	0.0579

[a] Average ± standard error of the mean at baseline
[b] Significance to placebo at baseline
[c] Average ± standard error of the mean at 1 hour
[d] Change from baseline at 1 hour
[e] Significance of change from baseline to 1 hour
[f] Significance to placebo at 1 hour
[g] Significance to placebo, percent change at 1 hour
[h] Average ± standard error of the mean at 2 hours
[i] Change from baseline at 2 hours
[j] Significance of change from baseline to 2 hours
[k] Significance to placebo at 2 hours
[l] Significance to placebo, percent change at 2 hours

Table 7. Mitochondrial volume under inflammatory stress, placebo subtracted, acute effects - levels of significance.

	N	Baseline average [a]	One-hour average [b]	P-value [c]	Two-hour average [d]	P-value [e]	P-value [f]
Lymphocytes							
Lifewave X39	12	0 ± 0	1.7 ± 4.6	0.5682	0.5 ± 5.4	0.8723	0.5829
Monocytes							
Lifewave X39	12	0 ± 0	6.9 ± 4.5	0.0535	1.4 ± 4.7	0.6920	0.0451
Neutrophils							
Lifewave X39	12	0 ± 0	1.8 ± 1.7	0.2369	3.8 ± 3.2	0.0867	0.2290

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at 1 hour

[c] Significance of difference in percent change from baseline to 1 hour

[d] Average ± standard error of the mean at 2 hours

[e] Significance of difference in percent change from baseline to 2 hours

[f] Significance of difference in percent change from 1 hour to 2 hours

Table 8. Mitochondrial volume under inflammatory stress, long-term effects - levels of significance.

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one Average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
Lymphocytes											
Lifewave X39	12	-3.7 ± 3.6	12.1 ± 4.5	Increase	0.0001	15 ± 3.6	Increase	0.0000	22.2 ± 7.2	Increase	0.0001
Monocytes											
Lifewave X39	12	-4.4 ± 4.4	6.8 ± 4.6	Increase	0.0027	9.4 ± 3.1	Increase	0.0004	19.9 ± 7.7	Increase	0.0000
Neutrophils											
Lifewave X39	12	-7.2 ± 3.1	1.9 ± 3.3	Increase	0.0046	5.1 ± 3.7	Increase	0.0007	10.7 ± 5.9	Increase	0.0003

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at day 1

[c] Change from baseline at day 1

[d] Significance of difference in percent change from baseline to day 1

[e] Average ± standard error of the mean at week 1

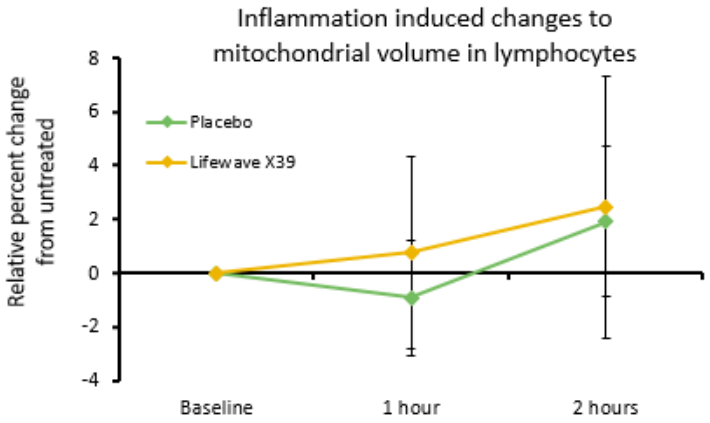
[f] Change from baseline at week 1

[g] Significance of difference in percent change from baseline to week 1

[h] Average ± standard error of the mean at week 4

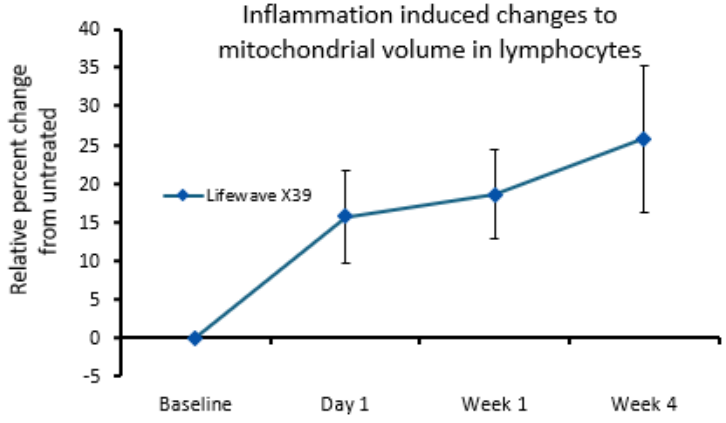
[i] Change from baseline at week 4

[j] Significance of difference in percent change from baseline to week 4



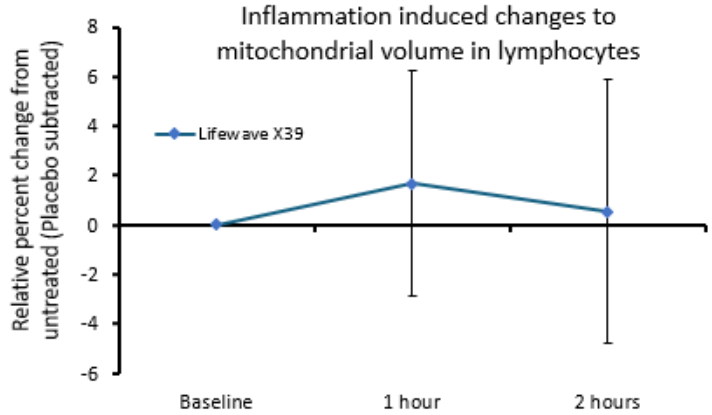
Statistical Comparison

Lifewave X39 - Placebo		



Statistical Comparison

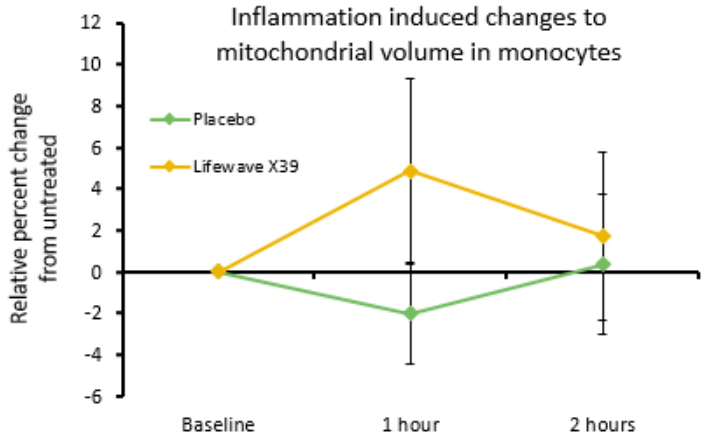
Baseline to		**	**	**
Day 1 to				*
Week 1 to				



Statistical Comparison

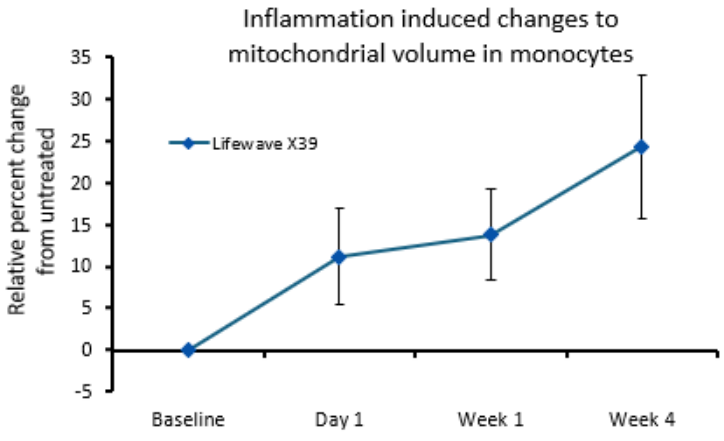
Baseline to			
1 hour to			

Figure 14. ***Top left****: Acute changes to mitochondrial volume in lymphocytes in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to inflammation ex vivo.* ***Bottom left****: The change in mitochondrial volume in lymphocytes in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in inflammation-induced changes to mitochondrial volume in lymphocytes in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*



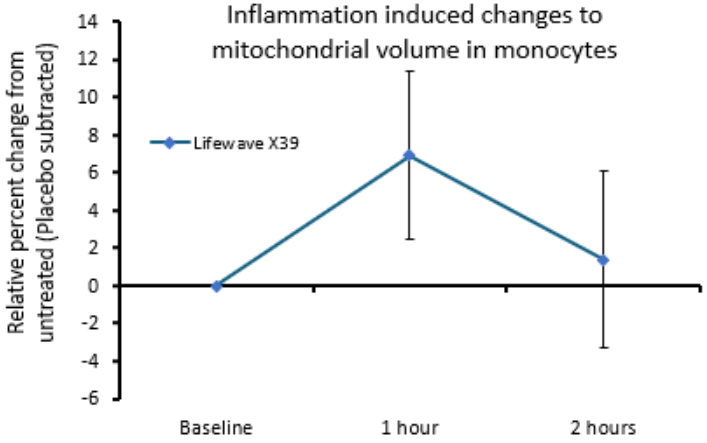
Statistical Comparison

Lifewave X39 - Placebo		*	



Statistical Comparison

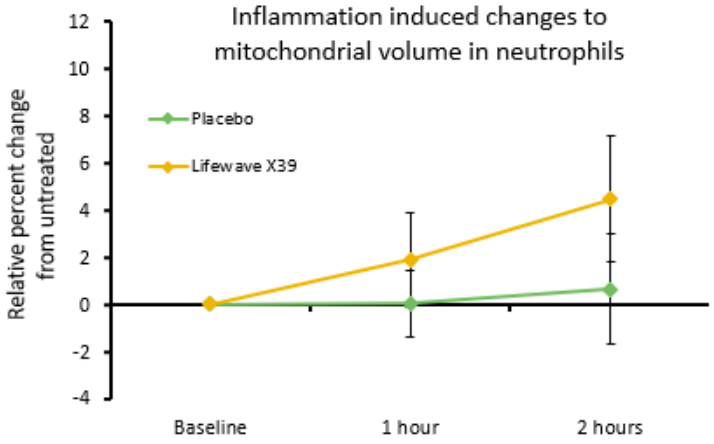
Baseline to		**	**	**
Day 1 to				**
Week 1 to				(*)



Statistical Comparison

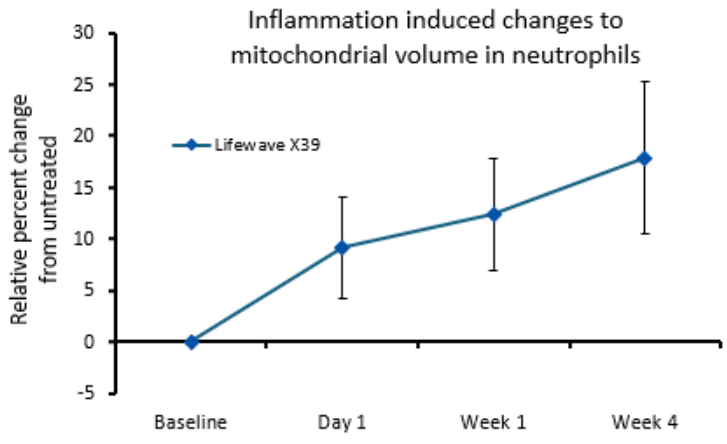
Baseline to		(*)	
1 hour to			*

Figure 15. ***Top left****: Acute changes to mitochondrial volume in monocytes in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to inflammation ex vivo.* ***Bottom left****: The change in mitochondrial volume in monocytes in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in inflammation-induced changes to mitochondrial volume in monocytes in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*



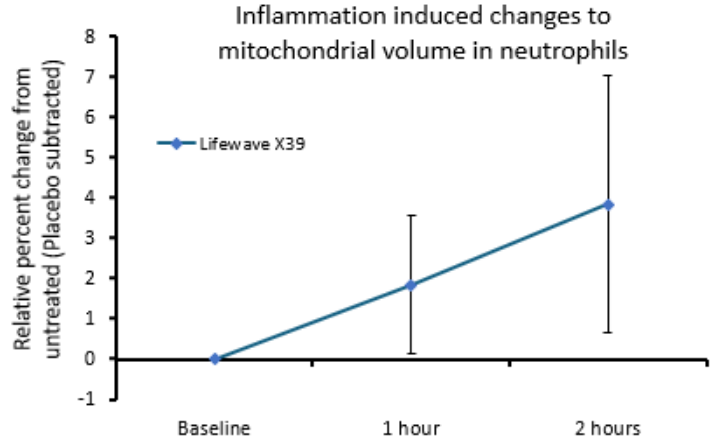
Statistical Comparison

Lifewave X39 - Placebo			(*)



Statistical Comparison

Baseline to		**	**	**
Day 1 to				*
Week 1 to				



Statistical Comparison

Baseline to			(*)
1 hour to			

Figure 16. ***Top left****: Acute changes to mitochondrial volume in neutrophils in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to inflammation ex vivo.* ***Bottom left****: The change in mitochondrial volume in neutrophils in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in inflammation-induced changes to mitochondrial volume in neutrophils in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*

6.1.5 Mitochondrial Membrane Potential

The relative mitochondrial membrane potential is evaluated using the JC-1 reporter dye, which has unique properties where, in the presence of a high electrical potential, the JC-1 molecules will cluster and emit fluorescence in the orange spectrum. The method for evaluation of Mitochondrial Membrane Potential is based on tetraethylbenzimidazolylcarbocyanine iodide (JC-1), a cationic dye that accumulates in energized mitochondria. At low concentrations (due to low mitochondrial membrane potential), JC-1 is predominantly a monomer that yields green fluorescence with emission of 530±15 nm. At high concentrations (due to high mitochondrial membrane potential), the dye aggregates yielding a red to orange colored emission (590±17.5 nm). Therefore, a decrease in the aggregate fluorescent count is indicative of depolarization whereas an increase is indicative of hyperpolarization (Figure 17).

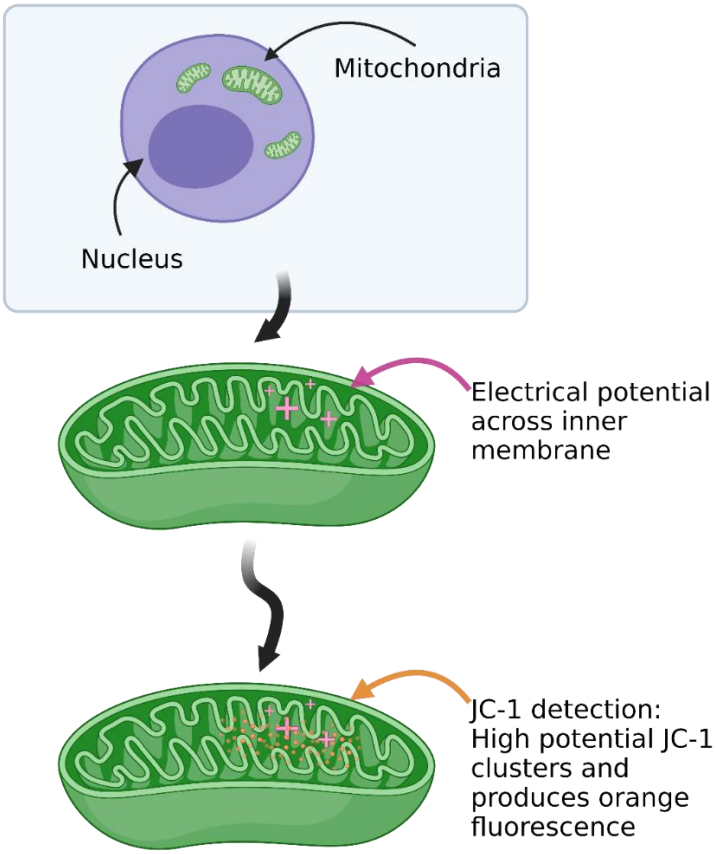


*Figure 17. Mitochondria have an inner and outer membrane. An electrical charge - also called the **mitochondrial membrane potential** – drives part of the cellular energy production, through the electron transport chain. Mitochondria were labeled with the JC-1 fluorescent reporter dye that clusters in the presence of a high electrical potential, and under those conditions emits orange fluorescence.*

For the clinical trial, white blood cells were isolated and cultured briefly in the lab under:

- No stress,
- Oxidative stress,
- Inflammatory stress.

The cell cultures were stained and monitored by **flow cytometry.** This allows electronic gating on the three major types of which blood cells (i.e., circulating immune cells): Mononuclear phagocytes (monocytes), polymorphonuclear phagocytes (granulocytes), and lymphocytes. These cell types have different metabolic states and respond differently to oxidative and inflammatory stress. During the flow cytometry analysis, electronic gating is used to simultaneously examine the orange fluorescence properties of each cell type (Figure 18).

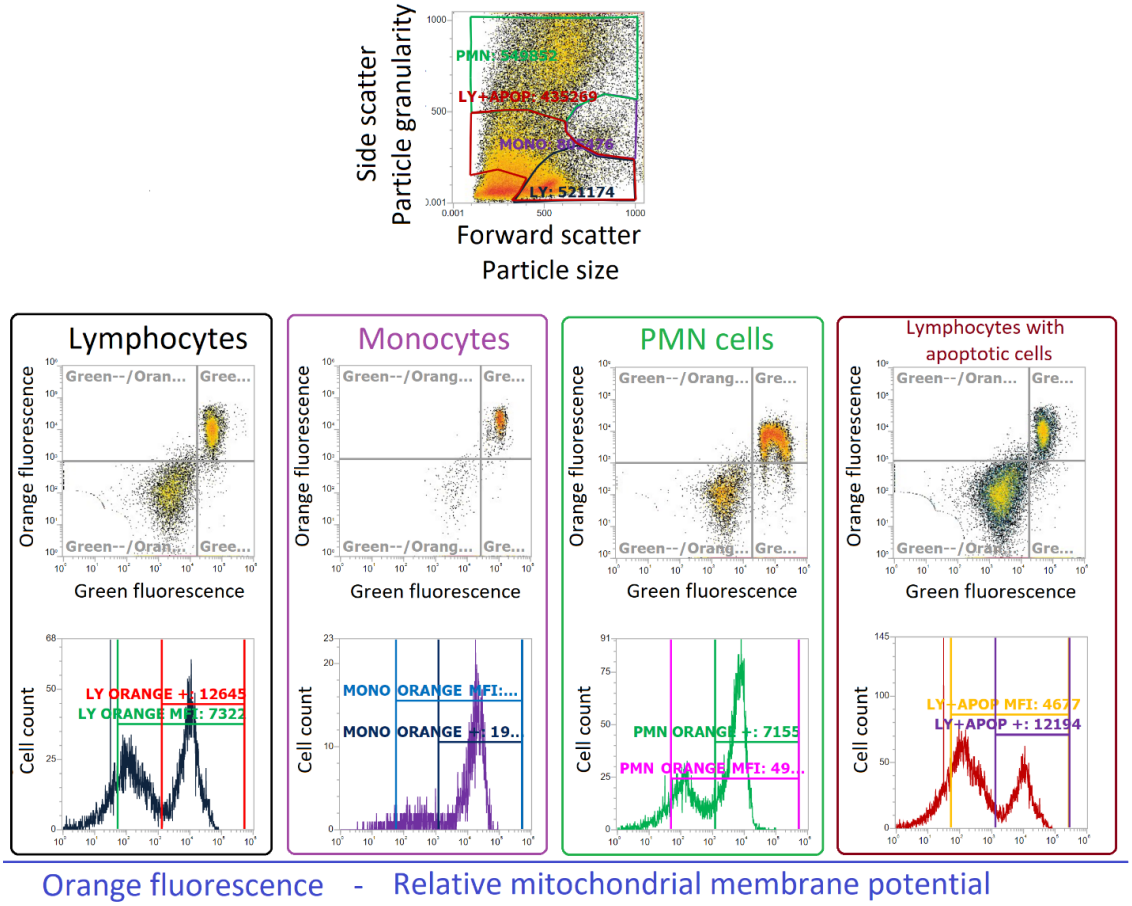
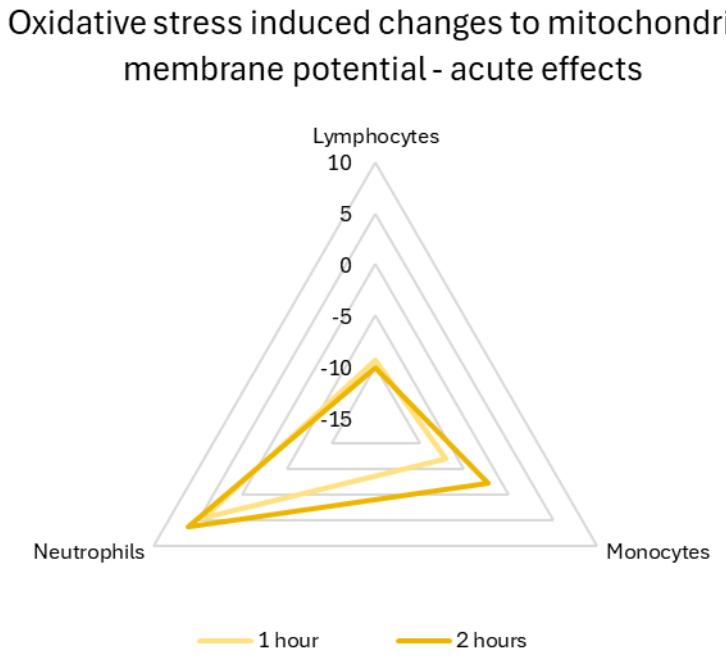


Figure 18. Gating strategy for the analysis of JC-1 orange fluorescence is shown.

