Clinical Papers & Press Releases for SL-NAD+ 100mg & LumeniX 100mg

Property of Entity Health, Inc
3500 South DuPont Highway
Dover, Delaware 19901

ēntity

Content Page: SL-NAD+ 100mg & LumeniX 100mg Information

(Co. Reg. No: 200405621W)

NEWS RELEASE

Breakthrough Sublingual Wafers Raise NAD+ Levels by 76% and Enhance Well-Being in Human Trial

- ✓ SL-NAD+ wafers demonstrated in human trial to:
  - ➢ significantly increase blood NAD+ levels by 59% over two weeks and 76% over six weeks compared to baseline
  - ➢ improve energy levels, mood, sleep quality, mental clarity, and physical strength
  - ➢ be safe and well tolerated

Singapore, 9 July 2024 — iX Biopharma Ltd (the "**Company**"), a specialty pharmaceutical company specializing in drug delivery systems and a leader in innovative healthspan nutraceuticals, announced today the top-line results from an open-label, pilot study conducted in London, United Kingdom by NAD Laboratory Ltd, evaluating the effects of its novel sublingual NAD+ (nicotinamide adenine dinucleotide) wafer (SL-NAD+) on NAD+ levels in nine healthy individuals.

The study showed a significant increase in blood NAD+ levels, with an average rise of 59% at two weeks and 76% at six weeks compared to baseline.

In addition to boosting NAD+ levels, the study showed that SL-NAD+ wafers also improved various aspects of health and wellness in the participants. According to a self-reported questionnaire, participants reported enhancements in energy levels, mood, sleep quality, mental clarity, and/or physical strength, which were sustained throughout the six-week study period. Moreover, SL-NAD+ wafers were safe and well tolerated.

These results add to a growing body of evidence that the supplementation of NAD is a good way to improve the quality of life as one ages. However, SL-NAD+ is the only scientifically proven consumer product that delivers pure NAD+ via the oral mucosa to effectively increase NAD+ levels, making it the most direct and efficient way to boost NAD+ levels and enjoy its benefits. Its advantages include:

- ✓ effective sublingual delivery for better bioavailability and quicker uptake;
- ✓ consistent dosing with no need for conversion to NAD+ compared to NR (nicotinamide riboside) and NMN (nicotinamide mononucleotide) precursor supplements; and
- ✓ in comparison to NAD+ IV drips, it is more practical, convenient, affordable, and readily available, providing a way for continuous supplementation to maintain constant, optimal NAD+ levels.

"We are very pleased with the outcome of this study, which now confirms that SL-NAD+ wafers can significantly raise NAD+ levels when measured in plasma and within red blood cells," said **Dr Janakan Krishnarajah, Chief Operating Officer and Chief Medical Officer** of the Company. *"This achievement is made possible by the Company's proprietary freeze-drying process, which stabilises NAD+, and our patented wafer formulation, ensuring rapid disintegration, release, and increased absorption, unlocking the full benefits of this critical molecule. This is a breakthrough for the field of longevity science and for millions of people who want to optimise their cellular health."*

About NAD+ and SL-NAD+

NAD (nicotinamide adenine dinucleotide) is a critical molecule in our body responsible for vital cellular functions in the body. It is crucial for energy production, cellular metabolism, DNA repair, regulating sleep cycles and promoting healthy aging. NAD+ levels decrease as we age, with levels typically dropping to half by the time we reach 50. The decline in NAD+ levels with age is linked to various age-related health concerns and metabolic disorders. In recent years, NAD+ has become an important focus in scientific research on aging, with maintenance of adequate NAD+ levels being linked to healthy aging and longevity. Clinical trials have also been conducted to investigate the potential of NAD+ in treating various age-related diseases, such as Type 2 Diabetes, Non-Alcoholic Fatty Liver Disease, neurodegenerative diseases like Parkinson's disease, cardiovascular and skeletal muscle diseases.

Despite its potential, NAD+ has been challenging to utilise effectively, other than through IV. Alternative ways to boost NAD+ levels with NAD precursors, like NMN and NR, may be inefficient due to bioavailability and other issues, such as inefficient conversion to NAD+ due to age-related declines in enzyme activity.

SL-NAD+ is a novel sublingual wafer that delivers NAD+ directly into the bloodstream, bypassing the digestive system to ensure higher bioavailability and significantly boosting intracellular NAD+ levels. The Company's proprietary freeze-drying process and patented wafer formulation stabilises NAD+ and delivers them as nanoparticles, ensuring rapid disintegration, release, and absorption through the sublingual mucosa. SL-NAD+ is available for purchase on https://entity-health.com/product/sl-nad/ and through select specialist clinics in Singapore.

About iX Biopharma Ltd

iX Biopharma is a specialty pharmaceutical and nutraceutical company listed on the Catalist board of the Singapore Exchange Securities Trading Limited (SGX-ST), operating a fully integrated business model from drug development to manufacturing and supply, with facilities in Australia. The Group is focused on the development and commercialisation of pharmaceutical drugs and innovative nutraceuticals using novel, patent-protected formulations for sublingual delivery.

iX Biopharma has developed a number of patented drug delivery platform technologies, including WaferiX, WaferlogiX and NADiX, which deliver small molecule and biologics sublingually via the mucosa for better absorption, faster onset of action and predictable effect. The drug delivery platforms are particularly useful for drug repurposing, where existing approved drugs are developed into new drugs targeting different indications or a different route of administration, at a lower development cost and risk. iX Biopharma's portfolio includes among others, ketamine, dexmedetomidine, sublingual vaccine delivery, and healthspan products.

Contact for media:

Eva Tan
Chief Commercial Officer
T: +65 6235 3212
E: eva.tan@ixbiopharma.com

Protocol Title:

An open label, pilot study to evaluate the effects of a novel sublingual NAD+ wafer (SL-NAD+) in healthy individuals.

Aim:

Primary objective: To determine the effect of SL-NAD+ wafers on the NAD+ (nicotinamide adenine dinucleotide) levels in whole blood.