6.1.6 Mitochondrial membrane potential under oxidative stress

Mitochondria are vulnerable to oxidative stress and can respond to such stress by increasing the mitochondrial membrane potential to facilitate continued and improved cellular energy production under stressed conditions. We calculated this by comparing the mitochondrial membrane potential in unstressed cell cultures to the mitochondrial membrane potential in H_2O_2-mediated oxidatively stressed cultures.

- In the acute phase of the study, where we documented rapid mitochondrial biogenesis with significantly increased mitochondrial volume per cell, we observed cell type-dependent changes to the mitochondrial membrane potential under oxidative stress conditions, in blood samples drawn when wearing the X39 patch, compared to blood samples drawn when wearing the placebo patch:
    - Lymphocytes: The mitochondrial membrane potential was reduced, compared to placebo. The 10% reduction was highly significant at both 1 and 2 hours.
    - Monocytes: the mitochondrial membrane potential was reduced, compared to placebo. The mild reduction did not reach statistical significance at either 1 or 2 hours.
    - Neutrophils: The mitochondrial membrane potential was increased, compared to placebo. The 4-6% increase was significant at both 1 and 2 hours.
- The long-term effects for the different cell types showed increased mitochondrial membrane potential in all three cell types:
    - Lymphocytes: The increase reached a statistical trend at Day 1, and statistical significance at Week 1, but lost the significance at Week 4 after wearing the X39 patch.
    - Monocytes: The increase reached a statistical trend at Day 1, and a high level of statistical significance at Week 1, but lost the significance at Week 4 after wearing the X39 patch.
    - Neutrophils: The increase reached a high level of statistical significance at Day 1 and Week 1, but lost the significance at Week 4 after wearing the X39 patch.



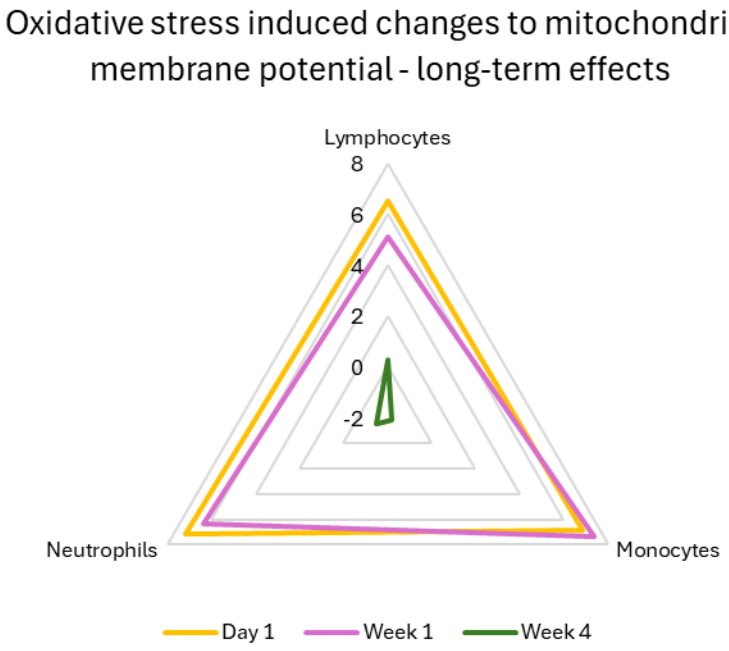


Figure 19. Oxidative stress-induced changes to mitochondrial membrane potential after wearing the X39 patch. ***Top****: Acute effects to the oxidative stress-induced change, compared to wearing the placebo patch.* ***Bottom****: Long-term changes when compared to baseline.*

Table 9. Mitochondrial membrane potential under oxidative stress, acute effects - levels of significance.

	N	Baseline average [a]	P-value [b]	One-hour average [c]	Change [d]	P-value [e]	P-value [f]	P-value [g]	Two-hour average [h]	Change [i]	P-value [j]	P-value [k]	P-value [l]
Lymphocytes													
Placebo	12	-3.6 ± 2.7		6.3 ± 3.2	Increase	0.0000			8.2 ± 3.6	Increase	0.0000		
Lifewave X39	12	2.9 ± 4.9	0.0481	3.5 ± 3.9	Increase	0.4964	0.2017	0.0010	4.7 ± 3.8	Increase	0.5342	0.1466	0.0020
Monocytes													
Placebo	12	-4.8 ± 1.8		1.3 ± 3.1	Increase	0.0373			-2.7 ± 2.6	Increase	0.3444		
Lifewave X39	12	-5.9 ± 3.6	0.7252	-6.9 ± 3.5	Decrease	0.7265	0.0413	0.1049	-6.2 ± 3.1	Decrease	0.9134	0.1396	0.3855
Neutrophils													
Placebo	12	4.6 ± 2.5		3.2 ± 1.7	Decrease	0.4844			4.3 ± 2.4	Decrease	0.9159		
Lifewave X39	12	0.2 ± 2.2	0.0622	3.2 ± 3.6	Increase	0.0622	0.9220	0.0618	6.1 ± 2.8	Increase	0.0008	0.6204	0.0147

[a] Average ± standard error of the mean at baseline
[b] Significance to placebo at baseline
[c] Average ± standard error of the mean at 1 hour
[d] Change from baseline at 1 hour
[e] Significance of change from baseline to 1 hour
[f] Significance to placebo at 1 hour
[g] Significance to placebo, percent change at 1 hour
[h] Average ± standard error of the mean at 2 hours
[i] Change from baseline at 2 hours
[j] Significance of change from baseline to 2 hours
[k] Significance to placebo at 2 hours
[l] Significance to placebo, percent change at 2 hours

Table 10. Mitochondrial membrane potential under oxidative stress, placebo subtracted, acute effects - levels of significance.

	N	Baseline average [a]	One-hour average [b]	P-value [c]	Two-hour average [d]	P-value [e]	P-value [f]
Lymphocytes							
Lifewave X39	12	0 ± 0	-9.3 ± 4.3	0.0047	-10 ± 5.2	0.0028	0.6298
Monocytes							
Lifewave X39	12	0 ± 0	-7.1 ± 5.8	0.1834	-2.4 ± 3.1	0.3497	0.3456
Neutrophils							
Lifewave X39	12	0 ± 0	4.4 ± 3.5	0.0447	6.1 ± 3.5	0.0129	0.4457

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at 1 hour

[c] Significance of difference in percent change from baseline to 1 hour

[d] Average ± standard error of the mean at 2 hours

[e] Significance of difference in percent change from baseline to 2 hours

[f] Significance of difference in percent change from 1 hour to 2 hours

Table 11. Mitochondrial membrane potential under oxidative stress, long-term effects - levels of significance.

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one Average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
Lymphocytes											
Lifewave X39	12	-4.6 ± 3.4	1.9 ± 3.8	Increase	0.0637	0.5 ± 2.7	Increase	0.0249	-4.3 ± 4.4	Increase	0.9252
Monocytes											
Lifewave X39	12	-6.8 ± 3.2	0 ± 5.6	Increase	0.0665	0.6 ± 2.1	Increase	0.0049	-8.7 ± 2.6	Decrease	0.4935
Neutrophils											
Lifewave X39	12	1.9 ± 2.4	9.1 ± 3.2	Increase	0.0017	8.3 ± 2.8	Increase	0.0035	0.4 ± 2.5	Decrease	0.4305

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at day 1

[c] Change from baseline at day 1

[d] Significance of difference in percent change from baseline to day 1

[e] Average ± standard error of the mean at week 1

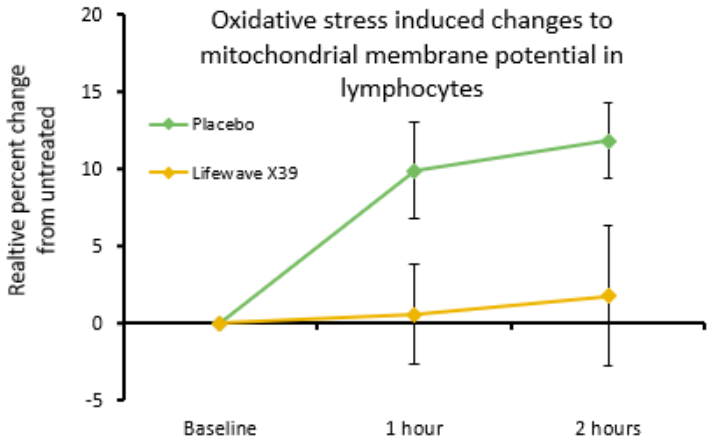
[f] Change from baseline at week 1

[g] Significance of difference in percent change from baseline to week 1

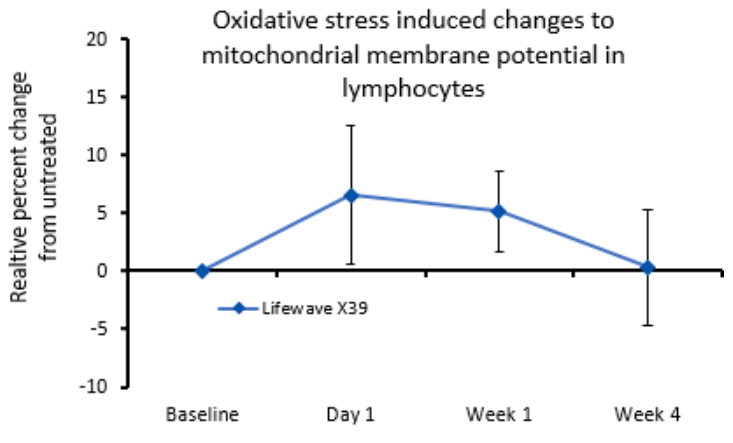
[h] Average ± standard error of the mean at week 4

[i] Change from baseline at week 4

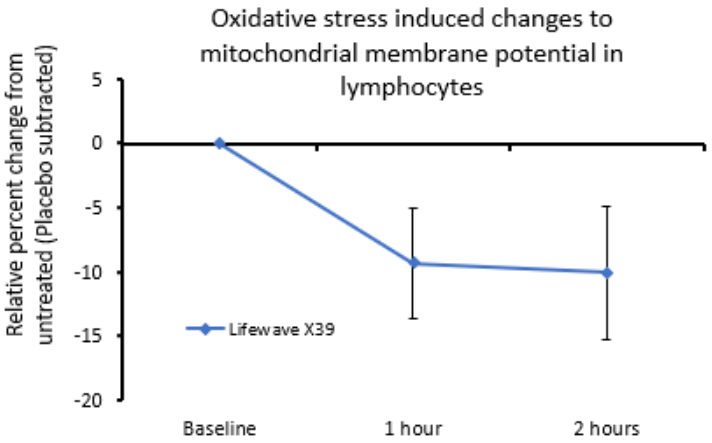
[j] Significance of difference in percent change from baseline to week 4



Statistical Comparison			
Lifewave X39 - Placebo		**	**

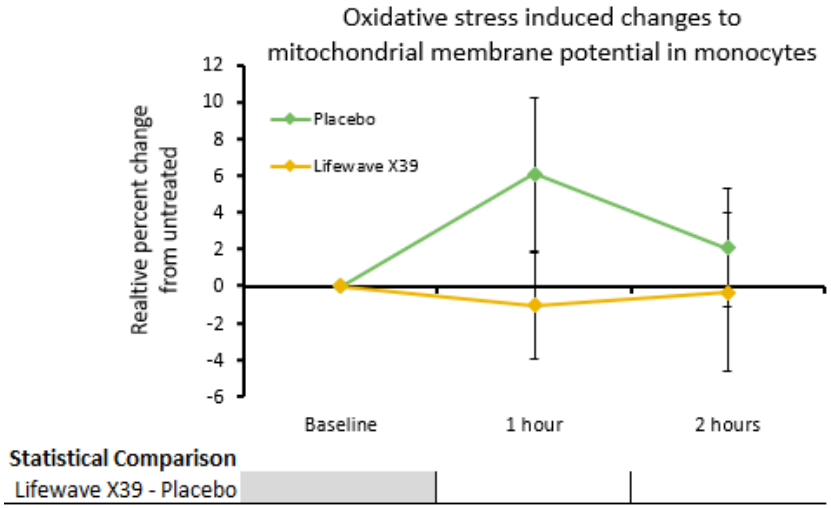


Statistical Comparison				
Baseline to		(*)	*	
Day 1 to				(*)
Week 1 to				



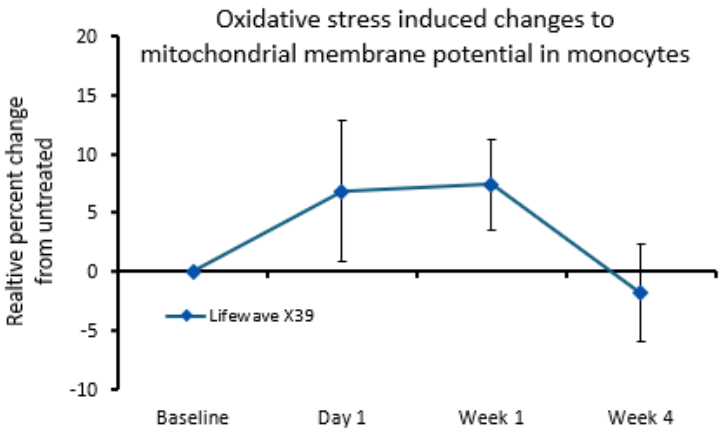
Statistical Comparison			
Baseline to		**	**
1 hour to			

Figure 20. ***Top left****: Acute changes to mitochondrial membrane potential in lymphocytes in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to oxidative stress ex vivo.* ***Bottom left****: The change in mitochondrial membrane potential in lymphocytes in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in oxidative stress-induced changes to mitochondrial membrane potential in lymphocytes in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*



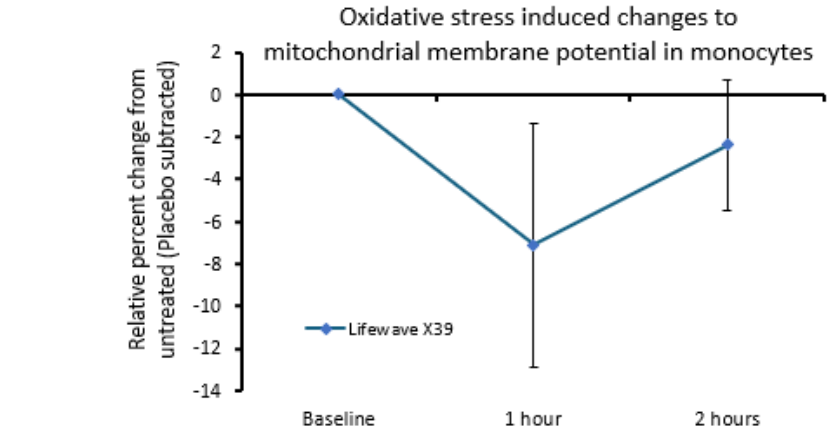
Statistical Comparison

Lifewave X39 - Placebo			



Statistical Comparison

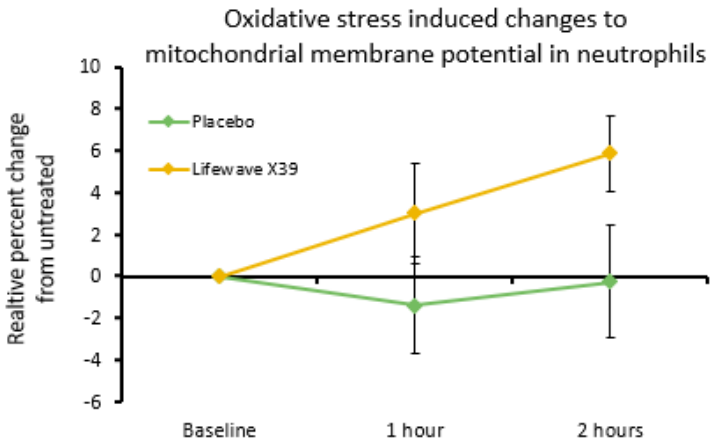
Baseline to		(*)	**	
Day 1 to				(*)
Week 1 to				**



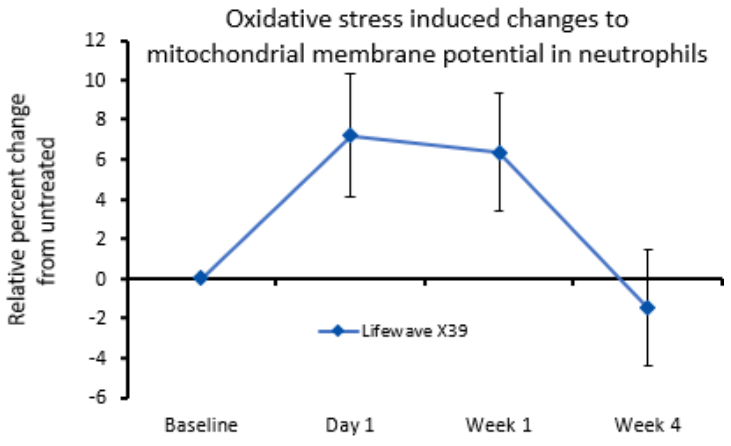
Statistical Comparison

Baseline to			
1 hour to			

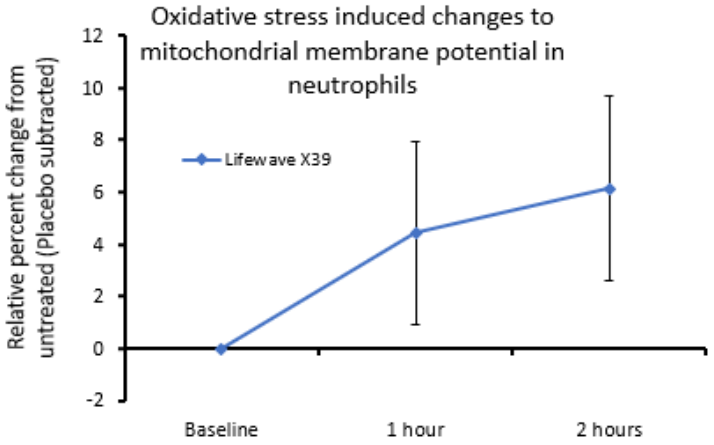
Figure 21. ***Top left****: Acute changes to mitochondrial membrane potential in monocytes in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to oxidative stress ex vivo.* ***Bottom left****: The change in mitochondrial membrane potential in monocytes in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in oxidative stress-induced changes to mitochondrial membrane potential in monocytes in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*



Statistical Comparison			
Lifewave X39 - Placebo		(*)	*



Statistical Comparison				
Baseline to		**	**	
Day 1 to				**
Week 1 to				**



Statistical Comparison			
Baseline to		*	*
1 hour to			

Figure 22. ***Top left****: Acute changes to mitochondrial membrane potential in neutrophils in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to oxidative stress ex vivo.* ***Bottom left****: The change in mitochondrial membrane potential in neutrophils in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in oxidative stress-induced changes to mitochondrial membrane potential in neutrophils in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*

6.1.7 Mitochondrial membrane potential under inflammatory stress

Mitochondria are sensitive to inflammatory stress and can respond to such stress by increasing the mitochondrial membrane potential to facilitate continued and improved cellular energy production under stressed conditions. We calculated this by comparing the mitochondrial membrane potential in unstressed cell cultures to the mitochondrial membrane potential in cell cultures inflamed with the bacterial toxin LPS.

- In the acute phase of the study, we documented rapid changes to the mitochondrial membrane potential. We observed cell type-dependent changes to the mitochondrial membrane potential under inflammatory stress conditions, in blood samples drawn when wearing the X39 patch, compared to blood samples drawn when wearing the placebo patch:
    - Lymphocytes: The mitochondrial membrane potential was reduced, compared to placebo. The 10% reduction was highly significant at 1 hour and significant at 2 hours.
    - Monocytes: the mitochondrial membrane potential showed a mild increase at 2 hours; this change did not reach statistical significance.
    - Neutrophils: The mitochondrial membrane potential was not significantly altered at 1 or 2 hours.
- The long-term effects for the different cell types showed increased mitochondrial membrane potential in all three cell types:
    - Lymphocytes: The increase reached a high level of statistical significance at Day 1 and Week 1 but lost the significance at Week 4 after wearing the X39 patch.
    - Monocytes: The increase reached a high level of statistical significance at Day 1 and Week 1 but lost the significance at Week 4 after wearing the X39 patch.
    - Neutrophils: The increase reached a high level of statistical significance at Day 1 and Week 1 but lost the significance at Week 4 after wearing the X39 patch.

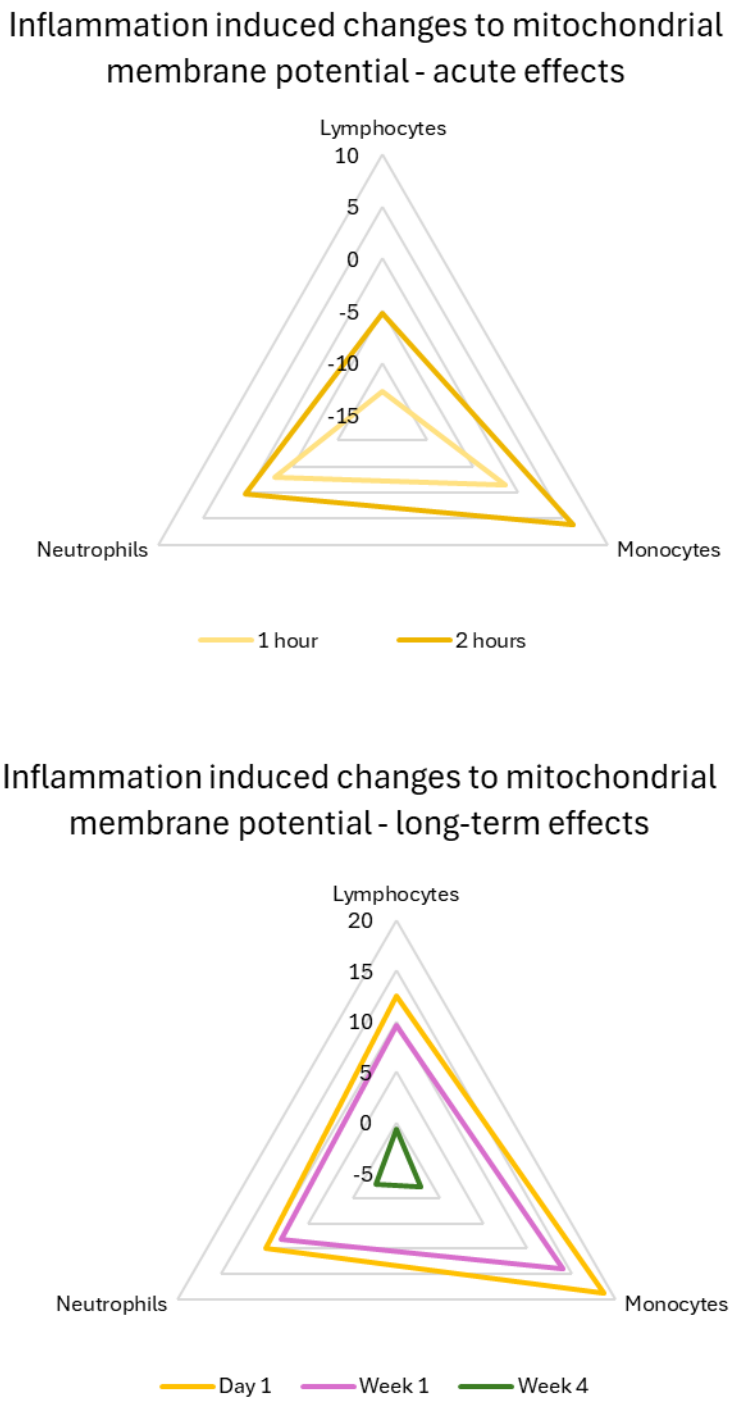


Figure 23. Inflammation-induced changes to mitochondrial membrane potential after wearing the X39 patch. ***Top****: Acute effects to the inflammation-induced change, compared to wearing the placebo patch.* ***Bottom****: Long-term changes when compared to baseline*

Table 12. Mitochondrial membrane potential under inflammatory stress, acute effects - levels of significance.

	N	Baseline average [a]	P-value [b]	One-hour average [c]	Change [d]	P-value [e]	P-value [f]	P-value [g]	Two-hour average [h]	Change [i]	P-value [j]	P-value [k]	P-value [l]
Lymphocytes													
Placebo	12	-0.3 ± 2.7		6.5 ± 3.7	Increase	0.0311			0.8 ± 2.4	Increase	0.6169		
Lifewave X39	12	6 ± 2.9	0.0142	0.1 ± 3.3	Decrease	0.0055	0.0898	0.0003	1.8 ± 1.9	Decrease	0.1171	0.6556	0.0304
Monocytes													
Placebo	12	8.9 ± 4.7		15.3 ± 6.1	Increase	0.0308			9 ± 7.1	Increase	0.9796		
Lifewave X39	12	2.4 ± 7.4	0.2231	7.4 ± 7.4	Increase	0.0363	0.1867	0.6033	8.6 ± 7.5	Increase	0.0746	0.9395	0.0953
Neutrophils													
Placebo	12	-5.2 ± 2.7		-3.4 ± 2.1	Increase	0.3630			-2.5 ± 3.2	Increase	0.2583		
Lifewave X39	12	-7.2 ± 1.9	0.4528	-8.4 ± 2.6	Decrease	0.5657	0.0254	0.2760	-4.3 ± 4.2	Increase	0.2773	0.5473	0.9466

[a] Average ± standard error of the mean at baseline
[b] Significance to placebo at baseline
[c] Average ± standard error of the mean at 1 hour
[d] Change from baseline at 1 hour
[e] Significance of change from baseline to 1 hour
[f] Significance to placebo at 1 hour
[g] Significance to placebo, percent change at 1 hour
[h] Average ± standard error of the mean at 2 hours
[i] Change from baseline at 2 hours
[j] Significance of change from baseline to 2 hours
[k] Significance to placebo at 2 hours
[l] Significance to placebo, percent change at 2 hours

Table 13. Mitochondrial membrane potential under inflammatory stress, placebo subtracted, acute effects - levels of significance.

	N	Baseline average [a]	One-hour average [b]	P-value [c]	Two-hour average [d]	P-value [e]	P-value [f]
Lymphocytes							
Lifewave X39	12	0 ± 0	-12.7 ± 4.7	0.0001	-5.3 ± 3.1	0.0361	0.0175
Monocytes							
Lifewave X39	12	0 ± 0	-1.4 ± 3.4	0.5986	6.1 ± 5.1	0.1030	0.0166
Neutrophils							
Lifewave X39	12	0 ± 0	-3 ± 3.9	0.2604	0.2 ± 6	0.9475	0.2743

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at 1 hour

[c] Significance of difference in percent change from baseline to 1 hour

[d] Average ± standard error of the mean at 2 hours

[e] Significance of difference in percent change from baseline to 2 hours

[f] Significance of difference in percent change from 1 hour to 2 hours

Table 14. Mitochondrial membrane potential under inflammatory stress, long-term effects - levels of significance.

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one Average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
Lymphocytes											
Lifewave X39	12	5.2 ± 2.9	17.7 ± 3.9	Increase	0.0006	14.9 ± 4.9	Increase	0.0041	4.5 ± 4.6	Decrease	0.8337
Monocytes											
Lifewave X39	12	6.7 ± 7.2	25.4 ± 11.3	Increase	0.0024	20.7 ± 4.7	Increase	0.0013	4.5 ± 4.8	Decrease	0.6975
Neutrophils											
Lifewave X39	12	-2.8 ± 2.4	7.1 ± 4.2	Increase	0.0003	5.3 ± 4.3	Increase	0.0041	-5.5 ± 3.7	Decrease	0.4397

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at day 1

[c] Change from baseline at day 1

[d] Significance of difference in percent change from baseline to day 1

[e] Average ± standard error of the mean at week 1

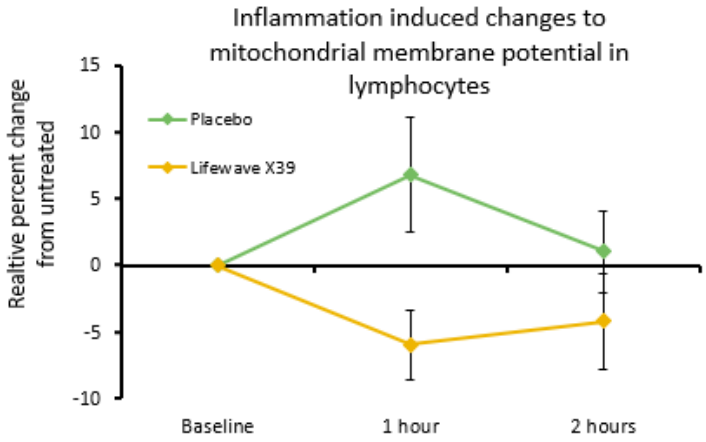
[f] Change from baseline at week 1

[g] Significance of difference in percent change from baseline to week 1

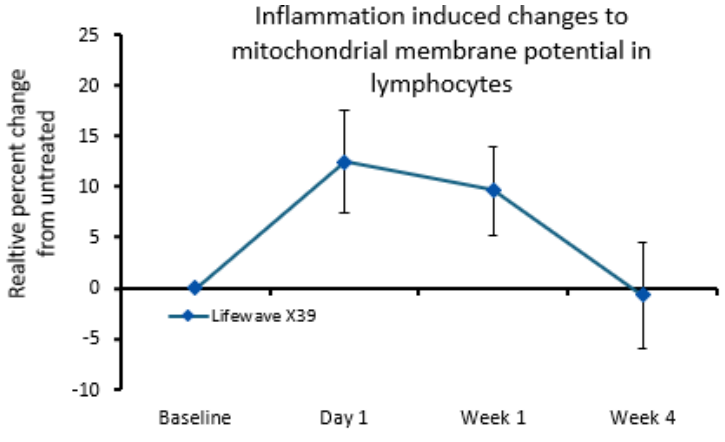
[h] Average ± standard error of the mean at week 4

[i] Change from baseline at week 4

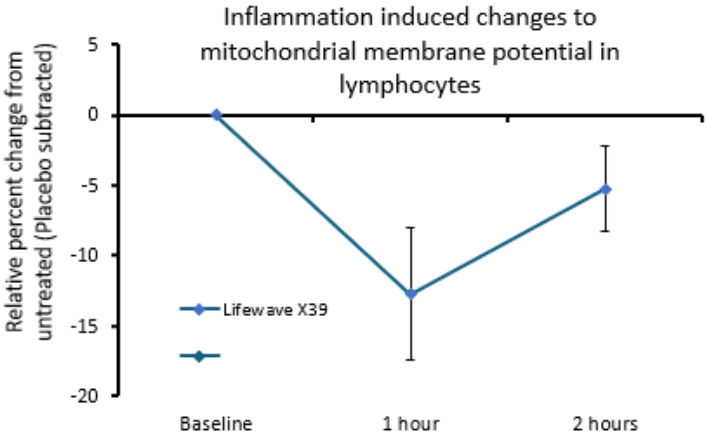
[j] Significance of difference in percent change from baseline to week 4



Statistical Comparison			
Lifewave X39 - Placebo		**	*

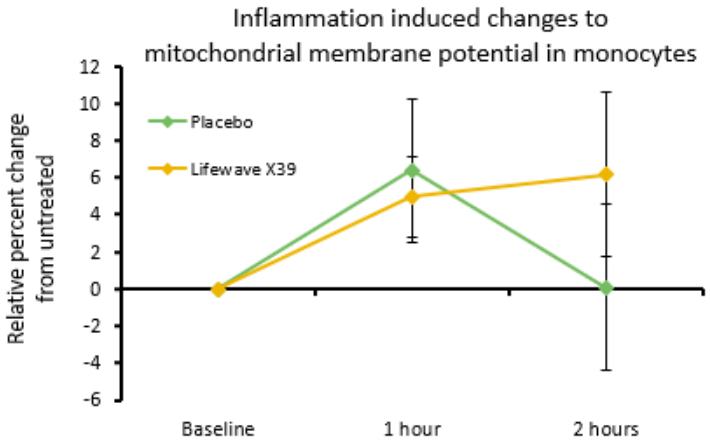


Statistical Comparison				
Baseline to		**	**	
Day 1 to				**
Week 1 to				*



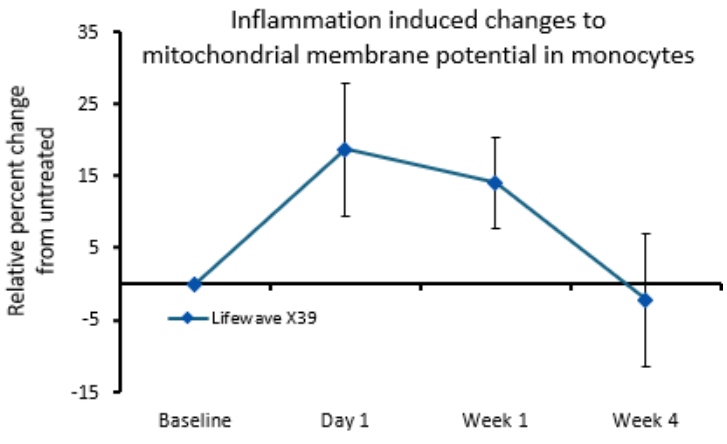
Statistical Comparison			
Baseline to		**	*
1 hour to			*

Figure 24. ***Top left****: Acute changes to mitochondrial membrane potential in lymphocytes in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to inflammation ex vivo.* ***Bottom left****: The change in mitochondrial membrane potential in lymphocytes in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in inflammation-induced changes to mitochondrial membrane potential in lymphocytes in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*



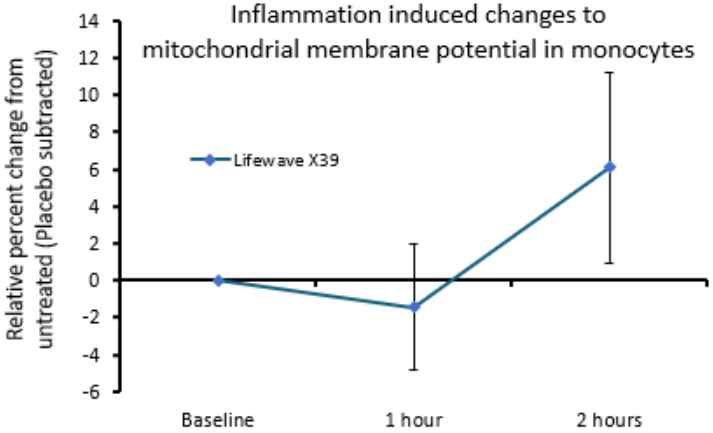
Statistical Comparison

Lifewave X39 - Placebo			(*)



Statistical Comparison

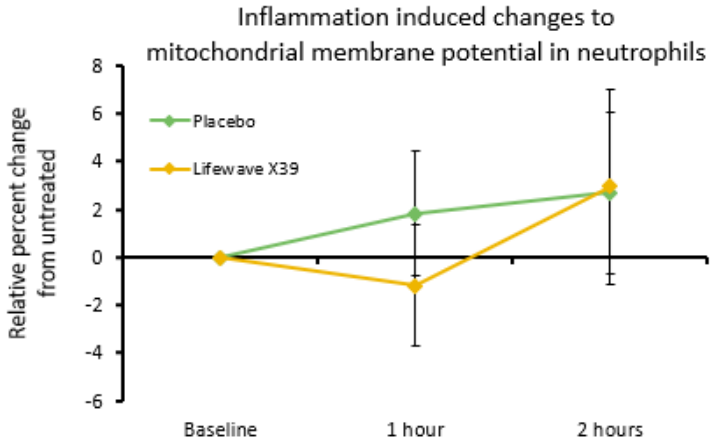
Baseline to		**	**	
Day 1 to				*
Week 1 to				**



Statistical Comparison

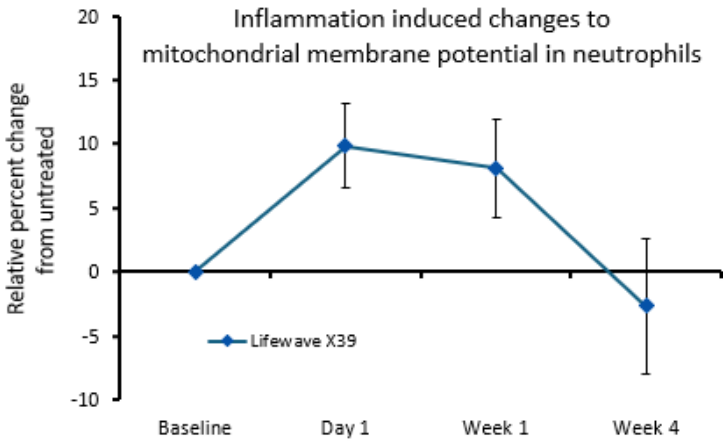
Baseline to			
1 hour to			*

Figure 25. ***Top left****: Acute changes to mitochondrial membrane potential in monocytes in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to inflammation ex vivo.* ***Bottom left****: The change in mitochondrial membrane potential in monocytes in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in inflammation-induced changes to mitochondrial membrane potential in monocytes in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*



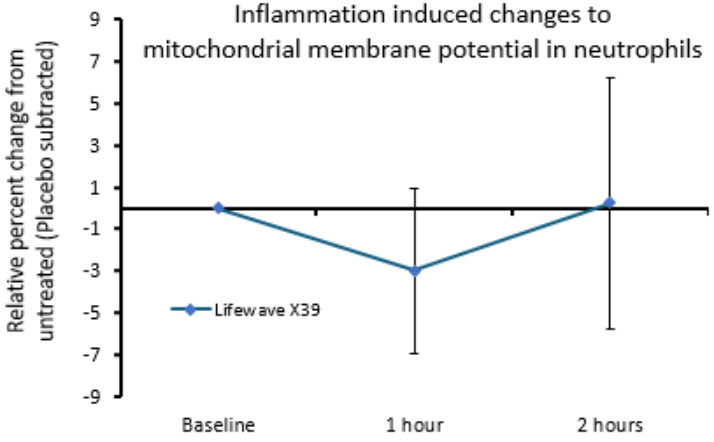
Statistical Comparison

Lifewave X39 - Placebo			



Statistical Comparison

Baseline to		**	**	
Day 1 to				**
Week 1 to				**



Statistical Comparison

Baseline to			
1 hour to			

Figure 26. ***Top left****: Acute changes to mitochondrial membrane potential in neutrophils in blood circulation when wearing the Lifewave X39 or placebo patch, exposed to inflammation ex vivo.* ***Bottom left****: The change in mitochondrial membrane potential in neutrophils in blood circulation when wearing the X39 patch, adjusted by subtracting the changes when using the placebo patch.* ***Top right****: The relative percent change in inflammation-induced changes to mitochondrial membrane potential in neutrophils in blood circulation after wearing the Lifewave X39 patch over 4 weeks.*

6.2 Bodily Communication: Cytokines and Growth Factors

Serum was used to test **cytokine profile**, to document rapid changes to cytokine levels in each study participant, reflecting communication within the immune system. Testing involved a broad panel of **pro- and anti-inflammatory cytokines, anti-viral peptides, and regenerative growth factors**, using a 27-plex Luminex magnetic bead array and the MagPix® multiplexing system. The following markers are tested: IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, basic FGF, G-CSF, GM-CSF, IFN-gamma, IP-10, MCP-1 (MCAF), MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, and VEGF.