Secondary objectives: (i) To evaluate the effects of SL-NAD+ wafers on energy levels, mood, sleep, mental clarity and physical strength, (ii) To assess the safety and tolerability of SL-NAD+ wafers administered via sublingual route.

Method:

This is an open-label, pilot study in 9 healthy individuals (5 female, 4 male) with 7 included in the whole blood NAD+ analysis. Each participant self-administered SL-NAD+ wafers over a six week treatment period, 200mg NAD+ per day for the first 2 weeks (2 wafers a day), then 100mg NAD+ for the remaining 4 weeks period (1 wafer per day). Venous blood samples were collected at week 0 (baseline), week 2 and week 6 to determine whole blood NAD+ levels in plasma and within red blood cells by colorimetric assay.

Questionnaires captured data on lifestyle factors, modalities of special interest (energy levels, mood, sleep, mental clarity and physical strength) and adverse events at baseline, week 2, and week 6.

(SL-NAD+ uses a patented wafer matrix technology as the NAD+ carrier. The wafers were prepared by freeze-drying an aqueous dispersion of NAD+ in a patented matrix formulation, producing a highly porous, amorphous, non-ionic and non-crystalline solid dosage form. When placed under the tongue, the wafers disintegrate rapidly in contact with saliva, fully releasing NAD+ from the matrix to the sublingual mucosal membrane, enabling direct and increased sublingual uptake.)

Results and Discussions:

Figures 1 & 2 show the increase/ improvement in whole blood NAD+ concentration after taking sublingual NAD+ (SL-NAD+) for 6 weeks.

Figure 1 shows the average increase in whole blood NAD+ level after 6 weeks of sublingual administration. Figure 2 shows the blood NAD+ level for the seven individuals included in the whole blood NAD+ analysis

The graphs illustrate a significant elevation in whole blood NAD+ levels following sublingual administration of NAD+, with an average rise of 59% in the first two weeks and an increase of 76% over six weeks.

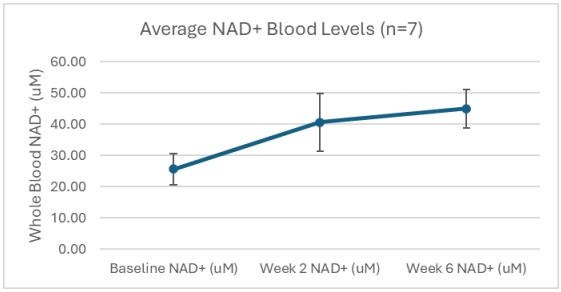


Figure 1: Average NAD+ Blood Levels (n=7)

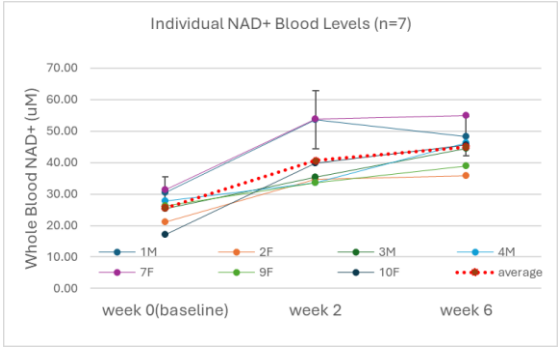


Figure 2: Individual NAD+ Blood Levels (n=7)

Figure 3 is the bar-graph version of both Figures 1 & 2. The horizontal lines reveal the average increase in whole blood NAD+ levels to 40.70 uM (after 2 weeks sublingual administration) and 45.00 uM (after 6 weeks sublingual administration) from a baseline whole blood NAD+ level of 25.60 uM.

The study confirms that NAD+ is absorbed sublingually into the bloodstream and subsequently intracellularly into the red blood cells.

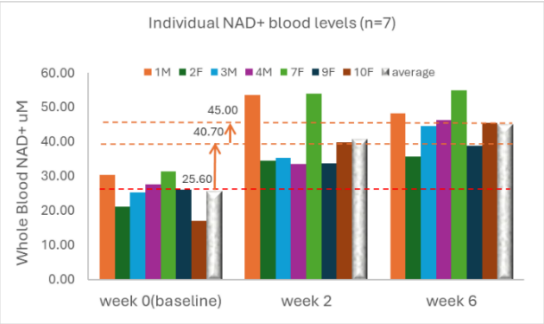


Figure 3: Individual NAD+ Blood Levels (n=7)

Figure 4 shows the differences between whole blood NAD+ levels between individuals who were younger than 50 years old (n=4) and those who were older than 50 years old (n=3). Despite a lower baseline NAD+ in the older age group, both groups reached a NAD+ whole blood level of approximately 45 uM after 6 weeks of daily supplementation.

In a published study, the plasma NAD+ level decreased from approximately 50 nM in young individuals (20–40 years) to approximately 10 nM in elderly subjects (60–87 years)[1].

The decline in NAD+ levels with age is associated with several factors[2] including metabolic changes, lifestyle factors (sedentary lifestyles, high fat/sugar diets, excessive alcohol intake, and immune challenges) and changes in cellular energy metabolism with dysregulation of cellular energy metabolism underpinning chronic age-related diseases, including dementia, cardiovascular disease, and cancer.

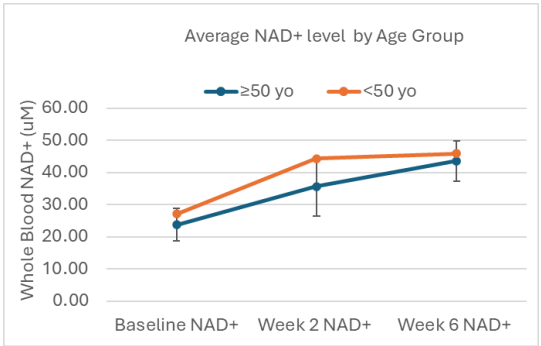


Figure 4: Average NAD+ level by Age Group

Figure 5 shows the differences between male and female whole blood NAD+ levels. The female participants (n=4) have lower baseline NAD+ whole blood level compared to the male participants (n=3). After 2 weeks and 6 weeks of sublingual administration of NAD+, both females and males had improved NAD+ whole blood concentrations to a similar average level.