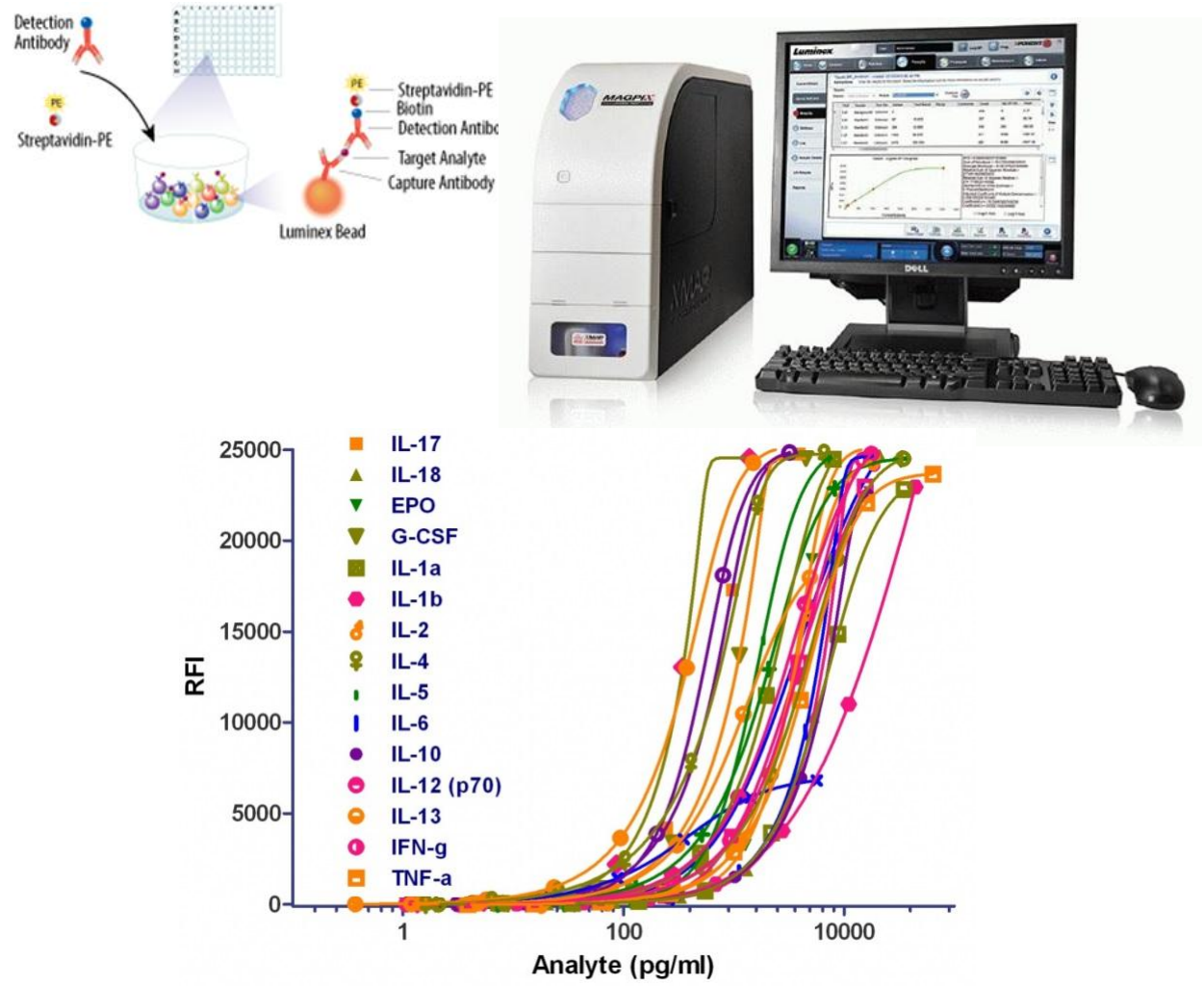


Figure 27. Luminex multiplex protein array using magnetic bead principles to capture and quantify multiple biomarkers in one small biological samples (in vitro or clinical).

Synopsis:

Cytokines, chemokines, and growth factors are the chemical signals our body use to communicate. Many cell types can produce cytokines, including immune cells, endothelial and epithelial cells, and muscle cells. All tissues and organs can respond to cytokines, including immune tissue, the vasculature, and the brain.

In both in the acute and the long-term phases of this study, we documented changes to cytokine levels when wearing the X39 patch.

- Acute effects: In blood samples drawn when wearing the X39 patch, compared to blood samples drawn when wearing the placebo patch, there were rapid and selective changes to specific cytokines:
    - The anti-inflammatory cytokine IL-1ra showed a mild increase at 1 hour, returning to similar levels as placebo at 2 hours. The change when compared to placebo did not reach statistical significance.
    - The chemokine IL-8 showed a mild decrease at 1 hour, reaching a statistical trend. The levels returned to similar levels as when wearing the placebo patch at 2 hours.
    - The chemokine RANTES and the immune-activating pro-inflammatory cytokine TNF-α were increased at 2 hours when compared to placebo. The increase in RANTES levels was highly significant, and the increase for TNF-α was significant.
    - The reparative growth factor PDGF-BB showed an increase at 2 hours above that of the placebo. This increase was significant when compared to placebo.
- Long-term effects: In blood samples taken from the participants as they were using the X39 patch for 4 weeks, there were selective changes to specific cytokines.
    - The anti-inflammatory cytokine IL-1ra showed a rapid increase at Day 1, lasting through Week 1, and returning to baseline levels at Week 4.
    - The chemokine IL-8 showed significant reduction at Day 1 and Week 4.
    - RANTES levels showed a very mild and insignificant increase at Week 1, deemed of no real physiological relevance.
    - The TNF-α levels showed a rapid reduction at Day 1, reaching a statistical trend. The effect was not visible at Week 1 or Week 4.
    - The levels of PDGF-BB did not change significantly long-term, suggesting that a short-lived pulse may happen every time a new patch is applied.

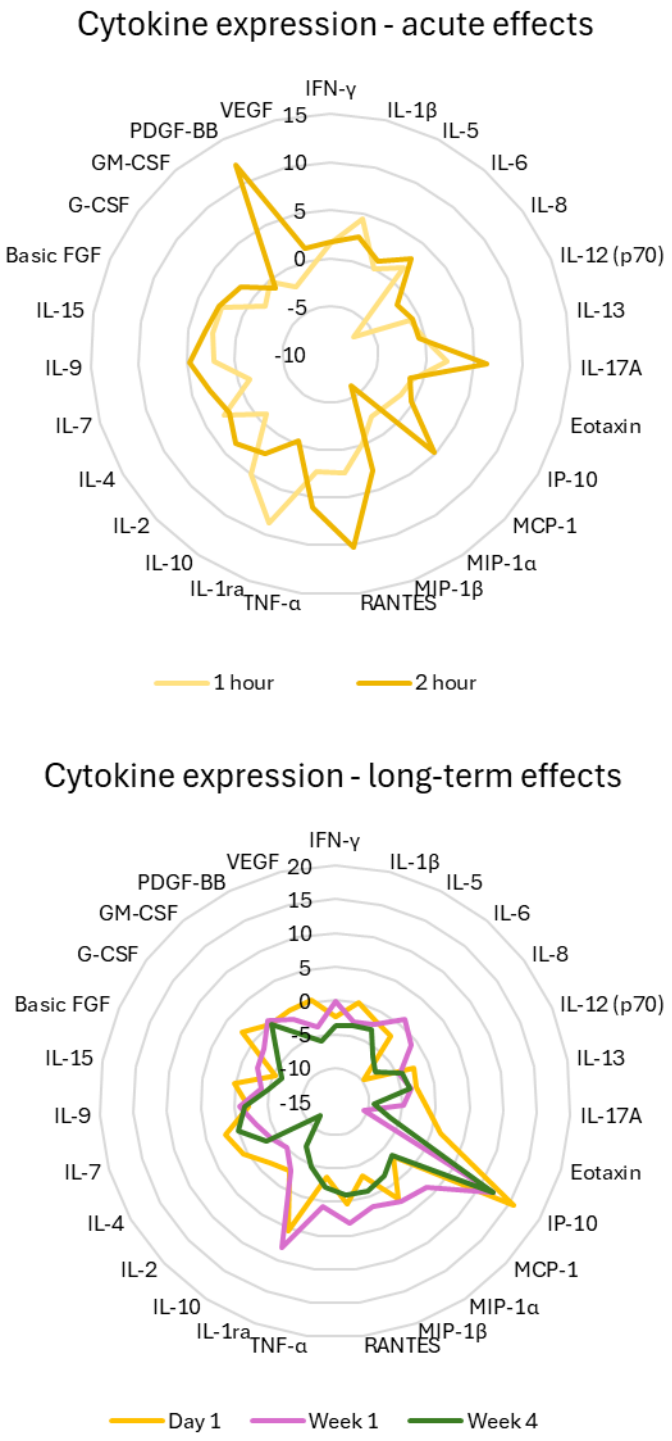


Figure 28. Radar map showing changes to cytokine levels of the 27 cytokines tested

Table 15. Pro-inflammatory cytokines, acute effects - levels of significance.

	N	Baseline average [a]	P-value [b]	One-hour average [c]	Change [d]	P-value [e]	P-value [f]	P-value [g]	Two-hour average [h]	Change [i]	P-value [j]	P-value [k]	P-value [l]
IFN-γ													
Placebo	12	21.8 ± 1.5		21.3 ± 1.5	Decrease	0.4434			20.8 ± 1.6	Decrease	0.1820		
Lifewave X39	12	21.5 ± 1.5	0.5779	21.2 ± 1.2	Decrease	0.5062	0.8473	0.6732	20.9 ± 1.4	Decrease	0.1317	0.9307	0.6433
IL-1β													
Placebo	12	14.6 ± 0.6		14 ± 0.5	Decrease	0.1876			14.1 ± 0.7	Decrease	0.3015		
Lifewave X39	12	14.4 ± 0.5	0.6418	14.6 ± 0.6	Increase	0.3473	0.0809	0.1284	14.4 ± 0.7	Decrease	0.9091	0.4431	0.5203
IL-5													
Placebo	12	19.3 ± 1.5		18.9 ± 1.5	Decrease	0.4154			19.1 ± 1.7	Decrease	0.7547		
Lifewave X39	12	19.5 ± 1.7	0.7361	18.9 ± 1.4	Decrease	0.1434	0.8947	0.9980	19.1 ± 1.3	Decrease	0.4306	1.0000	0.8177
IL-6													
Placebo	12	18.1 ± 1.3		17.3 ± 0.8	Decrease	0.1756			17.5 ± 1	Decrease	0.2400		
Lifewave X39	12	17.2 ± 0.5	0.0945	17.1 ± 0.8	Decrease	0.9052	0.5884	0.6701	17.3 ± 0.7	Increase	0.4373	0.6782	0.3371
IL-8													
Placebo	12	25.1 ± 1.4		24.3 ± 1.3	Decrease	0.3996			23.1 ± 1.4	Decrease	0.0672		
Lifewave X39	12	26.4 ± 1.8	0.2201	23.6 ± 1.4	Decrease	0.0023	0.4417	0.1012	24 ± 1.7	Decrease	0.0068	0.3549	0.7368

[a] Average ± standard error of the mean at baseline
[b] Significance to placebo at baseline
[c] Average ± standard error of the mean at 1 hour
[d] Change from baseline at 1 hour
[e] Significance of change from baseline to 1 hour
[f] Significance to placebo at 1 hour
[g] Significance to placebo, percent change at 1 hour
[h] Average ± standard error of the mean at 2 hours
[i] Change from baseline at 2 hours
[j] Significance of change from baseline to 2 hours
[k] Significance to placebo at 2 hours
[l] Significance to placebo, percent change at 2 hours

Table 16. Pro-inflammatory cytokines, acute effects - levels of significance.

	N	Baseline average [a]	P-value [b]	One-hour average [c]	Change [d]	P-value [e]	P-value [f]	P-value [g]	Two-hour average [h]	Change [i]	P-value [j]	P-value [k]	P-value [l]
IL-12 (p70)													
Placebo	12	18 ± 1.1		17.3 ± 0.7	Decrease	0.3872			17.6 ± 0.8	Decrease	0.6432		
Lifewave X39	12	17.8 ± 0.7	0.5259	17.2 ± 0.6	Decrease	0.4738	0.8269	0.8229	17.5 ± 0.7	Decrease	0.9574	0.7421	0.8604
IL-13													
Placebo	12	17.1 ± 0.6		16.8 ± 0.3	Decrease	0.4353			16.9 ± 0.5	Decrease	0.6674		
Lifewave X39	12	17 ± 0.5	0.8266	16.8 ± 0.5	Decrease	0.7903	1.0000	0.9556	16.8 ± 0.4	Decrease	0.5375	0.7688	0.7942
IL-17A													
Placebo	12	22.8 ± 3		20.4 ± 0.8	Decrease	0.3310			20.5 ± 0.9	Decrease	0.3437		
Lifewave X39	12	20.6 ± 1	0.3066	20.4 ± 0.7	Decrease	0.9429	0.9438	0.7341	21.1 ± 0.8	Increase	0.3006	0.3848	0.3941
Eotaxin													
Placebo	12	418 ± 48.8		418 ± 50.6	Increase	0.9836			435 ± 53.8	Increase	0.1720		
Lifewave X39	12	425 ± 52.5	0.6077	422 ± 53.6	Decrease	0.7716	0.7682	0.7775	434 ± 53.8	Increase	0.3796	0.9570	0.6908
IP-10													
Placebo	12	242 ± 96.6		230 ± 85.3	Decrease	0.2738			236 ± 81.1	Decrease	0.7094		
Lifewave X39	12	228 ± 81.5	0.3373	211 ± 66.7	Decrease	0.1790	0.1971	0.7955	210 ± 55.9	Decrease	0.3389	0.1687	0.9585

[a] Average ± standard error of the mean at baseline
[b] Significance to placebo at baseline
[c] Average ± standard error of the mean at 1 hour
[d] Change from baseline at 1 hour
[e] Significance of change from baseline to 1 hour
[f] Significance to placebo at 1 hour
[g] Significance to placebo, percent change at 1 hour
[h] Average ± standard error of the mean at 2 hours
[i] Change from baseline at 2 hours
[j] Significance of change from baseline to 2 hours
[k] Significance to placebo at 2 hours
[l] Significance to placebo, percent change at 2 hours

Table 17. Pro-inflammatory cytokines, acute effects - levels of significance.

	N	Baseline average [a]	P-value [b]	One-hour average [c]	Change [d]	P-value [e]	P-value [f]	P-value [g]	Two-hour average [h]	Change [i]	P-value [j]	P-value [k]	P-value [l]
MCP-1													
Placebo	12	54.6 ± 6.4		51.2 ± 5.6	Decrease	0.0140			49.9 ± 6	Decrease	0.0630		
Lifewave X39	12	53.8 ± 7	0.5699	48.1 ± 5.3	Decrease	0.0154	0.0560	0.5138	50.6 ± 5.3	Decrease	0.1386	0.7237	0.2132
MIP-1α													
Placebo	12	15.1 ± 0.7		14.8 ± 0.6	Decrease	0.5708			15.6 ± 0.7	Increase	0.3021		
Lifewave X39	12	15.7 ± 0.7	0.3562	15.1 ± 0.6	Decrease	0.1252	0.3667	0.5666	15.1 ± 0.5	Decrease	0.1757	0.2289	0.0725
MIP-1β													
Placebo	12	1,239 ± 27.5		1,185 ± 38.7	Decrease	0.1138			1,196 ± 27.2	Decrease	0.1471		
Lifewave X39	12	1,274 ± 29.6	0.2826	1,217 ± 31.9	Decrease	0.0134	0.3148	0.9676	1,266 ± 24.4	Decrease	0.7848	0.0101	0.3705
RANTES													
Placebo	12	5,678 ± 152		5,460 ± 164	Decrease	0.0958			5,427 ± 139	Decrease	0.0187		
Lifewave X39	12	5,540 ± 132	0.2831	5,465 ± 136	Decrease	0.4873	0.9674	0.3438	5,858 ± 114	Increase	0.0086	0.0011	0.0010
TNF-α													
Placebo	12	57.3 ± 1.7		54.2 ± 1.9	Decrease	0.0126			53.6 ± 1.8	Decrease	0.0022		
Lifewave X39	12	57.4 ± 1.6	0.9509	55.5 ± 1.4	Decrease	0.0775	0.2530	0.3449	56.9 ± 1.5	Decrease	0.6648	0.0086	0.0324

[a] Average ± standard error of the mean at baseline
[b] Significance to placebo at baseline
[c] Average ± standard error of the mean at 1 hour
[d] Change from baseline at 1 hour
[e] Significance of change from baseline to 1 hour
[f] Significance to placebo at 1 hour
[g] Significance to placebo, percent change at 1 hour
[h] Average ± standard error of the mean at 2 hours
[i] Change from baseline at 2 hours
[j] Significance of change from baseline to 2 hours
[k] Significance to placebo at 2 hours
[l] Significance to placebo, percent change at 2 hours

Table 18. Anti-inflammatory cytokines, acute effects - levels of significance

	N	Baseline average [a]	P-value [b]	One-hour average [c]	Change [d]	P-value [e]	P-value [f]	P-value [g]	Two-hour average [h]	Change [i]	P-value [j]	P-value [k]	P-value [l]
IL-1ra													
Placebo	12	14.3 ± 1.1		13.9 ± 0.6	Decrease	0.4290			14.1 ± 0.5	Decrease	0.7597		
Lifewave X39	12	14 ± 0.6	0.6623	15 ± 1	Increase	0.1863	0.0747	0.1991	14 ± 0.6	Increase	0.9006	0.8652	0.9348
IL-10													
Placebo	12	14.6 ± 1.2		13.2 ± 0.4	Decrease	0.0817			13.6 ± 0.5	Decrease	0.2053		
Lifewave X39	12	13.9 ± 0.7	0.4005	13.5 ± 0.4	Decrease	0.3087	0.1925	0.2224	13.5 ± 0.4	Decrease	0.3701	0.7298	0.6395

[a] Average ± standard error of the mean at baseline
[b] Significance to placebo at baseline
[c] Average ± standard error of the mean at 1 hour
[d] Change from baseline at 1 hour
[e] Significance of change from baseline to 1 hour
[f] Significance to placebo at 1 hour
[g] Significance to placebo, percent change at 1 hour
[h] Average ± standard error of the mean at 2 hours
[i] Change from baseline at 2 hours
[j] Significance of change from baseline to 2 hours
[k] Significance to placebo at 2 hours
[l] Significance to placebo, percent change at 2 hours

Table 19. Regulating cytokines, acute effects- levels of significance

	N	Baseline average [a]	P-value [b]	One-hour average [c]	Change [d]	P-value [e]	P-value [f]	P-value [g]	Two-hour average [h]	Change [i]	P-value [j]	P-value [k]	P-value [l]
IL-2													
Placebo	12	13.5 ± 2.5		11.4 ± 0.5	Decrease	0.3124			11.9 ± 0.8	Decrease	0.4348		
Lifewave X39	12	12 ± 0.8	0.3707	11.4 ± 0.4	Decrease	0.5431	1.0000	0.9090	12.2 ± 0.6	Increase	0.4315	0.5379	0.7105
IL-4													
Placebo	12	14 ± 0.8		13.5 ± 0.7	Decrease	0.3435			13.7 ± 0.7	Decrease	0.5561		
Lifewave X39	12	13.5 ± 0.9	0.3959	13.4 ± 0.6	Decrease	0.8269	0.8464	0.4508	13.5 ± 0.6	Increase	0.9536	0.5255	0.5635
IL-7													
Placebo	12	14.2 ± 0.6		13.8 ± 0.5	Decrease	0.2587			13.6 ± 0.5	Decrease	0.1028		
Lifewave X39	12	14.2 ± 0.6	0.9562	13.6 ± 0.5	Decrease	0.0323	0.5712	0.6620	14 ± 0.5	Decrease	0.3881	0.0838	0.2316
IL-9													
Placebo	12	382 ± 6.1		358 ± 10.9	Decrease	0.0209			360 ± 10.1	Decrease	0.0283		
Lifewave X39	12	379 ± 8.3	0.7616	363 ± 9.2	Decrease	0.0378	0.6031	0.4456	374 ± 8.1	Decrease	0.5616	0.0368	0.0657
IL-15													
Placebo	12	15.3 ± 0.5		14.7 ± 0.5	Decrease	0.1442			14.8 ± 0.6	Decrease	0.2785		
Lifewave X39	12	15.1 ± 0.5	0.5783	14.8 ± 0.4	Decrease	0.4200	0.5279	0.3957	15 ± 0.5	Decrease	0.9036	0.4678	0.2780

[a] Average ± standard error of the mean at baseline
[b] Significance to placebo at baseline
[c] Average ± standard error of the mean at 1 hour
[d] Change from baseline at 1 hour
[e] Significance of change from baseline to 1 hour
[f] Significance to placebo at 1 hour
[g] Significance to placebo, percent change at 1 hour
[h] Average ± standard error of the mean at 2 hours
[i] Change from baseline at 2 hours
[j] Significance of change from baseline to 2 hours
[k] Significance to placebo at 2 hours
[l] Significance to placebo, percent change at 2 hours

Table 20. Growth factors, acute effects - levels of significance

	N	Baseline average [a]	P-value [b]	One-hour average [c]	Change [d]	P-value [e]	P-value [f]	P-value [g]	Two-hour average [h]	Change [i]	P-value [j]	P-value [k]	P-value [l]
Basic FGF													
Placebo	12	22.5 ± 1.3		21.6 ± 1.4	Decrease	0.1908			21.9 ± 1.5	Decrease	0.3879		
Lifewave X39	12	22.5 ± 1.7	0.9419	22.1 ± 1.4	Decrease	0.4071	0.3133	0.4972	22.4 ± 1.5	Decrease	0.7247	0.2732	0.4795
G-CSF													
Placebo	12	20.3 ± 1.1		19.8 ± 0.8	Decrease	0.5578			19.7 ± 0.8	Decrease	0.5137		
Lifewave X39	12	20.3 ± 0.9	0.9236	19.7 ± 0.7	Decrease	0.0997	0.9063	0.6901	20.3 ± 0.8	Decrease	1.0000	0.3708	0.7558
GM-CSF													
Placebo	12	22 ± 0.5		22 ± 0.5	No change	NC			22.2 ± 0.5	Increase	0.2127		
Lifewave X39	12	22 ± 0.5	0.3277	22 ± 0.5	Decrease	0.3277	NC	0.1617	22 ± 0.5	Decrease	0.3277	0.2127	0.1955
PDGF-BB													
Placebo	12	280 ± 37.5		285 ± 40.8	Increase	0.5529			278 ± 41.4	Decrease	0.8682		
Lifewave X39	12	282 ± 44.8	0.8446	267 ± 35.4	Decrease	0.1993	0.1615	0.5384	307 ± 48.6	Increase	0.1072	0.0621	0.0253
VEGF													
Placebo	12	23.9 ± 1.3		23.9 ± 1.3	Increase	0.9683			24.1 ± 1.7	Increase	0.8398		
Lifewave X39	12	23.9 ± 1.6	0.9234	23.6 ± 1.3	Decrease	0.4670	0.3502	0.7459	24 ± 1.3	Increase	0.7177	0.9117	0.7080