There is a difference in NAD+ blood levels between men and women as shown in some studies. One study found that the whole blood NAD+ contents in men were significantly higher than that in women (34.5 vs. 31.3 μmol/L)[1]. This finding suggests that sex can influence NAD+ levels.

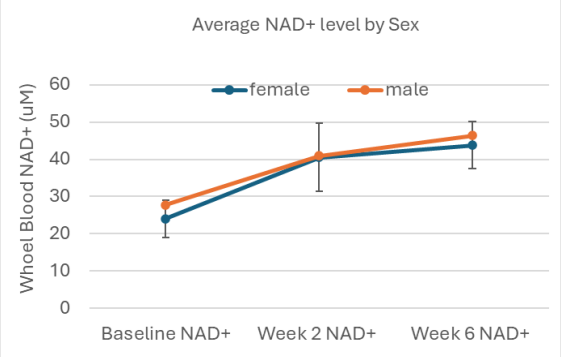


Figure 5: Average NAD+ level by Sex

Self-reported Questionnaire:

All 9 participants reported improvement in at least one modality of interest. Improvements were reported in the following modalities at week 2 and maintained throughout the study to week 6: Energy levels (6 out of 9 participants), mood (5 out of 9 participants), sleep quality (4 out of 9 participants), mental clarity (4 out of 9 participant) and physical strength (3 out of 9 participants).

Safety and Tolerability:

SL-NAD+ wafers were safe and well tolerated by all participants. Only one adverse event (mild headache) was reported by one participant during the 6 week period and was considered only possibly related to wafer administration. It resolved without intervention.

Conclusion:

This study demonstrated that sublingual administration of SL-NAD+ wafers significantly increased whole blood NAD+ levels (combined plasma and intracellular red blood cell levels) in healthy individuals, with an average increase of 59% after 2 weeks and 76% after 6 weeks. The study aligned with published data showing that NAD+ levels decrease with age, highlighting the potential of SL-NAD+ wafers to counteract this decline. This study also observed lower baseline NAD+ whole blood levels in female volunteers compared to males, suggesting that sex can influence NAD+ levels.

The SL-NAD+ wafers were found to be safe and well-tolerated by the participants over the 6-week treatment period.

The findings of this study highlight the high bioavailability of SL-NAD+ wafers through sublingual administration and support the potential benefits of NAD+ supplementation.

References:

1. Fan Yang et al. Association of Human Whole Blood NAD+ Contents With Aging. Frontiers in Endocrinology. March 2022 | Volume 13.

2. Hassina Massudi et al. Age-Associated Changes In Oxidative Stress and NAD metabolism In Human Tissue. PLoS ONE 7(7): e42357. https://doi.org/10.1371/journal.pone.0042357.

(Co. Reg. No: 200405621W)

NEWS RELEASE

New Study Demonstrates that SL-NAD+ delivers NAD+ into Cells

- ✓ Sublingual NAD+ is the new gold standard for daily NAD+ supplementation, without the need for precursors

Singapore, 2 September 2024 — iX Biopharma Ltd. (the "**Company**"), a specialty pharmaceutical company specializing in drug delivery systems and a leader in innovative healthspan nutraceuticals, has announced groundbreaking results from a pharmacokinetic study evaluating the sublingual absorption of a novel NAD+ wafer, SL-NAD+. This study is the first to provide compelling evidence that NAD+ can directly enter cells, offering a promising new approach to NAD+ supplementation.

The study consisted of 3 parts: 2 single-dose PK studies and a multiple-dose PK study, conducted in 18 Sprague-Dawley rats, examining the plasma and red blood cell NAD+ levels following sublingual administration of NAD+. Assessment of NAD+ levels were done via the LC-MS/MS method, one of the most advanced and reliable methods to test for NAD+.

The results revealed several important findings:

1) First evidence of direct cellular entry**:** The study strongly suggests that NAD+ can be transported in and out of cells directly (NAD+ flux), most probably via connexin 43 hemichannels and other solute carrier channels. This is the first in-vivo study to provide evidence supporting this capability.

2) Rapid sublingual absorption: Mean peak plasma concentration, a 2-fold increase in plasma NAD+ levels, was achieved within 10 minutes of dosing. This underscores the effectiveness of sublingual delivery through the mucosa.

3) Significant bioavailability**:** The findings suggest a sublingual bioavailability of SL-NAD+ at 22% compared to intravenous (IV) administration. This provides a promising alternative to IV NAD+ therapy, with the potential for more convenient and sustained NAD+ delivery.

Dr. Janakan Krishnarajah, Chief Operating Officer and Chief Medical Officer of iX Biopharma, said: "This study's findings provide strong evidence that our innovative sublingual freeze-dried technology delivers NAD+ rapidly into plasma and then directly into cells, challenging the previously held belief that its large size prevents cellular penetration. Along with the positive data from our recent human clinical study, this breakthrough positions direct NAD+ supplementation as the new gold standard for boosting NAD+ levels, removing the need for precursors."

About NAD+ and SL-NAD+

NAD (nicotinamide adenine dinucleotide) is a critical molecule in our body responsible for vital cellular functions in the body. It is crucial for energy production, cellular metabolism, DNA repair, regulating sleep cycles and promoting healthy aging. NAD+ levels decrease as we age, with levels typically dropping to half by the time we reach 50. The decline in NAD+ levels with age is linked to various age-related health concerns and metabolic disorders. In recent years, NAD+ has become an important focus in scientific research on aging, with maintenance of adequate NAD+ levels being linked to healthy aging and longevity. Clinical trials have also been conducted to investigate the potential of NAD+ in treating various age-related diseases, such as Type 2 Diabetes, Non-Alcoholic Fatty Liver Disease, neurodegenerative diseases like Parkinson's disease, cardiovascular and skeletal muscle diseases.

Despite its potential, NAD+ has been challenging to utilise effectively, other than through IV. Alternative ways to boost NAD+ levels with NAD precursors, like NMN and NR, may be inefficient due to bioavailability and other issues, such as inefficient conversion to NAD+ due to age-related declines in enzyme activity.

SL-NAD+ is a novel sublingual wafer that delivers NAD+ directly into the bloodstream, bypassing the digestive system to ensure higher bioavailability and significantly boosting intracellular NAD+ levels. The Company's proprietary freeze-drying process and patented wafer formulation stabilises NAD+ and delivers them as nanoparticles, ensuring rapid disintegration, release, and absorption through the sublingual mucosa. SL-NAD+ is available for purchase on https://entity-health.com/product/sl-nad/ and through selected specialist clinics in Singapore.

About iX Biopharma Ltd

iX Biopharma is a specialty pharmaceutical and nutraceutical company listed on the Catalist board of the Singapore Exchange Securities Trading Limited (SGX-ST), operating a fully integrated business model from drug development to manufacturing and supply, with facilities in Australia. The Group is focused on the development and commercialisation of pharmaceutical drugs and innovative nutraceuticals using novel, patent-protected formulations for sublingual delivery.

iX Biopharma has developed a number of drug delivery platform technologies, including WaferiX, WaferlogiX and NADiX, which deliver small molecule and biologics sublingually via the mucosa for better absorption, faster onset of action and predictable effect. The drug delivery platforms are particularly useful for drug repurposing, where existing approved drugs are developed into new drugs targeting different indications or a different route of administration, at a lower development cost and risk. iX Biopharma's portfolio includes among others, ketamine, dexmedetomidine, medicinal cannabis and nutraceuticals designed to improve healthspan and longevity.

(Co. Reg. No: 200405621W)

Contact for media:

Eva Tan
Chief Commercial Officer
T: +65 6235 3212
E: eva.tan@ixbiopharma.com

NAD-R01 SL NAD+:
Pharmacokinetic study of
SL- NAD+ sublingual wafers in Sprague-Dawley rats

Protocol Title:

A pharmacokinetic study to evaluate the sublingual absorption following single and multiple dosing of a novel sublingual NAD+ wafer (SL-NAD+) in Sprague-Dawley rats.

Aim:

Primary objective: To determine the sublingual absorption profile following single and multiple dosing of SL-NAD+ wafers on the NAD+ levels in plasma and red blood cells (RBC) of rats.

Secondary objectives: To evaluate the sublingual bioavailability of SL-NAD+ wafers in rats.

Method:

This is a pharmacokinetic study in 20 Sprague-Dawley rats examining the plasma and RBC NAD+ levels of sublingually administered NAD+. Each rat was anaesthetised prior to each wafer dose and administered SL-NAD+ wafers in a single 100mg dose or multiple doses (400mg and 800mg dose over 4 hours). Venous blood samples were collected at 2.5 min, 5 min, 7.5 min, 10 min, 15 min, 30 min, 45 min, 1h, 1.5h, 2h, 4 h, 5 h, 6 h and 8h, to determine the NAD+ levels in plasma and RBCs by the LC-MS/MS method for the quantification of NAD+.

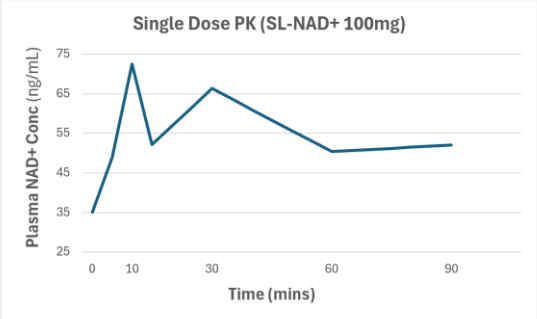
The plasma and RBC pharmacokinetic parameters for NAD+ were calculated using standard non-compartmental analysis (Phoenix® software, version 8.3, Certara Corporation, Mountain View, California 94040/USA) using linear trapezoidal method with linear interpolation.

SL-NAD+ uses a patented wafer matrix technology as the NAD+ carrier. The wafers were prepared by freeze-drying an aqueous dispersion of NAD+ in a patented matrix formulation, producing a highly porous, amorphous and non-ionic solid dosage form. When placed under the tongue, the wafers disintegrate rapidly in contact with saliva, fully releasing NAD+ from the matrix to the sublingual mucosal membrane, enabling direct systemic absorption.

Results and Discussion:

1. Single dose PK in Sprague-Dawley rats. Plasma NAD+ concentrations were obtained following administration of sublingual NAD+ 100mg wafer.

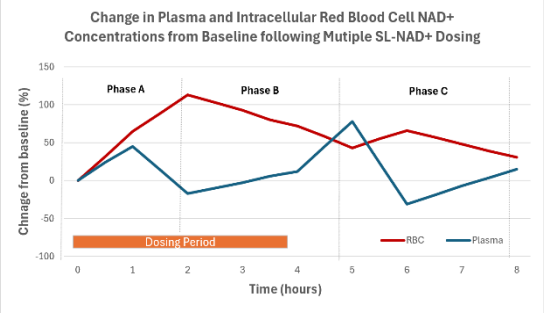
Figure 1



Sublingual administration of SL-NAD+ wafers resulted in rapid absorption of NAD+ directly into plasma via the sublingual mucosa. Mean peak plasma concentration of 72.4ng/mL was reached within 10 minutes of administration, a 2-fold increase from the mean baseline level of 35.1ng/mL.

2. Multiple dose PK study in Sprague-Dawley rats. Rats were administered either SL-NAD+ 400mg or 800mg over a 4-hour dosing period. Mean change (%) in plasma and intracellular RBC concentrations were assessed from baseline over 8 hours and demonstrated NAD+ flux into plasma and subsequently between plasma and intracellular compartments.