[a] Average ± standard error of the mean at baseline
[b] Significance to placebo at baseline
[c] Average ± standard error of the mean at 1 hour
[d] Change from baseline at 1 hour
[e] Significance of change from baseline to 1 hour
[f] Significance to placebo at 1 hour
[g] Significance to placebo, percent change at 1 hour
[h] Average ± standard error of the mean at 2 hours
[i] Change from baseline at 2 hours
[j] Significance of change from baseline to 2 hours
[k] Significance to placebo at 2 hours
[l] Significance to placebo, percent change at 2 hours

Table 21. Pro-inflammatory cytokines, placebo subtracted, acute effects - levels of significance

	N	Baseline average [a]	One-hour average [b]	P-value [c]	Two-hour average [d]	P-value [e]	P-value [f]
IFN-γ							
Lifewave X39	12	0 ± 0	1.6 ± 4.8	0.6649	1.7 ± 4.8	0.6324	0.9393
IL-1β							
Lifewave X39	12	0 ± 0	4.5 ± 3.6	0.1022	2.6 ± 5.2	0.5297	0.5214
IL-5							
Lifewave X39	12	0 ± 0	0 ± 4.3	0.9980	0.9 ± 5.3	0.8176	0.6865
IL-6							
Lifewave X39	12	0 ± 0	1.8 ± 5.6	0.3293	3 ± 4.1	0.1757	0.6440
IL-8							
Lifewave X39	12	0 ± 0	-7 ± 5.5	0.0960	-1.4 ± 5.5	0.7304	0.1105
IL-12 (p70)							
Lifewave X39	12	0 ± 0	-0.9 ± 5.7	0.8303	-0.7 ± 5.4	0.7590	0.9127
IL-13							
Lifewave X39	12	0 ± 0	-0.1 ± 3	0.8651	-0.7 ± 3.5	0.9794	0.8107

[a] Average ± standard error of the mean at baseline
[b] Average ± standard error of the mean at 1 hour
[c] Significance of difference in percent change from baseline to 1 hour
[d] Average ± standard error of the mean at 2 hours
[e] Significance of difference in percent change from baseline to 2 hours
[f] Significance of difference in percent change from 1 hour to 2 hours

Table 22. Pro-inflammatory cytokines, placebo subtracted, acute effects - levels of significance

	N	Baseline average [a]	One-hour average [b]	P-value [c]	Two-hour average [d]	P-value [e]	P-value [f]
IL-17A							
Lifewave X39	12	0 ± 0	2.2 ± 8.6	0.5094	6.3 ± 10.3	0.2886	0.2437
Eotaxin							
Lifewave X39	12	0 ± 0	-1.1 ± 5.4	0.7791	-1.4 ± 4.8	0.6825	0.9040
IP-10							
Lifewave X39	12	0 ± 0	-1.5 ± 8	0.7948	-0.3 ± 8.1	0.9580	0.7140
MCP-1							
Lifewave X39	12	0 ± 0	-2.1 ± 4.4	0.5125	4.9 ± 5.3	0.2175	0.0543
MIP-1α							
Lifewave X39	12	0 ± 0	-2.2 ± 5.2	0.5502	-6.2 ± 4.4	0.0788	0.1724
MIP-1β							
Lifewave X39	12	0 ± 0	-0.1 ± 3.3	0.9650	2.9 ± 4.2	0.3876	0.3205
RANTES							
Lifewave X39	12	0 ± 0	2.4 ± 3.3	0.3730	10.2 ± 3.4	0.0011	0.0013
TNF-α							
Lifewave X39	12	0 ± 0	2.3 ± 3.1	0.3356	6 ± 3	0.0194	0.1102

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at 1 hour

[c] Significance of difference in percent change from baseline to 1 hour

[d] Average ± standard error of the mean at 2 hours

[e] Significance of difference in percent change from baseline to 2 hours

[f] Significance of difference in percent change from 1 hour to 2 hours

Table 23. Anti-inflammatory cytokines, placebo subtracted, acute effects - levels of significance

	N	Baseline average [a]	One-hour average [b]	P-value [c]	Two-hour average [d]	P-value [e]	P-value [f]
IL-1ra							
Lifewave X39	12	0 ± 0	8.6 ± 9.1	0.2019	-0.4 ± 6.3	0.9334	0.0465
IL-10							
Lifewave X39	12	0 ± 0	5 ± 5.6	0.2294	2.4 ± 7	0.6452	0.2446

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at 1 hour

[c] Significance of difference in percent change from baseline to 1 hour

[d] Average ± standard error of the mean at 2 hours

[e] Significance of difference in percent change from baseline to 2 hours

[f] Significance of difference in percent change from 1 hour to 2 hours

Table 24. Regulating cytokines, placebo subtracted, acute effects levels of significance

	N	Baseline average [a]	One-hour average [b]	P-value [c]	Two-hour average [d]	P-value [e]	P-value [f]
IL-2							
Lifewave X39	12	0 ± 0	-0.9 ± 10.7	0.7682	3.5 ± 13.1	0.5132	0.3863
IL-4							
Lifewave X39	12	0 ± 0	2.7 ± 4.7	0.4332	2.2 ± 4.9	0.5557	0.8061
IL-7							
Lifewave X39	12	0 ± 0	-1.2 ± 3.6	0.6696	3.1 ± 3.3	0.2075	0.0344
IL-9							
Lifewave X39	12	0 ± 0	2.2 ± 3.7	0.4384	4.7 ± 3.1	0.0957	0.4446
IL-15							
Lifewave X39	12	0 ± 0	2.5 ± 3.9	0.3957	3.2 ± 3.9	0.3102	0.7280

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at 1 hour

[c] Significance of difference in percent change from baseline to 1 hour

[d] Average ± standard error of the mean at 2 hours

[e] Significance of difference in percent change from baseline to 2 hours

[f] Significance of difference in percent change from 1 hour to 2 hours

Table 25. Growth factors, placebo subtracted acute effects - levels of significance

Basic FGF							
Lifewave X39	12	0 ± 0	2.4 ± 4.4	0.3745	2.6 ± 4.6	0.4724	0.9061
G-CSF							
Lifewave X39	12	0 ± 0	-1.5 ± 5.1	0.7551	1.7 ± 7.4	0.6735	0.3563
GM-CSF							
Lifewave X39	12	0 ± 0	-0.2 ± 0.2	0.3277	-1 ± 1	0.1700	0.1617
PDGF-BB							
Lifewave X39	12	0 ± 0	-2.1 ± 4.6	0.5531	12.1 ± 7.1	0.0227	0.0010
VEGF							
Lifewave X39	12	0 ± 0	-0.9 ± 3.7	0.7443	1.3 ± 4.8	0.7050	0.2959

[a] Average ± standard error of the mean at baseline
[b] Average ± standard error of the mean at 1 hour
[c] Significance of difference in percent change from baseline to 1 hour
[d] Average ± standard error of the mean at 2 hours
[e] Significance of difference in percent change from baseline to 2 hours
[f] Significance of difference in percent change from 1 hour to 2 hours

Table 26. Pro-inflammatory cytokines, long-term effects - levels of significance

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one Average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
IFN-γ											
Lifewave X39	12	22 ± 1.6	21.3 ± 1.6	Decrease	0.3011	21.6 ± 1.6	Decrease	0.6334	20.8 ± 1.5	Decrease	0.1262
IL-1β											
Lifewave X39	12	14.8 ± 0.6	14.8 ± 0.7	Increase	0.9608	14.2 ± 0.7	Decrease	0.3296	14.2 ± 0.7	Decrease	0.2386
IL-5											
Lifewave X39	12	19.6 ± 1.7	19.2 ± 1.6	Decrease	0.4236	19.1 ± 1.8	Decrease	0.4309	18.8 ± 1.4	Decrease	0.1083
IL-6											
Lifewave X39	12	18 ± 1.2	17.4 ± 0.9	Decrease	0.3401	17.8 ± 0.9	Decrease	0.8083	16.6 ± 0.7	Decrease	0.0278
IL-8											
Lifewave X39	12	26.6 ± 1.8	23.7 ± 1.6	Decrease	0.0138	25.9 ± 2.5	Decrease	0.6802	24 ± 1.2	Decrease	0.0262
IL-12 (p70)											
Lifewave X39	12	18.5 ± 1.1	17.7 ± 0.7	Decrease	0.3972	17.4 ± 1	Decrease	0.1157	17.3 ± 0.8	Decrease	0.3082
IL-13											
Lifewave X39	12	17.4 ± 0.6	16.8 ± 0.5	Decrease	0.2355	16.7 ± 0.5	Decrease	0.0525	16.7 ± 0.5	Decrease	0.0874

[a] Average ± standard error of the mean at baseline
[b] Average ± standard error of the mean at day 1
[c] Change from baseline at day 1
[d] Significance of difference in percent change from baseline to day 1
[e] Average ± standard error of the mean at week 1
[f] Change from baseline at week 1
[g] Significance of difference in percent change from baseline to week 1
[h] Average ± standard error of the mean at week 4
[i] Change from baseline at week 4
[j] Significance of difference in percent change from baseline to week 4

Table 27. Pro-inflammatory cytokines, long-term effects - levels of significance

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one Average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
IL-17A											
Lifewave X39	12	23.5 ± 3	21.5 ± 1	Decrease	0.3882	21.1 ± 1.3	Decrease	0.2151	19.5 ± 0.9	Decrease	0.1297
Eotaxin											
Lifewave X39	12	409 ± 47.4	417 ± 50.9	Increase	0.4212	360 ± 43.8	Decrease	0.0502	389 ± 57.6	Decrease	0.2888
IP-10											
Lifewave X39	12	223 ± 81.6	235 ± 67.4	Increase	0.6099	229 ± 78.9	Increase	0.8303	254 ± 96.9	Increase	0.0313
MCP-1											
Lifewave X39	12	55 ± 7	53.4 ± 7.6	Decrease	0.4407	55.2 ± 6.7	Increase	0.9387	52.2 ± 6.7	Decrease	0.1862
MIP-1α											
Lifewave X39	12	15.4 ± 0.7	15.6 ± 0.5	Increase	0.6726	15.7 ± 0.9	Increase	0.7032	15 ± 0.8	Decrease	0.4885
MIP-1β											
Lifewave X39	12	1,274 ± 25.9	1,228 ± 28.5	Decrease	0.1390	1,289 ± 23.7	Increase	0.6449	1,262 ± 24.7	Decrease	0.6081
RANTES											
Lifewave X39	12	5,653 ± 113	5,652 ± 102	Decrease	0.9926	5,805 ± 118	Increase	0.2374	5,582 ± 104	Decrease	0.3733
TNF-α											
Lifewave X39	12	58.1 ± 1.7	55.9 ± 2.1	Decrease	0.0596	58.1 ± 1.6	Increase	0.9891	56.6 ± 1.6	Decrease	0.1712

[a] Average ± standard error of the mean at baseline
[b] Average ± standard error of the mean at day 1
[c] Change from baseline at day 1
[d] Significance of difference in percent change from baseline to day 1
[e] Average ± standard error of the mean at week 1
[f] Change from baseline at week 1
[g] Significance of difference in percent change from baseline to week 1
[h] Average ± standard error of the mean at week 4
[i] Change from baseline at week 4
[j] Significance of difference in percent change from baseline to week 4

Table 28. Anti-inflammatory cytokines, long term effects - levels of significance

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one Average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
IL-1ra											
Lifewave X39	12	14.5 ± 1.1	14.8 ± 0.9	Increase	0.7630	15.2 ± 1.5	Increase	0.5481	13.4 ± 0.5	Decrease	0.0813
IL-10											
Lifewave X39	12	15 ± 1.3	14.1 ± 0.6	Decrease	0.2674	14 ± 0.7	Decrease	0.2710	13.3 ± 0.4	Decrease	0.0631

[a] Average ± standard error of the mean at baseline
[b] Average ± standard error of the mean at day 1
[c] Change from baseline at day 1
[d] Significance of difference in percent change from baseline to day 1
[e] Average ± standard error of the mean at week 1
[f] Change from baseline at week 1
[g] Significance of difference in percent change from baseline to week 1
[h] Average ± standard error of the mean at week 4
[i] Change from baseline at week 4
[j] Significance of difference in percent change from baseline to week 4

Table 29 Regulating cytokines, long term effects - levels of significance

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one Average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
IL-2											
Lifewave X39	12	14.2 ± 2.5	12.4 ± 0.8	Decrease	0.3612	12.3 ± 0.9	Decrease	0.2643	10.7 ± 0.3	Decrease	0.1204
IL-4											
Lifewave X39	12	13.9 ± 1	13.7 ± 0.7	Decrease	0.7205	13 ± 0.7	Decrease	0.0782	13.1 ± 0.6	Decrease	0.1804
IL-7											
Lifewave X39	12	14.3 ± 0.7	14.4 ± 0.5	Increase	0.7953	13.8 ± 0.6	Decrease	0.2117	14.1 ± 0.4	Decrease	0.6188
IL-9											
Lifewave X39	12	381 ± 6.6	373 ± 9.5	Decrease	0.3315	378 ± 10.1	Decrease	0.7538	375 ± 9.7	Decrease	0.4269
IL-15											
Lifewave X39	12	15.4 ± 0.6	15.4 ± 0.6	Decrease	0.9637	14.8 ± 0.7	Decrease	0.1078	14.6 ± 0.5	Decrease	0.0732

[a] Average ± standard error of the mean at baseline
[b] Average ± standard error of the mean at day 1
[c] Change from baseline at day 1
[d] Significance of difference in percent change from baseline to day 1
[e] Average ± standard error of the mean at week 1
[f] Change from baseline at week 1
[g] Significance of difference in percent change from baseline to week 1
[h] Average ± standard error of the mean at week 4
[i] Change from baseline at week 4
[j] Significance of difference in percent change from baseline to week 4

Table 30. Growth factors, long term effects - levels of significance

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one Average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
Basic FGF											
Lifewave X39	12	23.2 ± 1.7	21.8 ± 1.6	Decrease	0.0857	22.4 ± 1.6	Decrease	0.2277	21.5 ± 1.5	Decrease	0.0285
G-CSF											
Lifewave X39	12	20.5 ± 1.1	20.7 ± 1.1	Increase	0.9536	19.8 ± 1.2	Decrease	0.2014	19.3 ± 0.9	Decrease	0.1700
GM-CSF											
Lifewave X39	12	22 ± 0.5	22 ± 0.5	No change	1.0000	22.2 ± 0.5	Increase	0.1853	22 ± 0.5	Decrease	0.3277
PDGF-BB											
Lifewave X39	12	279 ± 40.8	277 ± 39.8	Decrease	0.8215	272 ± 39.8	Decrease	0.6360	270 ± 42.7	Decrease	0.4039
VEGF											
Lifewave X39	12	24.6 ± 1.7	24.5 ± 1.6	Decrease	0.8833	23.5 ± 1.7	Decrease	0.0967	23 ± 1.6	Decrease	0.0167

[a] Average ± standard error of the mean at baseline
[b] Average ± standard error of the mean at day 1
[c] Change from baseline at day 1
[d] Significance of difference in percent change from baseline to day 1
[e] Average ± standard error of the mean at week 1
[f] Change from baseline at week 1
[g] Significance of difference in percent change from baseline to week 1
[h] Average ± standard error of the mean at week 4
[i] Change from baseline at week 4
[j] Significance of difference in percent change from baseline to week 4

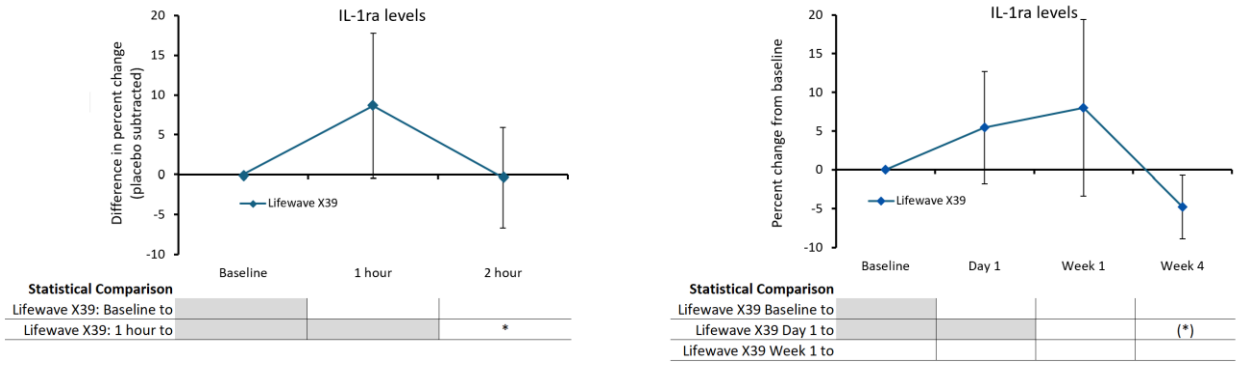


Figure 29. Interleukin-1 receptor antagonist (IL-1ra) levels in blood circulation while wearing the Lifewave X39 patch. ***Left:*** *The percent change in IL-1ra levels in blood circulation while wearing the Lifewave X39 patch, adjusted by subtracting the changes while wearing the placebo patch.* ***Right****: The percent change in IL-1ra levels in blood circulation after wearing the Lifewave X39 patch daily for 4 weeks.*

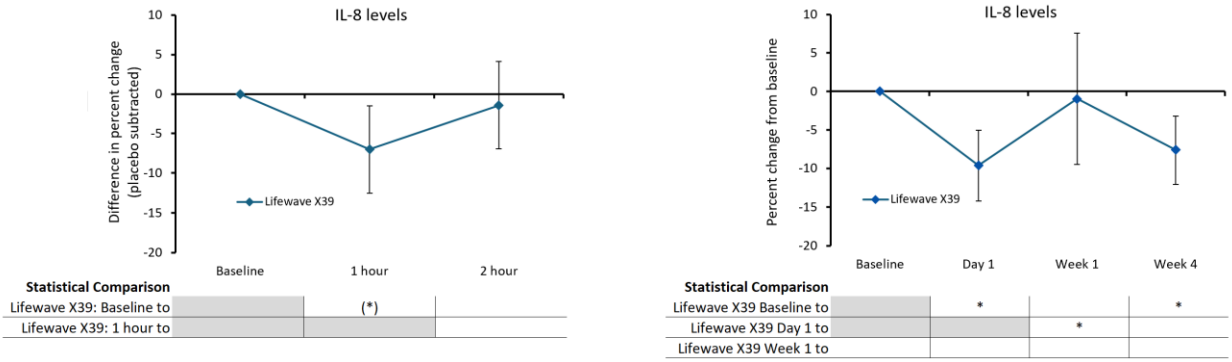


Figure 30. Interleukin-8 (IL-8) levels in blood circulation while wearing the Lifewave X39 patch. ***Left:*** *The percent change in IL-8 levels in blood circulation while wearing the Lifewave X39 patch, adjusted by subtracting the changes while wearing the placebo patch.* ***Right****: The percent change in IL-8 levels in blood circulation after wearing the Lifewave X39 patch daily for 4 weeks.*

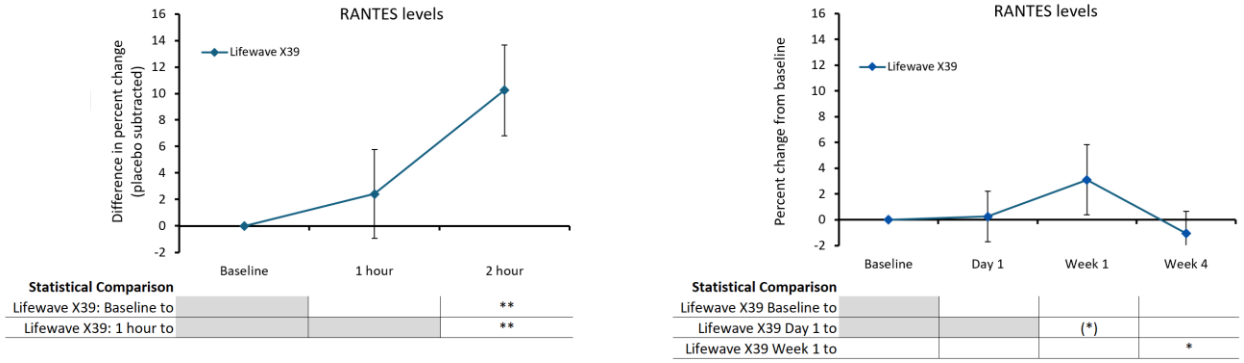


Figure 31. RANTES levels in blood circulation after wearing the Lifewave X39 patch. ***Left:*** *The percent change in RANTES levels in blood circulation while wearing the Lifewave X39 patch, adjusted by subtracting the changes while wearing the placebo patch.* ***Right****: The percent change in RANTES levels in blood circulation after wearing the Lifewave X39 patch daily for 4 weeks.*