Figure 2

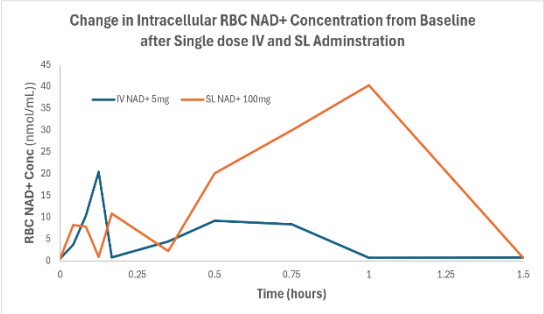


NAD+ flux during this experiment can be described across 3 phases. Phase A – NAD+ is absorbed directly into systemic circulation across the sublingual mucosa with initial peak plasma NAD+ concentration reached within 1 hour. NAD+ concurrently enters the RBCs from plasma reaching peak intracellular RBC NAD+ levels (2-fold increase from baseline) within 1 hour of the peak plasma concentration. Phase B – Intracellular NAD+ levels decline due to consumption and potentially efflux from the RBCs to maintain intracellular NAD+ homeostasis. Plasma NAD+ levels rise with continuing dosing. Phase C – Dosing complete. NAD+ resumes net influx into the cell with decline in plasma NAD+ levels, prior to reversing to net efflux out of the cells for the remainder of the period.

The study provides supporting data that NAD+ can enter the cell directly. The mechanism for NAD+ transport into and out of the cell is most probably through transportation via connexin 43 hemichannels and other solute carrier channels.[1,2,3]

3. Single dose PK (bioavailability study) in Sprague-Dawley rats. This study demonstrated changes in intracellular RBC NAD+ levels from baseline. Rats were administered either IV NAD+ 5mg or SL-NAD+ 100mg wafers.

Figure 3



Standardised for dose, SL-NAD+ wafers produced an increase in RBC NAD+ levels [AUC, area under the curve] of 22% of the increase produced by IV NAD+ administration. As far as we are aware, this is the first bioavailability study for sublingual NAD+ in mammals. This study aligned with published data showing that extracellularly delivered NAD+ can increase intracellular NAD levels.[4]

Conclusion:

This study demonstrated that sublingual administration of SL-NAD+ wafers significantly increased plasma and red blood cell levels in healthy Sprague-Dawley rats. A 2-fold increase in plasma NAD+ levels was observed within 10 minutes of dosing.

The findings of this study suggest a sublingual bioavailability of SL-NAD+ of 22% compared to IV administration. This supports sublingual delivery of NAD+ with SL-NAD+ wafers as an effective approach to increase intracellular NAD+ levels.

References:

1. Santina Bruzzone et al. A Self-restricted CD38-connexin 43 cross-talk Affects NAD and Cyclic ADP-ribose Metabolism and Regulates Intracellular Calcium in 3T3 Fibroblasts. The Journal of Biological Chemistry Vol. 276, No. 51, Issue of December 21, pp. 48300–48308, 2001

2. Elena Zocchi et al. Ligand-induced internalization of CD38 results in intracellular Ca^{2+} mobilization: role of NAD+ transport across cell membranes. FASEB J. 1999, 13, 273–283

3. J Clement, M Wong, A Poljak, P Sachdev and N Braidy. The Plasma NAD+ Metabolome Is Dysregulated in "Normal" Aging. Rejuvenation Research. Volume 22, Number 2, 2019

4. Richard A. Billington et al. Characterization of NAD Uptake in mammalian Cells. The Journal of Biological Chemistry. 2008, 283, 10, 6367–6374.

A RANDOMISED, DOUBLE-BLIND, MULTIPLE-DOSE TRIAL OF THE EFFICACY OF A GLUTATHIONE WAFER AS A THERAPEUTIC SKIN HEALTH SUPPLEMENT

Stephen Lim PhD [1] and Janakan Krishnarajah MD[1]

Results summary

After 14 days

- ✓ Significant increase in skin luminosity and gloss by up to 60%
- ✓ Significant reduction in eye wrinkles and fine lines by up to 51%

After 28 days

- ✓ Significant increase in skin elasticity by up to 226%

After 56 days

- ✓ Significant increase in skin smoothness with decrease in skin roughness by up to 71%

Based on a clinical trial of 34 female participants with signs of skin ageing on a regime of 200mg sublingual GSH wafers daily for 4 weeks followed by 100mg sublingual GSH wafers for 8 weeks

ABSTRACT

Glutathione (GSH) is a potent master antioxidant and plays a crucial role in various physiological processes within the body, including maintaining skin health and appearance[1,2].

Its powerful antioxidant function neutralizes free radicals and reduces oxidative stress in the skin. Free radicals damage skin cells, collagen and elastin fibres, leading to roughness and premature aging. By neutralizing these free radicals, glutathione may help protect the skin and maintain its smoothness.[3]

Glutathione also stimulates the synthesis of collagen, a protein that provides structure and elasticity to the skin. Increased collagen production improves skin firmness and smoothness, reducing the appearance of fine lines and wrinkles.[4]

Glutathione is involved in the pathway of detoxification processes, in the elimination of toxins and harmful substances. The detoxification helps achieve clearer, healthier skin, contributing to a smoother complexion.[5,6]

GSH is a naturally occurring tripeptide. Literature reports show that GSH can improve skin gloss and luminosity. Several routes of supplementing GSH for these skin treatment purposes are available including intravenous (IV), oral and topical administration.

We investigated a novel patented sublingual wafer containing GSH marketed under the name MeltMed Radiance in a randomised, double-blind, multi-dose trial for efficacy as a therapeutic skin health supplement.

Objectives: The primary objectives of this study were to determine the changes in skin gloss and luminosity, skin elasticity, eye wrinkles and fine lines and skin roughness following the sublingual administration of GSH wafers.

Methods: The 12-week study was conducted on 34 healthy females aged 30 to 65 years old with Fitzpatrick skin type IV or V at one clinical site in Sydney, Australia.