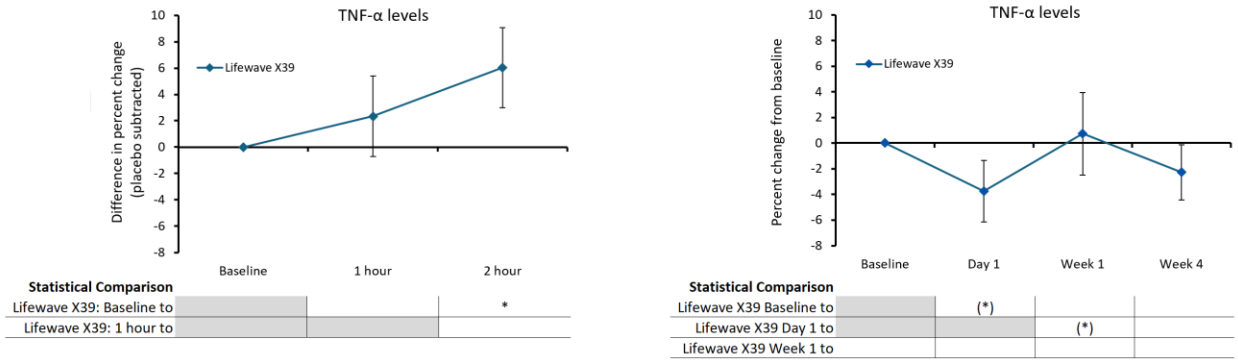


Figure 32. Tumor necrosis factor (TNF-α) levels in blood circulation after wearing the Lifewave X39 patch. ***Left:*** *The percent change in TNF-α levels in blood circulation while wearing the Lifewave X39 patch, adjusted by subtracting the changes while wearing the placebo patch.* ***Right****: The percent change in TNF-α levels in blood circulation after wearing the Lifewave X39 patch daily for 4 weeks.*

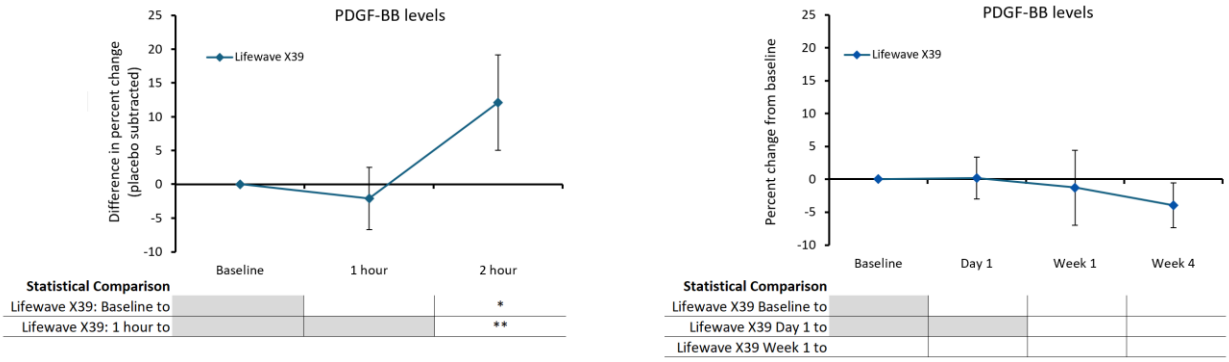


Figure 33. Platelet derived growth factor BB (PDGF-BB) levels in blood circulation after wearing the Lifewave X39 patch. ***Left:*** *The percent change PDGF-BB levels in blood circulation while wearing the Lifewave X39 patch, adjusted by subtracting the changes while wearing the placebo patch.* ***Right****: The percent change in PDGF-BB levels in blood circulation after wearing the Lifewave X39 patch daily for 4 weeks.*

6.3 Immune cell alertness and priming

In a clinical trial to monitor immune-activating events, we expected a cascade of events, starting by activation of immune cells in the skin, systemic changes to cytokine levels, changes to immune cell trafficking (enhanced immune surveillance), followed by immune surveillance in tissue throughout the body, and possibly re-entry of activated immune cells back into the blood circulation.

Blood samples offer a convenient window into the immune events happening after a product is consumed. We do not have convenient windows into what may happen at the initial gut activation, but we envision this is similar to events in vitro. We do not have windows into tissue and thus cannot monitor downstream events after immune cells migrate from blood into tissue to scavenge for microbial invaders and perform innate and adaptive types of immune responses. **Therefore, we mimic this by taking some of the blood samples and challenging the immune cells ex vivo (outside the body) with microbial mimetics.** This allows us to document **whether immune cells became more alert ("primed") after the patch was applied to the skin, and before the blood was drawn.** This does not reflect any mechanisms pertaining to a disease.

This testing can capture rapid changes in the responsiveness and status of immune cells in the blood circulation. This is a good indication that applying the patch may **trigger increased immune alertness/awareness.**

Three parallel sets of ex vivo cultures were made from each blood draw during this clinical trial to evaluate priming in vivo:

- Ex vivo addition of bacterial lipopolysaccharide (LPS);
- Ex vivo addition of a viral mimetic (Poly I:C);
- Untreated cells (control).

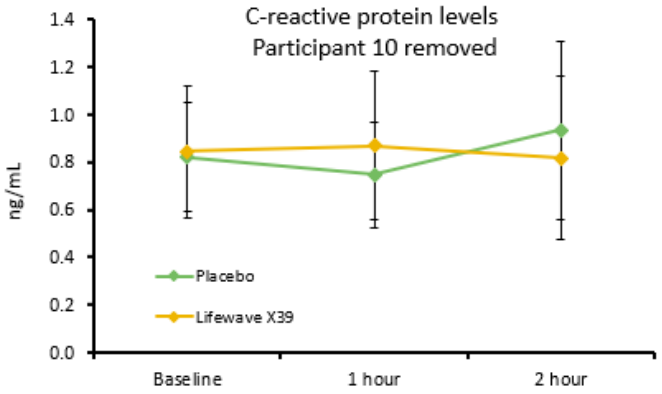
The cultures were incubated for 24 hours, after which the culture supernatants were harvested and banked frozen**. This will allow additional tests to be added at a later time (separate budget).** Such testing may include some or all of these pro- and anti-inflammatory cytokines, anti-viral peptides, and regenerative growth factors, selected from the 27-plex Luminex magnetic bead array and the MagPix® multiplexing system: *IL-1beta, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, Eotaxin, basic FGF, G-CSF, GM-CSF, IFN-gamma, IP-10, MCP-1 (MCAF), MIP-1alpha, MIP-1beta, PDGF-BB, RANTES, TNF-alpha, and VEGF.*

6.4 C-Reactive Protein

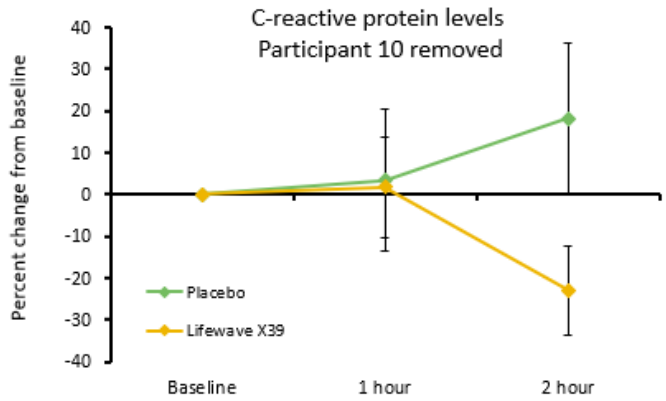
Blood samples were tested for C-reactive protein (CRP), a protein produced in the liver, linked to inflammation. A high-sensitivity test was used. Since study participants were in good health, we did not expect to see high levels of CRP at baseline. However, within the normal healthy range, we did observe minor changes during the study.

Synopsis:

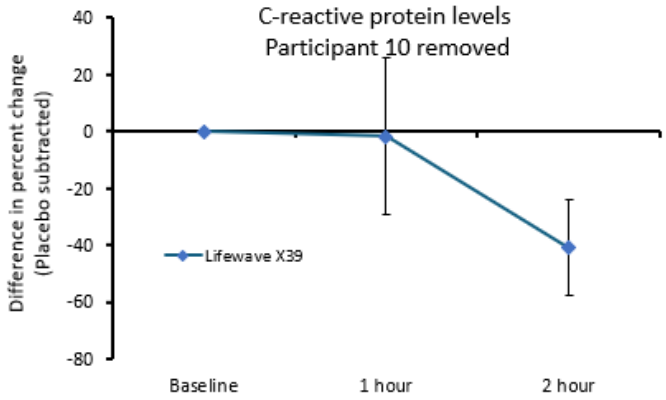
- The analysis of acute changes to CRP was done on 11 participants, with results from Participant 10 removed (see below):
    - When participants were wearing the X39 patch, the CRP levels were reduced over the 2-hour period, compared to the changes when they were wearing the placebo patch.
    - The reduction reached a high level of statistical significance.
- Long-term changes: The analysis of long-term changes to CRP was done on 11 participants, with results from Participant 10 removed (see below):
    - The average change in CRP levels was reduced from baseline to Day 1, reaching a high level of significance.
    - Over time, the data were more variable and Week 1 not showing a decrease, in contrast, a mild increase was seen, with a high variability among participants. This is expected, since CRP levels fluctuate rapidly in response to many influences.
    - The average CRP levels were further reduced at Week 4, and the reduction was significant compared to baseline.
- Participant 10:
    - This participant had higher CRP levels than the other participants, with some values above 10 ng/mL serum.
    - Her values fluctuated during the initial clinic visits, and her data were removed from the analysis of acute effects of wearing the X39 patch for 2 hours.
    - Her long-term results were positive: She showed a good response to wearing the X39 patch, with a significant reduction from 12 ng/mL on baseline, 10 ng/mL on Day 1, and 5 ng/mL on Week 1 and Week 4.



Statistical Comparison			
Placebo: Baseline to			
Placebo: 1 hour to			
Lifewave X39: Baseline to			
Lifewave X39: 1 hour to			
Lifewave X39 to Placebo			

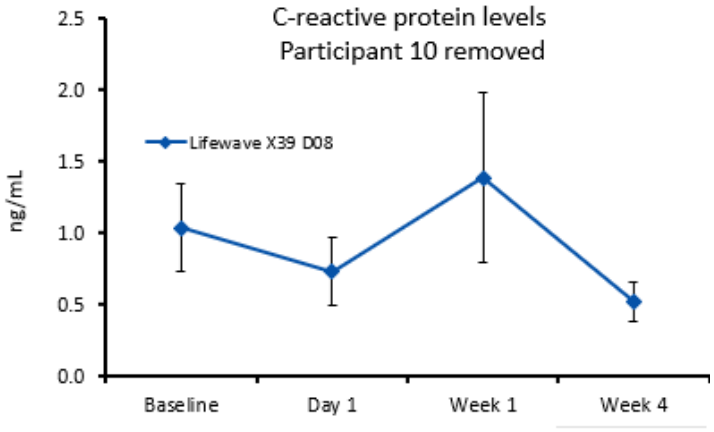


Statistical Comparison			
Lifewave X39 to Placebo			**

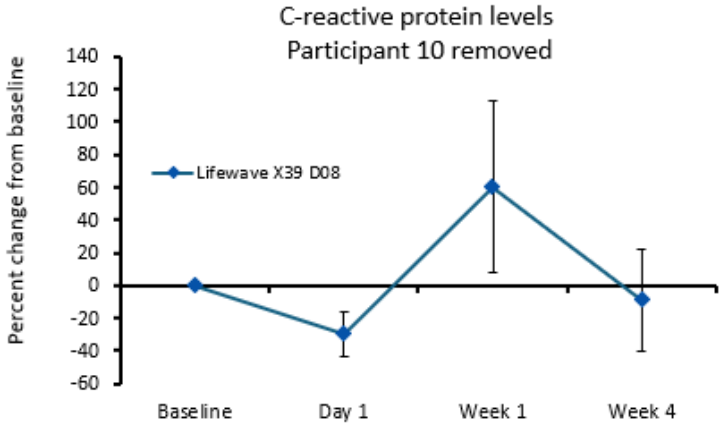


Statistical Comparison			
Lifewave X39: Baseline to			**
Lifewave X39: 1 hour to			**

Figure 34. ***Top left****: C-reactive protein levels in blood circulation after wearing the Lifewave X39 or placebo patch.* ***Top right****: The percent change in C-reactive protein levels in blood circulation after wearing the Lifewave X39 or placebo patch.* ***Bottom right****: The percent change in C-reactive protein levels in blood circulation after wearing the Lifewave X39 patch, adjusted by subtracting the changes after wearing the placebo patch. These graphs show averages ± standard error for eleven participants (data from participant 10 were removed).*

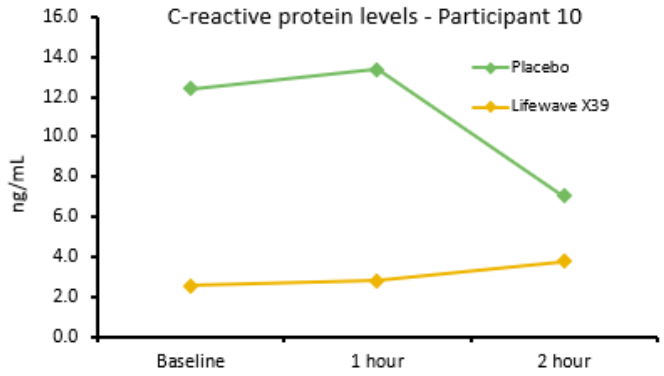


Statistical Comparison				
Lifewave X39 Baseline to		**		*
Lifewave X39 Day 1 to				
Lifewave X39 Week 1 to				*

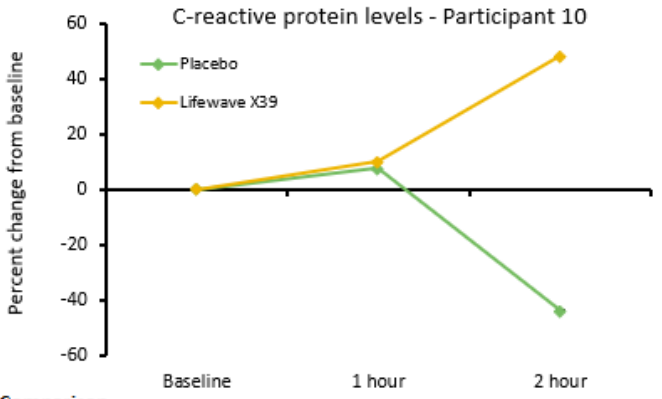


Statistical Comparison				
Lifewave X39 Baseline to		*		
Lifewave X39 Day 1 to			*	
Lifewave X39 Week 1 to				

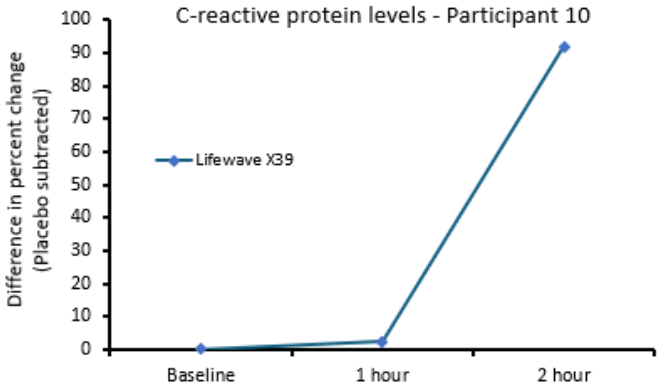
Figure 35. ***Top****: C-reactive protein levels at baseline, day 1, week 1, and week 4 for all participants (average change ± standard error of the mean) after wearing the Lifewave X39 patch.* ***Bottom****: Group average percent change from baseline in C-reactive protein levels for all participants at day 1, week 1, and week 4 after wearing the Lifewave X39 patch.*



Statistical Comparison			
Placebo: Baseline to			
Placebo: 1 hour to			
Lifewave X39: Baseline to			
Lifewave X39: 1 hour to			*
Lifewave X39 to Placebo			*

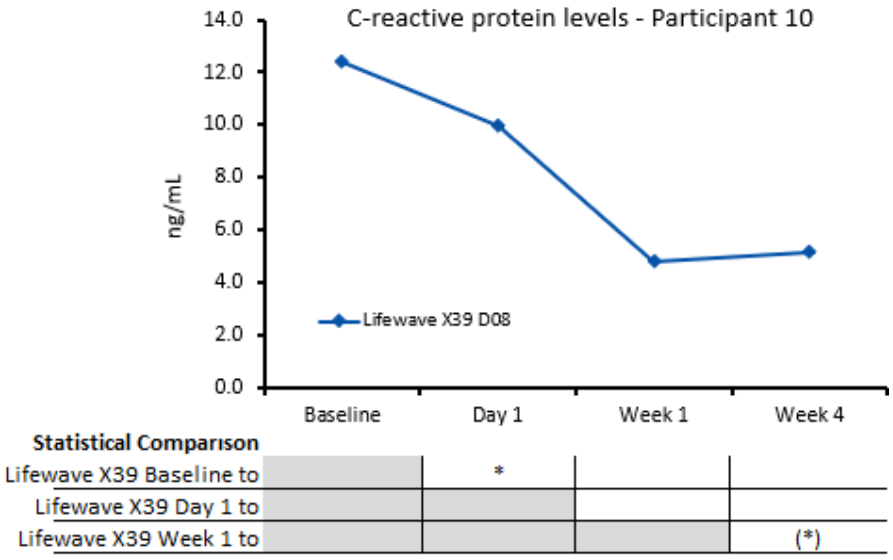


Statistical Comparison			
Lifewave X39 to Placebo			*

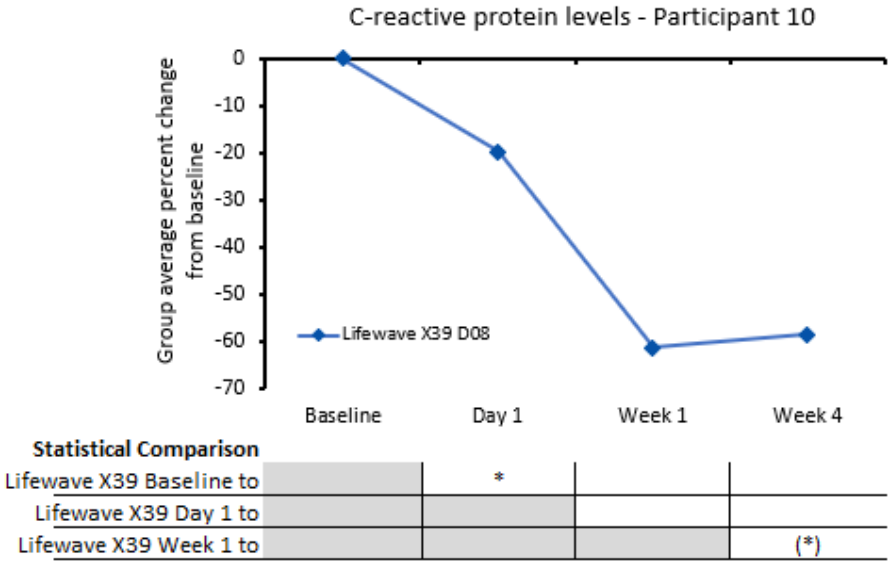


Statistical Comparison			
Lifewave X39: Baseline to			(*)
Lifewave X39: 1 hour to			**

Figure 36. ***Top left****: C-reactive protein levels in blood circulation after wearing the Lifewave X39 or placebo patch.* ***Top right****: The percent change in C-reactive protein levels in blood circulation after wearing the Lifewave X39 or placebo patch.* ***Bottom right****: The percent change in C-reactive protein levels in blood circulation after wearing the X39 patch, adjusted by subtracting the changes after wearing the placebo patch. These graphs show unique responses to participant 10 (P010) only.*



Statistical Comparison				
Lifewave X39 Baseline to		*		
Lifewave X39 Day 1 to				
Lifewave X39 Week 1 to				(*)



Statistical Comparison				
Lifewave X39 Baseline to		*		
Lifewave X39 Day 1 to				
Lifewave X39 Week 1 to				(*)

Figure 37. ***Top****: C-reactive protein levels at baseline, day 1, week 1, and week 4 after wearing the Lifewave X39 patch.* ***Bottom****: Percent change from baseline group average C-reactive protein levels at day 1, week 1, and week 4 after wearing the Lifewave X39 patch. These graphs show unique responses to participant 10 (P010) only.*

6.5 Effects on Energy /Focus – Questionnaire

As part of the acute phase of this study (the placebo-controlled cross-over phase), a brief 5-question questionnaire was asked immediately prior to each blood draw at baseline, 1, and 2 hours. The questions include levels of energy, focus, mental functioning, stress, and relaxation.

Synopsis:

- The self-reported level of energy showed an increase.
- This increase reached a statistical trend at Day 1, and statistical significance at Week 4.

Table 31. Energy and focus questionnaire responses, acute data – levels of significance

	N	Baseline average [a]	P-value [b]	One-hour average [c]	Change [d]	P-value [e]	P-value [f]	P-value [g]	Two-hour average [h]	Change [i]	P-value [j]	P-value [k]	P-value [l]
Energy level													
Placebo	12	68.9 ± 8.6		78.1 ± 8.2	Increase	0.0151			81.6 ± 6.2	Increase	0.0503		
Lifewave X39	12	68.9 ± 7.2	1.0000	80.3 ± 7.1	Increase	0.0029	0.4874	0.5946	82.3 ± 6.9	Increase	0.0000	0.9001	0.9055
How focused do you feel													
Placebo	12	86.3 ± 4.5		86.6 ± 5.4	Increase	0.8981			84.6 ± 6	Decrease	0.7296		
Lifewave X39	12	87.5 ± 4.5	0.7269	84.8 ± 6.5	Decrease	0.4527	0.6447	0.3337	90.7 ± 4.5	Increase	0.1012	0.0821	0.3388
Mental Functioning													
Placebo	12	85.4 ± 4.1		88.3 ± 4.4	Increase	0.2958			88.8 ± 4.3	Increase	0.3049		
Lifewave X39	12	84.2 ± 5.6	0.7227	88.2 ± 4.2	Increase	0.0740	0.9579	0.7361	88.8 ± 4.3	Increase	0.0673	1.0000	0.7876
Level of relaxation													
Placebo	12	93.1 ± 2.1		95.3 ± 1.5	Increase	0.2713			91.3 ± 3.6	Decrease	0.6982		
Lifewave X39	12	92.5 ± 2.7	0.8265	95 ± 1.6	Increase	0.0819	0.7639	0.9177	89.6 ± 4.2	Decrease	0.5480	0.7909	0.8922
Stress level													
Placebo	12	7.3 ± 2.4		5.7 ± 2.1	Decrease	0.1121			6.4 ± 2.1	Decrease	0.5259		
Lifewave X39	12	7.3 ± 2.6	0.9372	6.1 ± 2.2	Decrease	0.1911	0.3388	0.7709	6.1 ± 2.2	Decrease	0.1911	0.7153	0.7654

[a] Average ± standard error of the mean at baseline

[b] Significance compared to placebo at baseline

[c] Average ± standard error of the mean at 1 hour

[d] Change from baseline at 1 hour

[e] Significance of change from baseline to 1 hour

[f] Significance compared to placebo at 1 hour

[g] Significance to placebo , change at 1 hour

[h] Average ± standard error of the mean at 1 hour

[i] Change from baseline at 2 hours

[j] Significance compared to placebo at 2 hours

[k] Significance to placebo , change at 2 hours

Table 32. Energy and focus questionnaire responses, placebo subtracted – levels of significance

	N	Baseline average [a]	One-hour average [b]	P-value [c]	Two-hour average [d]	P-value [e]
Energy level						
Lifewave X39	12	0 ± 0	2.3 ± 4.1	0.5946	0.8 ± 6.2	0.9055
How focused do you feel						
Lifewave X39	12	0 ± 0	-3 ± 3	0.3337	4.8 ± 4.8	0.3388
Mental Functioning						
Lifewave X39	12	0 ± 0	1.2 ± 3.4	0.7361	1.3 ± 4.5	0.7876
Level of relaxation						
Lifewave X39	12	0 ± 0	0.3 ± 2.4	0.9177	-1.1 ± 7.8	0.8922
Stress level						
Lifewave X39	12	0 ± 0	0.3 ± 1.1	0.7709	-0.4 ± 1.4	0.7654

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at 1 hour

[c] Significance of change from baseline to 1 hour

[d] Average ± standard error of the mean at 2 hours

[e] Significance of change from baseline to 2 hours

Table 33. Energy and focus questionnaire responses, long-term, – levels of significance

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one Average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
Energy level											
Lifewave X39	12	64.1 ± 10.3	82.4 ± 8.1	Increase	0.0680	73.5 ± 9.6	Increase	0.1777	77.9 ± 6.7	Increase	0.0411
How focused do you feel											
Lifewave X39	12	83.3 ± 5.4	85.8 ± 7	Increase	0.7078	79.2 ± 8.1	Decrease	0.4474	90.4 ± 3.7	Increase	0.4854
Mental Functioning											
Lifewave X39	12	81.3 ± 6.2	85.8 ± 7.3	Increase	0.5426	83.8 ± 5.1	Increase	0.6404	85.4 ± 4.7	Increase	0.4226
Level of relaxation											
Lifewave X39	12	93.1 ± 2.1	91.7 ± 2.6	Decrease	0.6062	86.3 ± 5.7	Decrease	0.2892	85.8 ± 4	Decrease	0.5000
Stress level											
Lifewave X39	12	5.8 ± 2.2	7.8 ± 2.6	Increase	0.3645	14.5 ± 7.9	Increase	0.3176	10 ± 4	Increase	0.3949

[a] Average ± standard error of the mean at baseline

[b] Average ± standard error of the mean at day 1

[c] Change from baseline at day 1

[d] Significance of difference in percent change from baseline to day 1

[e] Average ± standard error of the mean at week 1

[f] Change from baseline at week 1

[g] Significance of difference in percent change from baseline to week 1

[h] Average ± standard error of the mean at week 4

[i] Change from baseline at week 4

[j] Significance of difference in percent change from baseline to week 4

6.6 Long-Term Effect on Wellness - Questionnaire

At the baseline, Day 1, Week 1, and Week 4 clinic visits for the long-term phase of the study, a wellness questionnaire was used to collect information regarding general health and wellness, designed and validated by our team to specifically capture changes in a person's perception of health and wellness, based on the World Health Organization's definition of "Health": "*Physical, mental, and social well-being, and not merely the absence of disease and infirmity*".[16] Our research team has used the Wellness questionnaire for 15 years in clinical trials focusing on fairly healthy individuals and documenting health improvements when consuming natural products, foods, juices, alkaline waters, and botanical extracts.[17]

Synopsis:

Using the X39 patch on a daily basis was associated with minor changes in overall wellness.

The noteworthy self-reported improvements to health included:

- Mental functioning (see Figure 38 below)
- Energy/vitality (see Figure 39 below)

These improvements were noticeable already at Day 1, but did not reach statistical significance over the 4-week study participation.

A few changes were at first glance not desirable; however, we suggest they are tied into the increased energy:

- Decreased emotional wellness, reaching statistical significance at Day 1, and losing significance after that;
- Increased stress, reaching a high level of statistical significance at Day 1, and losing significance after that;
- Decreased sleep quality at Week 4 (not significant).

The X39 patch being associated with increased energy and mental functioning may, for some people, have triggered a sense of unusual electrical charge that could be perceived as 'stress' in the beginning of the study, highest at Day 1. If we had asked different questions, targeted at how it can feel to wear the X39 patch, we may have captured this better. Also, the decreased sleep quality at Week 4 may be related to the increased energy.

Table 34. Wellness analysis – levels of significance

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
Wellness Score											
Lifewave X39	12	8.1 ± 0.4	8.1 ± 0.3	Decrease	0.8885	8.1 ± 0.4	Increase	0.8959	7.9 ± 0.4	Decrease	0.4014
Physical Functioning Score											
Lifewave X39	12	8.3 ± 0.4	8.4 ± 0.3	Increase	0.6178	8.4 ± 0.4	Increase	0.6723	8.1 ± 0.4	Decrease	0.5272
Mental Functioning Score											
Lifewave X39	12	7.5 ± 0.6	7.9 ± 0.5	Increase	0.2969	7.8 ± 0.5	Increase	0.3655	8 ± 0.4	Increase	0.3335
Emotional Wellbeing Score											
Lifewave X39	12	8.3 ± 0.5	7.7 ± 0.5	Decrease	0.0156	7.9 ± 0.5	Decrease	0.1494	7.9 ± 0.7	Decrease	0.2412
Stress Level Score											
Lifewave X39	12	1.7 ± 0.6	2.2 ± 0.6	Increase	0.0081	1.9 ± 0.6	Increase	0.4627	2 ± 0.8	Increase	0.4078
Social Functioning Score											
Lifewave X39	12	8 ± 0.5	7.8 ± 0.5	Decrease	0.7090	7.6 ± 0.8	Decrease	0.5470	7.6 ± 0.7	Decrease	0.2657
Sleep Score											
Lifewave X39	12	7.3 ± 0.6	7.5 ± 0.6	Increase	0.4590	7.4 ± 0.6	Increase	0.9103	6.7 ± 0.9	Decrease	0.2604
Energy/Vitality Score											
Lifewave X39	12	7.5 ± 0.5	7.9 ± 0.4	Increase	0.1360	7.8 ± 0.4	Increase	0.2674	7.8 ± 0.5	Increase	0.5863

[a] Average ± standard error of the mean at baseline
[b] Average ± standard error of the mean at day one
[c] Change from baseline at day one
[d] Significance of change from baseline to day one
[e] Average ± standard error of the mean at week one
[f] Change from baseline at week one
[g] Significance of change from baseline to week one
[h] Average ± standard error of the mean at week four
[i] Change from baseline at week four
[j] Significance of change from baseline to week four

Table 35. Wellness analysis – levels of significance P005, P010, P011 removed

	N	Baseline average [a]	Day one average [b]	Change [c]	P-value [d]	Week one average [e]	Change [f]	P-value [g]	Week four average [h]	Change [i]	P-value [j]
Wellness Score											
Lifewave X39	9	8.5 ± 0.3	8.5 ± 0.2	Decrease	0.6786	8.4 ± 0.4	Decrease	0.5481	8.4 ± 0.3	Decrease	0.7162
Physical Functioning Score											
Lifewave X39	9	8.5 ± 0.4	8.7 ± 0.3	Increase	0.2490	8.6 ± 0.4	Increase	0.6032	8.4 ± 0.4	Decrease	0.8156
Mental Functioning Score											
Lifewave X39	9	8.4 ± 0.5	8.4 ± 0.4	No change	1.0000	8.4 ± 0.5	Decrease	0.7539	8.4 ± 0.3	No change	1.0000
Emotional Wellbeing Score											
Lifewave X39	9	8.7 ± 0.4	8.2 ± 0.3	Decrease	0.0579	8.4 ± 0.5	Decrease	0.2721	8.7 ± 0.3	Decrease	0.9429
Stress Level Score											
Lifewave X39	9	1.3 ± 0.5	1.9 ± 0.4	Increase	0.0226	1.4 ± 0.6	Increase	0.7870	1.2 ± 0.4	Decrease	0.8067
Social Functioning Score											
Lifewave X39	9	8.5 ± 0.4	8.3 ± 0.5	Decrease	0.6224	7.7 ± 0.7	Decrease	0.0755	8.3 ± 0.5	Decrease	0.4260
Sleep Score											
Lifewave X39	9	7.9 ± 0.4	7.8 ± 0.5	Decrease	0.6454	7.6 ± 0.6	Decrease	0.3734	7.4 ± 0.7	Decrease	0.5080
Energy/Vitality Score											
Lifewave X39	9	8 ± 0.3	8 ± 0.3	No change	1.0000	8.1 ± 0.3	Increase	0.6454	8.2 ± 0.3	Increase	0.4979

[a] Average ± standard error of the mean at baseline
[b] Average ± standard error of the mean at day one
[c] Change from baseline at day one
[d] Significance of change from baseline to day one
[e] Average ± standard error of the mean at week one
[f] Change from baseline at week one
[g] Significance of change from baseline to week one
[h] Average ± standard error of the mean at week four
[i] Change from baseline at week four
[j] Significance of change from baseline to week four

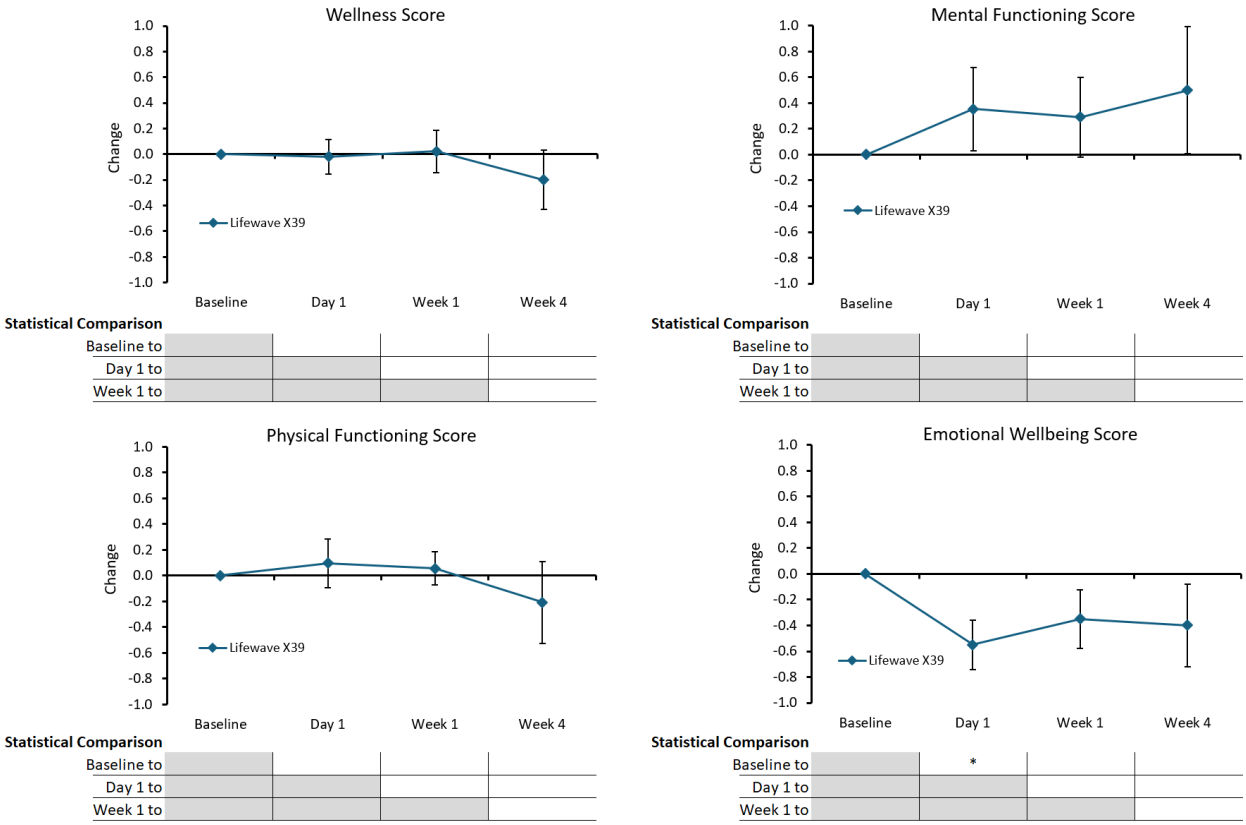


Figure 38. ***Top left****: The change in wellness score for all participants after wearing the Lifewave X39 patch.* ***Top right****: The change in mental functioning score for all participants after wearing the Lifewave X39 patch.* ***Bottom left****: The change in physical functioning score for all participants after wearing the Lifewave X39 patch.* ***Bottom right****: The change in emotional wellbeing score for all participants after wearing the Lifewave X39 patch.*

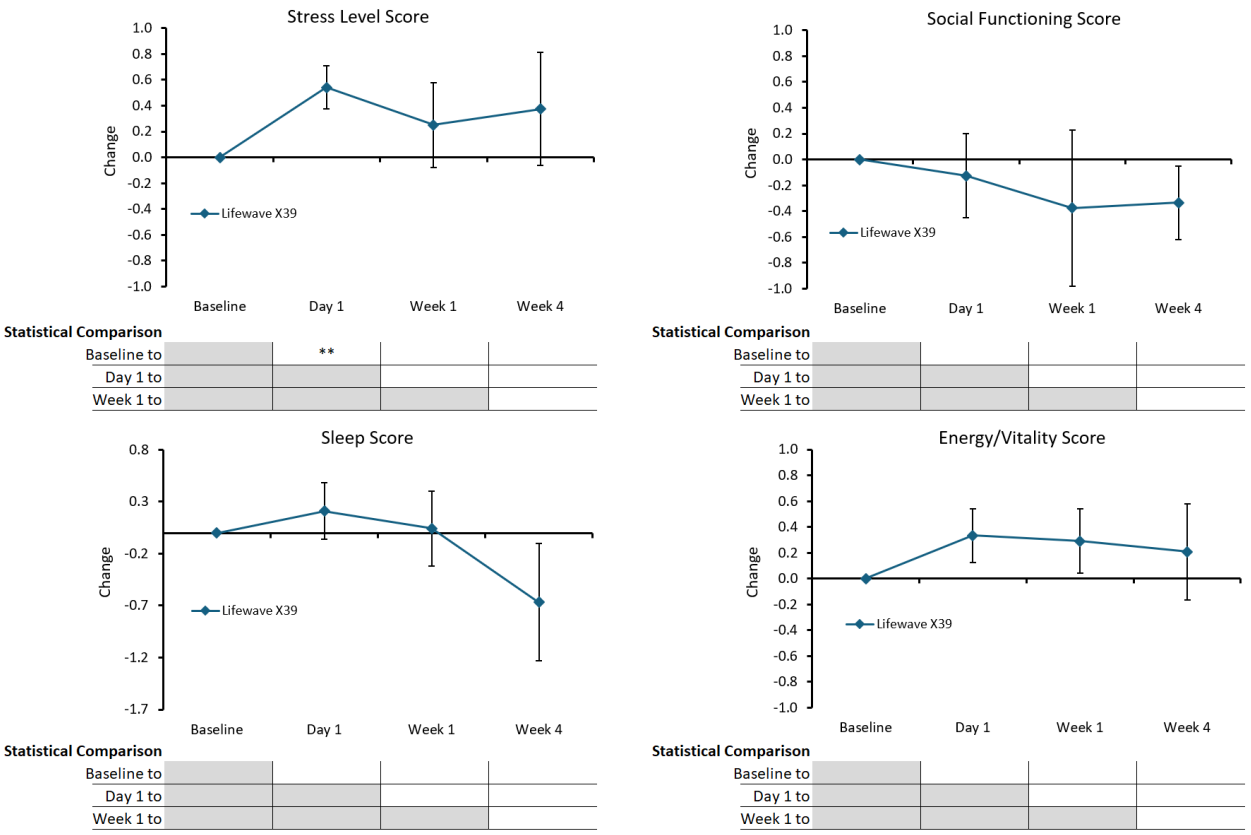


Figure 39. ***Top left****: The change in stress score for all participants after wearing the Lifewave X39 patch.* ***Top right****: The change in social functioning score for all participants after wearing the Lifewave X39 patch.* ***Bottom left****: The change in sleep score for all participants after wearing the Lifewave X39 patch.* ***Bottom right****: The change in energy/vitality score for all participants after wearing the Lifewave X39 patch.*

7 Conclusions

The use of the X39 patch was associated with a number of significant changes to cellular health, pro- and anti-inflammatory blood markers, and self-reported energy.

Effects on mitochondria

Wearing the X39 patch was associated with rapid and sustained increase in mitochondrial volume, indicating a direct effect on mitochondrial biogenesis.

Wearing the X39 patch was associated with increased mitochondrial resilience under oxidative and inflammatory stress, linked to the increased mitochondrial volume. This was initially associated with a decrease in mitochondrial membrane potential (1-2 hours), but already at Day 1 the mitochondrial membrane potential showed an increase; we interpret this as a temporary reduction in the membrane potential as the mitochondrial volume rapidly enlarged. The cells rapidly adjusted to the larger volume and were able to also increase the voltage across the inner membrane in the larger mitochondria.