Results: Significant improvements were observed in skin gloss and luminosity (up to 60%), skin elasticity (up to 226%), skin smoothness (up to 71%) and decrease in eye wrinkles and fine lines (up to 51%) within 14 to 56 days of commencing GSH therapy. GSH wafers were considered safe with no serious adverse events reported. All participants rated good to excellent for acceptability of wafers' smell, taste, after-taste as well as the sublingual disintegration rate of the GSH wafers

Conclusions: The authors concluded that the sublingual GSH wafers were safe, tolerable and clinically efficacious in healthy women with the ideal maintenance sublingual dose of 100mg GSH daily after a one month loading dose of 200mg GSH daily.

INTRODUCTION

According to a study by Yang et al,[7] nearly one-third of the 1,434 participants in their study felt unhappy with their skin during the COVID-19 pandemic. Aging skin is a significant and growing concern and the top age-related facial skin concerns include eye puffiness, loose skin, uneven tone, uneven texture, and dry skin. Also, video conferencing, mask wearing, and increased stress during the COVID-19 pandemic have exacerbated their skin concerns for many consumers. Their results revealed the top skin concerns were eye puffiness (86.5%), loose skin (85.1%), uneven tone (84.9%), uneven texture (83.5%), and dry skin (81.4%). Video conferencing (31%), wearing masks (23%), and increased stress (21%) during the COVID-19 pandemic affected how participants felt about their skin.

The study by Luebberding et al[11] revealed a progressive decline in the mechanical properties of skin with aging. These mechanical properties change differently in men and woman over their lifetimes. The obvious morphological sign of skin aging is the development of rhytides, commonly known as wrinkles. The development of facial wrinkles significantly affects men earlier and with greater severity than women. The elasticity of the skin, which influences formation of facial wrinkles and age-related skin laxity, is determined by collagen density and elastin fiber volume. The skin's elastic ability to recover after stretching, smoothness and firmness, are strongly affected by the aging process, the redox status and the GSH levels in the body. GSH has been used to enhance and support overall skin health.

The cellular redox state is altered in a number of pathological conditions, diabetes, cardiovascular diseases (eg atherosclerosis), inflammatory diseases, liver diseases (eg non-alcoholic fatty liver disease or NAFLD) and aging. GSH influences protein synthesis, with GSH deficiency causing changes to the functional and structural properties of cellular proteins, especially collagens. Intracellular redox potential influences the generation of collagen, and also influences gene transcription in mesangial cells, which is important for the functional and structural properties of cellular proteins.[4]

The catalytic activity of enzymes also contribute to collagen synthesis and its secretion are also influenced by the intracellular optimal redox state. GSH is important for maintaining homeostasis and acts as a "redox switch" to maintain this important optimal protein synthesis function. Hence, changes in the cellular redox state can influence collagen anabolism and secretion.[10]

Topical, oral, and IV GSH are available as nutraceutical products for skin health. Oral administration of GSH is not considered optimal due to its very poor bioavailability. The sublingual (SL) form is the most bioavailable compared to other oral forms (tablet, capsule) due to the sublingual absorption bypassing the liver first pass metabolism and the hostile environment of the GI tract. An in vivo study by Daniela Buonocore et al[8] revealed a rapid and efficient uptake of GSH into the blood via the oral mucosa resulting in higher bioavailability. In another in vivo study by Bernard Schmitt et al[9], these researchers

also showed increased plasma GSH levels in the SL group when compared to the oral GSH group. The differences between these two groups were statistically significant ($p < 0.05$).

The SL wafer in our study used a patented wafer matrix technology (WaferiX™) as the GSH carrier. This novel SL GSH wafer was prepared by freeze-drying an aqueous dispersion of GSH containing sodium carboxymethycellulose and amylopectin as the matrix formers. The novel wafer GSH formulation rapidly dissolves sublingually, releasing the GSH into the small saliva volume immediately, adjacent to the sublingual mucosal membranes, resulting in a direct SL absorption with higher bioavailability than other oral formulations.

This study aimed to explore the clinical effect of SL GSH on related skin conditions as a therapeutic skin health supplement.

METHODOLOGY

This study was a randomised, double-blind, multiple-dose study on the efficacy of a sublingual glutathione wafer (MeltMed Radiance™) as a therapeutic skin health supplement. The 12-week study was conducted on 34 healthy females aged 30 to 65 years old with Fitzpatrick skin type IV or V at one clinical site in Sydney, Australia.

The primary efficacy objectives included: (i) skin gloss and luminosity; (ii) skin elasticity; (iii) eye wrinkles/fine lines (crow's feet) and (iv) skin roughness.

Participants were instructed on how to administer the wafers under the tongue for maximum SL absorption. Food and drink were avoided within 10 minutes of administration and the wafers were administered twice daily (morning and evening).

Participants were blinded and randomised in a 1:1 ratio to one of the two cohorts each with seventeen (17) participants. Cohort 1 received 100mg GSH wafer (2 x 50mg GSH wafers plus 1 x placebo wafer) administered twice daily (total daily dose of 200mg) in Week 1 to Week 4. In Week 4 to Week 12, 50mg GSH wafer (1 x 50mg GSH wafer plus 1 x placebo wafer) were administered twice daily (total daily dose of 100mg). This will be referred to as the 200mg/100mg dosing regimen.

Cohort 2 received 150mg GSH wafer (3 x 50mg GSH wafers) administered twice daily (total daily dose of 300mg) in Week 1 to Week 4. In Week 4 to Week 12, 100mg GSH wafer (2 x 50mg GSH wafers) were administered twice daily (total daily dose of 200mg). This will be referred to as the 300mg/200mg dosing regimen.

Assessments were conducted at baseline (Day 0), Week 2 (Day 14), Week 4 (Day 28), Week 8 (Day 56) and Week 12 (Day 84) which was the end of study (+/- 3 days) or at early termination.