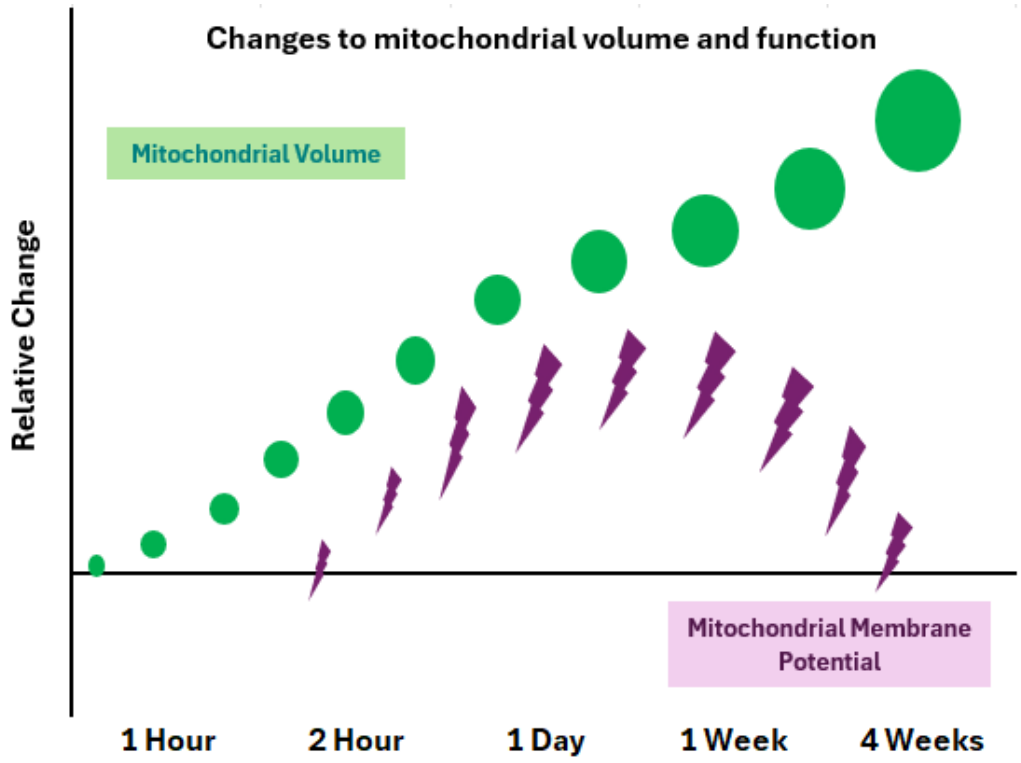


Figure 40. Interpretive diagram showing the events associated with wearing the X39 patch over 4 weeks. From 1 hour through the 4 weeks: Increased mitochondrial resilience to oxidative and inflammatory stress. From 2 hours to 1 week: Increased mitochondrial membrane potential before returning to baseline levels at 4 weeks.

Effects on blood markers for pro- and anti-inflammatory status

In both in the acute and the long-term phases of this study, we documented changes to cytokine levels when wearing the X39 patch. There was a rapid increase in anti-inflammatory markers and the restorative growth factor PDGF-BB and decreases to specific pro-inflammatory chemokines and cytokines (IL-8, TNF-α, RANTES). The levels of the anti-inflammatory cytokines stayed through Day 1 and Week 1, before returning to baseline values. It is important to note that the brief acute changes to pro-inflammatory markers were brief and transient, and there was no evidence of long-term increases to those markers.

C-reactive protein (CRP) levels showed a rapid decrease, reaching a high level of significance after 2 hours of wearing the X39 path when compared to placebo. The reduction seen already on Day 1 (after one day of using the patch) reached a high level of significance when compared to baseline. The reduced CRP levels at Week 4 remained statistically significant compared to baseline. Note: One participant had a higher level of CRP at baseline, and showed a mild reduction at Day 1, and further reduced levels at Week 1 and Week 4. Since her values fluctuated during the initial acute phase it is unknown whether her improvements were a natural resolution of an obscure problem or were solely due to wearing the X39 patch.

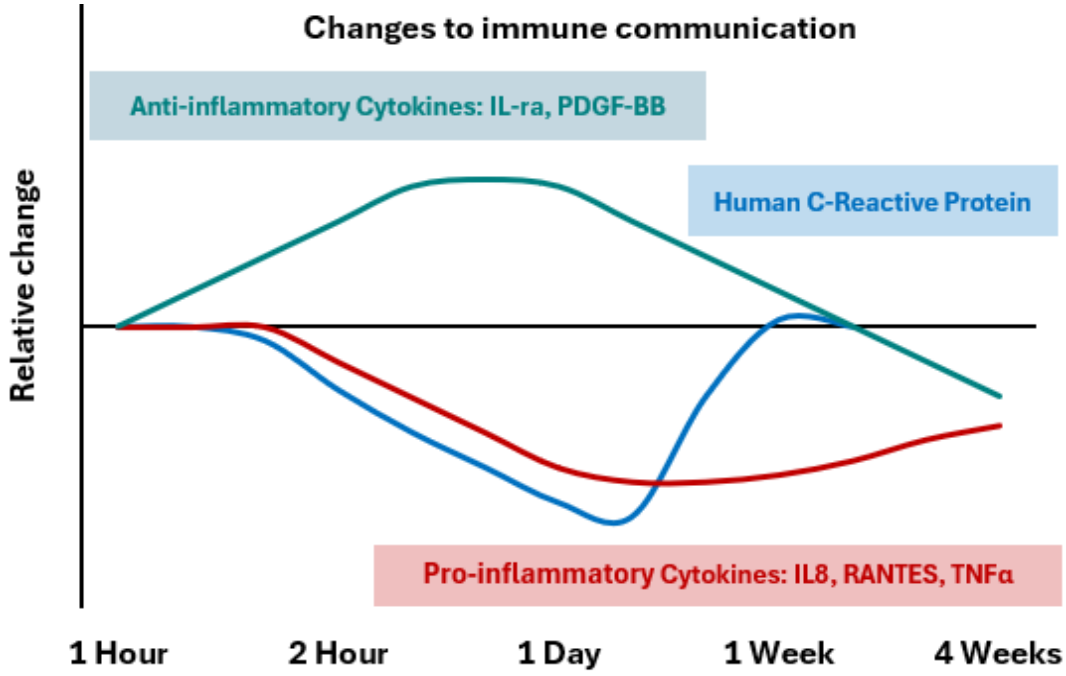


Figure 41. Interpretive diagram showing the events associated with wearing the X39 patch over 4 weeks. 1 hour to 1 week: Increased levels of anti-inflammatory cytokines IL-1ra and PDGF-BB. 2 hours through 4 weeks: decreased levels of pro-inflammatory cytokines IL-8, RANTES and TNFα. 2 hours to 1 week: degreased levels of Human C-Reactive Protein.

Questionnaires

The participants reported increased mental functioning and energy levels, seen already at Day 1, and continuing through the 4 weeks.

General conclusions

The use of the X39 patch generated results showing an orchestrated wave of events, starting with mitochondrial biogenesis, and continuing with increased mitochondrial membrane potential, increased anti-inflammatory cytokines, reduced pro-inflammatory biomarkers, and a subjective increase in self-perceived energy.

8 Further Work

This initial study may be followed by other types of work:

- Testing of the culture supernatants from the ex vivo immune cell challenges for cytokine profiling, including testing of exosome cytokines.
- Testing of banked frozen serum samples for additional biomarkers, such as cytochrome C oxidase, Superoxide dismutase, and other enzymes involved in energy and protection from oxidative stress.
- Testing of banked frozen serum samples for additional biomarkers, such as neuropeptides and antioxidant status.
- Considering publishing results in a PubMed-visible peer-reviewed journal.
- Conducting a larger placebo-controlled study, using the optimal parts of the current study design and measurements, and aiming for publishing a PubMed-visible peer-reviewed publication.
- Clinical study on energy and accelerated recovery from exercise-induced inflammation.
- Clinical study on chronic fatigue and pain.
- Clinical study on rapid changes to mood, mental state, and EEG patterns.
- In vitro work on isolated immune cells and inflammatory reactions.
- In vitro work on skin cells and inflammatory reactions.
- In vitro work on isolated mitochondria.

9 Appendix A. Cytokine Standards

Below is a table that shows the mean fluorescence intensity for each cytokine standard, added at a known amount indicated in picograms/milliliter (pg/mL). The testing used the Bio-Plex human cytokine/chemokine 27-plex cytokine protein array (Bio-Rad, Hercules, California, USA).

Table 36. Mean fluorescence intensity for the cytokine standards at a known dose -Run 1.

	Mean Fluorescence Intensity	pg/mL
Pro-inflammatory cytokines		
IFN-γ	13,519	32,873
IL-1β	10,144	8,630
IL-5	7,414	175,664
IL-6	15,036	7,023
IL-8	9,834	11,828
IL-12 (p70)	22,014	35,294
IL-13	13,957	4,631
IL-17A	24,420	60,959
Eotaxin	12,648	2,411
IP-10	13,957	15,690
MCP-1	12,466	8,808
MIP-1α	29,560	857
MIP-1β	13,603	6,737
RANTES	5,747	28,181
TNF-α	13,701	143,425
Anti-inflammatory cytokines		
IL-1ra	9,168	156,832
IL-10	8,207	29,333
Regulating cytokines		
IL-2	10,659	45,753
IL-4	13,934	3,454
IL-7	11,052	59,322
IL-9	5,754	79,136
IL-15	15,243	349,512
Growth factors		
Basic FGF	8,390	50,124
G-CSF	16,972	147,139
GM-CSF	24,662	6,993
PDGF-BB	7,461	53,744
VEGF	13,322	162,418

Table 37. Mean fluorescence intensity for the cytokine standards at a known dose -Run 2.

	Mean Fluorescence Intensity	pg/mL
Pro-inflammatory cytokines		
IFN-γ	16,890	32,873
IL-1β	13,901	8,630
IL-5	9,385	175,664
IL-6	14,025	7,023
IL-8	10,609	11,828
IL-12 (p70)	16,081	35,294
IL-13	13,392	4,631
IL-17A	26,180	60,959
Eotaxin	11,234	2,411
IP-10	13,392	15,690
MCP-1	17,999	8,808
MIP-1α	30,170	857
MIP-1β	14,341	6,737
RANTES	7,706	28,181
TNF-α	14,086	143,425
Anti-inflammatory cytokines		
IL-1ra	10,810	156,832
IL-10	11,696	29,333
Regulating cytokines		
IL-2	12,765	45,753
IL-4	16,095	3,454
IL-7	11,828	59,322
IL-9	7,586	79,136
IL-15	14,859	349,512
Growth factors		
Basic FGF	8,050	50,124
G-CSF	15,851	147,139
GM-CSF	25,860	6,993
PDGF-BB	4,871	53,744
VEGF	15,754	162,418

10 Appendix B. Cytokine Descriptions

Table 38. Pro-inflammatory cytokines.

Title	Abbreviation	Definition
Interferon gamma	IFN-γ	Important cytokine for innate and adaptive immunity against viral and some bacterial infections. Inhibits viral replication, due to its immunostimulatory and immunomodulatory effects. Produced by natural killer cells and natural killer T cells. Lesser quantities produced by T cells and innate lymphoid cells. IFN-Y treatment has shown significant anti-viral effects against the herpes simplex virus and may be an effective treatment in individuals with low T cells.
Interleukin-1 beta	IL-1β	Interleukin-1 beta (IL-1β) is a pro-inflammatory cytokine; crucial for host-defenses against infection and injury. IL-1β affects cell proliferation, differentiation, and apoptosis. IL-1β is a pivotal communication link between the immune system and the central nervous system, including fever induction. IL-1β is produced by activated monocytes and macrophages. Elevated levels of IL-1β may lead to inflammatory disorders in the gastrointestinal tract, bones, and other regions of the body.
Interleukin-5	IL-5	Stimulates B cell growth and increases immunoglobulin secretion, primarily IgA. IL-5 is an activator and chemoattractant of eosinophils. Produced by Type-2 T Helper cells and Mast cells. Elevated levels of IL-5 have been associated with inflammatory disorders, such as allergic rhinitis and eosinophilic esophagitis.
Interleukin-6	IL-6	Acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine, produced in response to infections and tissue injuries. IL-6 stimulates Acute Phase Response, hematopoiesis, and immune reactions. IL-6 expression is strictly controlled by transcription and post-transcriptional mechanisms. IL-6 is produced during acute inflammation by monocytes, macrophages, and endothelial cells. Elevated levels of IL-6 have been associated with inflammatory and auto-immune diseases, such as multiple sclerosis, diabetes, prostate cancer, and rheumatoid arthritis. Anti-IL-6 agents, such as tocilizumab, have been developed as a treatment for inflammatory diseases such as arthritis.
Interleukin-8	IL-8	Neutrophil chemotactic factor. Induces migration toward site of infection and stimulates phagocytosis. In a typical infection, macrophages see an antigen first, and are the first cells to release IL-8 to recruit other cells. Induces a series of physiological responses required for migration and phagocytosis, such as increases in intracellular Ca2+, exocytosis (e.g., histamine release), and respiratory burst. Elevated serum levels of IL-8 have been associated with the pathogenesis of cystic fibrosis.
Interleukin-12 (protein 70)	IL-12p70	Involved in the differentiation of naïve T cells into Th1 cells. Stimulates the production of IFN-γ and TNF-α from T cells and natural killer cells. Produced by activated antigen-presenting cells, such as dendritic cells, macrophages, neutrophils, and B-Lymphoblastoid cells. Elevated levels of IL-12 have been associated with the pathogenesis of autoimmune diseases.
Interleukin-13	IL-13	Central regulator of IgE synthesis, goblet cell hyperplasia, airway hyperresponsiveness, and mucus secretion. Mediator of allergic inflammation. Secreted by Type-2 T helper cells, CD4 cells, NKT cells, mast cells, basophils, and eosinophils. Dysregulated levels of IL-13 have been implicated in the pathogenesis of autoimmune diseases, such as systemic lupus and rheumatoid arthritis.

Interleukin-17A	IL-17A	Interleukin-17A (IL-17A) is a proinflammatory cytokine that plays a key role in the induction of innate immune defenses. IL-17A is important for host defense against infections caused by extracellular bacteria and fungi. IL-17A is produced by CD4+ T cells (Th17 cells), when activated by IL-23. High levels of IL-17A are associated with several chronic inflammatory disease including rheumatoid arthritis, psoriasis, and multiple sclerosis.
Eosinophil chemotactic protein (CCL11)	Eotaxin	Eotaxins are a CC chemokine subfamily of eosinophil chemotactic proteins. In humans, there are three types of Eotaxins: CCL11, CCL24, and CCL26. Eotaxins selectively recruit eosinophils via chemotaxis. Produced by activated endothelial cells, eosinophils, monocytes, and dermal fibroblasts. Higher blood plasma concentrations are seen in patients with schizophrenia, asthma, and those who suffered from an allergic response. Elevated levels of Eotaxins have been found in cannabis users.
Interferon gamma-induced protein 10 (CXCL10)	IP-10	Chemoattractant for monocytes, macrophages, T and NK cells, and dendritic cells. Involved in the promotion of T cell adhesion to endothelial cells, antitumor activity, and inhibition of bone marrow colony formation and angiogenesis. Secreted by endothelial cells, monocytes, and fibroblasts in response to binding of IFN-y.
Monocyte chemotactic protein 1 (CCL2)	MCP-1	Released at sites of inflammation due to injury or infection. Regulates the migration and infiltration of monocytes and macrophages. Produced by endothelial, fibroblasts, epithelial, and microglial cells. Elevated levels of MCP-1 are implicated in the pathogenesis of several diseases characterized by monocyte infiltrates, such as psoriasis, atherosclerosis, and rheumatoid arthritis.
Macrophage Inflammatory protein 1 alpha (CCL3)	MIP-1α	Proinflammatory cytokine crucial for immune response to infection and inflammation. Activates neutrophils and induces the release of pro-inflammatory cytokines. Produced by macrophages and monocytes following stimulation with bacterial endotoxins.
Macrophage Inflammatory protein 1 beta (CCL4)	MIP-1β	Proinflammatory cytokine crucial for immune response to infection and inflammation. Activates neutrophils and induces the release of pro-inflammatory cytokines. Produced by macrophages and monocytes following stimulation with bacterial endotoxins.
Regulated on Activation, Normal T cell Expressed and Secreted (CCL5)	RANTES	Proinflammatory chemokine responsible for recruiting leukocytes to sites of inflammation. Chemotactic for T cells, eosinophils, basophils, monocytes, natural killer cells, dendritic cells, and mastocytes. Induces the proliferation and activation of NK cells. Produced by T cells and Monocytes. Elevated levels of RANTES is associated with viral infections and cancer.
Tumor necrosis factor alpha	TNF-α	Cytokine and adipokine, responsible for regulating immune cells. TNF-α can induce fever, apoptotic cell death, weight loss, inflammation, inhibit tumorigenesis and viral replication, and respond to sepsis via IL-1 and IL-6-producing cells. Produced by activated macrophages, T lymphocytes, and Natural Killer cells. Dysregulation of TNF-α production is implicated in Alzheimer's disease, cancer, major depression, psoriasis, and inflammatory bowel disease.

Table 39. Anti-inflammatory cytokines.

Title	Abbreviation(s)	Definition
Interleukin-1 receptor antagonist	IL-1ra	Natural inhibitor of the pro-inflammatory effects of IL-1a and IL-1β. Secreted by various types of cells, including epithelial cells, adipocytes, and immune cells. A polymorphism of the IL-1ra gene is associated with an increased risk of osteoporotic fractures and gastric cancer.
Interleukin-10	IL-10	Interleukin 10 (IL-10) is an anti-inflammatory cytokine that helps contain a pro-inflammatory immune defense reaction towards pathogens, prevents tissue damage, and maintains normal tissue homeostasis. IL-10 enhances B cell survival, proliferation, and antibody production, and can block NF-kB activity. IL-10 is produced by T helper cells, monocytes, macrophages, and dendritic cells. Recombinant IL-10 is used in treatment of chronic gut inflammatory illnesses, demonstrating the importance of IL-10 for counteracting a hyperactive immune response in the human body.

Table 40. Regulating cytokines.

Title	Abbreviation(s)	Definition
Interleukin -2	IL-2	Signaling cytokine necessary for the growth, proliferation, and differentiation of T cells. Regulates the activity of leukocytes and lymphocytes and plays a part in the body's natural response to microbial infections through the discrimination between self and foreign molecules. Released by CD4+ T cells and activated CD8+ T cells. Elevated levels of IL-2 may be associated with itchy psoriasis.
Interleukin -4	IL-4	Induces differentiation of naive helper T cells (Th0 cells) to Th2 cells, which subsequently produce additional IL-4 in a positive feedback loop. IL-4 is produced primarily by mast cells, Th2 cells, eosinophils and basophils. IL-4 stimulates proliferation of activated B and T cells, and differentiation of B cells into plasma cells. IL-4 induces B cell class switching to IgE.
Interleukin -7	IL-7	Cytokine responsible for stimulating the differentiation of multipotent hemopoietic stem cells into lymphoid progenitor cells. IL-7 also stimulates the proliferation of cells in the lymphoid lineage (T cells, NK cells, and B cells). Secreted by stromal cells in the bone marrow and thymus. Elevated levels of IL-7 have been associated with patients with viral infections, such as HIV. IL-7 is currently being studied in clinical trials as an immunotherapy agent for treating HIV infections.
Interleukin -9	IL-9	Stimulates cell proliferation and prevents apoptosis in hematopoietic cells. Signal transducer and activator of the STAT proteins, specifically STAT1, STAT3, and STAT5. Produced by mast cells, natural killer T cells and CD4 T helper cells. Elevated levels of IL-9 have been associated with Crohn's disease.
Interleukin -15	IL-15	Regulates the activation and proliferation of T and natural killer cells. Secreted by mononuclear phagocytes following infection by virus(es). Suppression of IL-15 may be a potential treatment for celiac disease.

Table 41. Growth Factors.

Title	Abbreviation	Definition
Basic Fibroblast Growth Factor	Basic FGF	Growth factor and signaling protein, with broad mitogenic and cell survival activities. Involved in angiogenesis, morphogenesis, tissue repair, embryonic development, and tumor growth and invasion. In normal tissue, Basic FGF is present in basement membranes and in the subendothelial extracellular matrix of blood vessels.
Granulocyte-Colony Stimulating Factor	G-CSF	Stimulates bone marrow to produce granulocytes and stem cells for release into the bloodstream. Stimulates the proliferation, survival, and differentiation of neutrophil precursors and mature neutrophils. Functionally, G-CSF, is both a cytokine and hormone. Produced by endothelium, macrophages, and several other immune cells. G-CSF injections are an FDA approved treatment used to increase stem cells in blood circulation after trauma
Granulocyte-Macrophage Colony Stimulating Factor	GM-CSF	Stimulates stem cells to produce granulocytes, such as neutrophils, eosinophils, basophils, and monocytes. Protective role against intestinal infection. Stimulates the proliferation of intestinal mucosal myeloid cells in response to bacterial invasion. Produced by T cells, macrophages, endothelial cells, intestinal epithelial cells, and fibroblasts. Part of the immune/inflammatory cascade, leading to further immune activation and GM-CSF release. Elevated levels of GM-CSF have been associated with autoimmune inflammatory diseases, such as arthritis and encephalitis.
Platelet-Derived Growth Factor subunit Beta	PDGF-BB	Growth factor involved in angiogenesis and is a potent mitogen for cells of mesenchymal origin. Important for wound healing. PDGF is synthesized and stored in the alpha granules of platelets and is released upon platelet activation. Produced by activated macrophages, smooth muscle cells, and endothelial cells.
Vascular Endothelial Growth Factor	VEGF	Growth factor protein that stimulates vasculogenesis and angiogenesis. Stimulates new blood vessel growth during embryonic development, after vessel injury, and to bypass blocked vessels. Produced by macrophages, platelets, keratinocyte, renal mesangial cells, and tumors. Elevated serum levels of VEGF are seen in patients with bronchial asthma and diabetes mellitus.

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