Participants were allowed to continue their current skincare regimen without introducing any new or different skincare products or facial treatments. Participants were also asked to avoid sun exposure or to use appropriate sun protection when outdoors (sunscreen, hat, protective clothing). Each participant was provided with product instructions (for use and storage) and a diary to keep track of product use. At each visit participants' compliance and any changes to concomitant medications were recorded.

INSTRUMENTAL MEASUREMENTS

Skin elasticity was measured by Cutometer® Dual MPA 580 (Courage + Khazaka electronic GmbH) and skin luminosity (gloss) was measured by Skin Glossymeter® GL 200 (Courage + Khazaka electronic GmbH).

The skin properties were analysed by digital photographs of the face (front and side facial photographs) using a custom-made digital photography equipment (Canon EOS 60D DSLR camera). Each image was cropped and resized in GIMP to facilitate image analysis. After images were processed, they were imported into Image Pro Premier (IPP) for image analysis (assessment including eye wrinkles, and skin roughness). The IPP provides values in percentage change ("change from baseline" and "percentage change from baseline").

STATISTICAL ANALYSIS METHODS

The instrumental and photos measures were transferred to SAS for analysis. Descriptive statistics were calculated and averaged over participants for each measure and visit. These averages were analysed inferentially using repeated measures analysis of covariance within a mixed model framework. Inferential statistical analysis was performed in order to assess if each parameter (the dependent measure) varied linearly over time. The Baseline value (Visit 1) of the measure, in addition to other demographic variables, were used as covariates within each measure.

Adjusted means, calculated as Least Squared Means in the analyses, are presented in the tables with probabilities less than 0.05 indicating significant differences between selected means. The adjusted means between the first and last visit were compared using a t-test to see if there was an overall change at the end of the study.

Change from Baseline was calculated, and descriptive statistics were also calculated for each measure. This includes number of observations (n), mean, percentage change, standard deviation (SD), minimum, median, and maximum values for each parameter's value and its change from Baseline.

STUDY POPULATION

A total of thirty-four (34) healthy female participants with Fitzpatrick Skin Type IV or V and with some signs of skin ageing (crow's feet, and uneven skin texture) whose written informed consent had been obtained, were screened and enrolled in the study.

RESULTS AND DISCUSSIONS

The average age of the thirty-four (34) female participants enrolled was 44 years (SD = 9.5), ranging from 31 years to 64 years old.

88% of participants had an ethnicity from Asia, 3% from the South Pacific/Papua with 9% from other regions. 24% of participants had normal skin, 18% had dry skin, 11% had oily skin and 47% had combination skin.

56% had 'Phototype IV' skin (olive, moderate brown; burns minimally, always tans well); 44% had 'Phototype V' skin (brown, dark brown; rarely burns, tans profusely).

The following efficacy parameters showed a statistically significant improvement compared to baseline: skin luminosity and gloss (p = 0.04), eye wrinkles (p = 0.04), and skin roughness (p = 0.04).

High positive responder rates across all parameters

We analysed the percentage of participants who had a positive result to GSH therapy within the study period. The study showed an overall high positive responder rate of greater than 70% across all parameters. 100% of participants on the 200mg/100mg regime showed improvements to skin roughness and elasticity within 12 weeks (84 days), while only 100% of participants on the 300mg/200mg regime showed improvements to skin roughness within 12 weeks (84 days).

Due to the high levels of positive response seen in participants on the 200mg/100mg regime across the parameters studied, we postulate that 200mg/100mg regime provides the body with the best optimal homeostatic GSH level. More supplementations (eg 300/200mg dosage regime) may not be necessary, as excessive GSH may be broken down or rechannelled to other cells. GSH has a very complicated pattern of involvement in diverse biological processes/activities.[12]

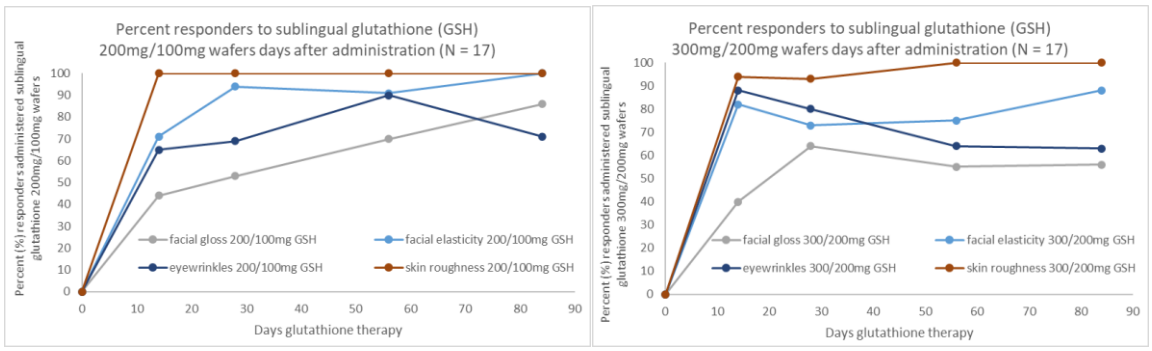


Fig 1. Graph of responder rates (%) from baseline throughout end of study for each efficacy parameter (Left graph = 200mg/100mg sublingual GSH dosage; Right graph = 300/200mg sublingual GSH dosage).

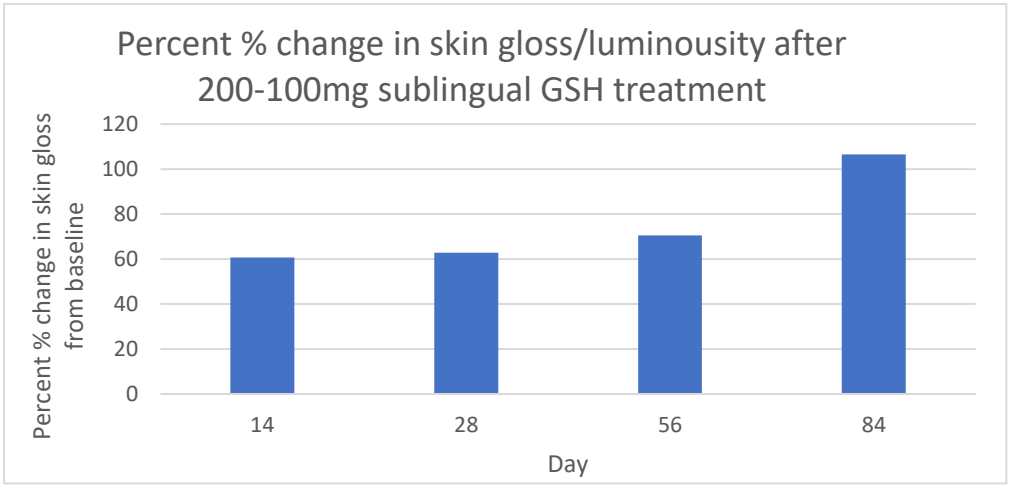


Fig 2. Graph of maximum percent changes from baseline for skin gloss/luminosity for the sublingual 200/100 mg GSH treatment group

Skin gloss and luminosity:

SL GSH therapy resulted in a statistically significant improvement to skin gloss and luminosity in participants compared to baseline (p = 0.04) (see Fig. 2).

Participants on the 200mg/100mg regime achieved rapid and marked increase in skin luminosity and gloss of up to 66% after 4 weeks, increasing up to a remarkable 106% after 12 weeks. By 12 weeks, increased skin luminosity and gloss was observed in 84% of participants.

Based on the mean data showing that maximum improvement was achieved after 12 weeks, continuous SL GSH therapy beyond the study period is expected to result in greater increase in skin luminosity and gloss.

The improvement in skin gloss in this study is in line with other GSH clinical studies[13, 14]. The mode of action of improvement in skin gloss is possibly by GSH inhibiting the tyrosinase enzyme as well as by reducing free radicals damaging the skin cells. Uneven skin gloss can be treated by reducing cells damage caused by UV radiation.

Skin elasticity

SL GSH therapy resulted in a statistically significant improvement in skin elasticity in participants compared to baseline (p = 0.03) (see Fig. 3)

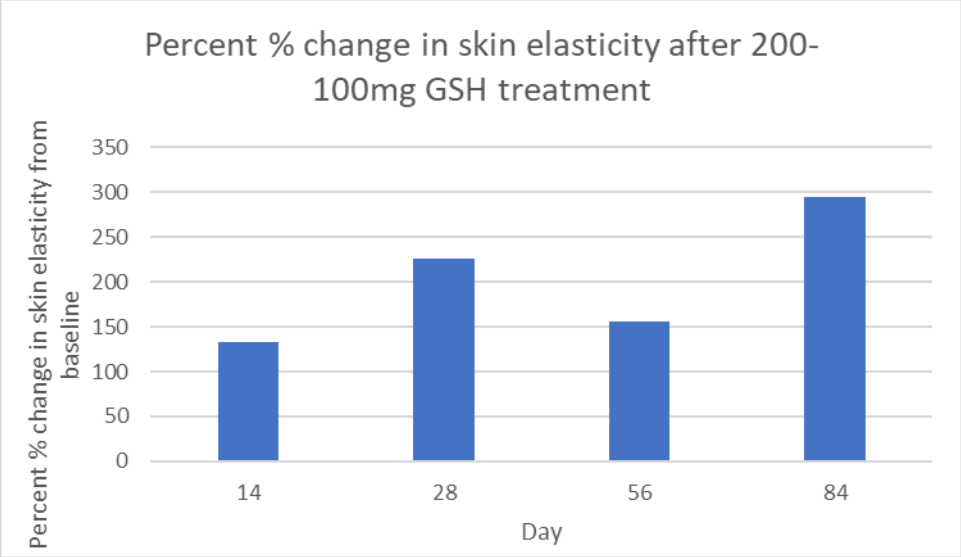


Fig 3. Graph of maximum percent changes from baseline for skin elasticity for the sublingual 200/100 mg GSH treatment group.

Participants on the 200mg/100mg GSH regime achieved significant increase in skin elasticity of up to 226% after 4 weeks and 295% after 12 weeks. By 12 weeks, increased skin elasticity was observed in 100% of participants. Elastin is a fibrous protein found in the dermal skin layer and is responsible for skin elasticity. This study showed that GSH can increase the skin elasticity, most probably due to its antioxidant effect. Weschawalit et al[15] in their study, reported that GSH improves skin elasticity and reduces skin wrinkles in either sun-exposed or sun-protected areas and that GSH is superior to placebo in reducing skin wrinkles.

The primary triggering factor of skin aging and/or skin non-elasticity is oxidative cellular damage caused by increased oxidative stress.[16,17]

Oxidative stress results from an imbalance between reactive oxygen species (ROS) synthesis and defence mechanisms that remove ROS. Enzymes that remove ROS, such as glutathione (GSH), are representative of defence mechanisms against oxidative stress.[18]

Eye wrinkles

SL GSH therapy resulted in a statistically significant reduction in eye wrinkles and fine lines around the eyes compared to baseline ($p = 0.04$) (see Fig. 4).

In the 200mg/100mg regime group, 90% of participants responded positively to treatment at the 8-week mark. The greatest maximum change from baseline in this cohort was 51%, observed at day 14 of the study.

This study demonstrated that glutathione supplementation yields cosmetic benefits such as improvements to skin elasticity and reduction in skin wrinkles, especially around the eyes.

Free radical formation in the cells (cause by UV radiation), if left unchecked or not neutralised (by GSH supplementation) will damage the cells and tissues and may cause inflammation and skin wrinkles, especially around the eye. GSH being a powerful and most important intracellular antioxidant, reduces free radicals and inflammation, resulting in the improvement of the skin wrinkles and skin complexion as shown in this study. This confirms the finding that GSH is effective in reducing facial wrinkles in another study by Weschawalit et al.[15]

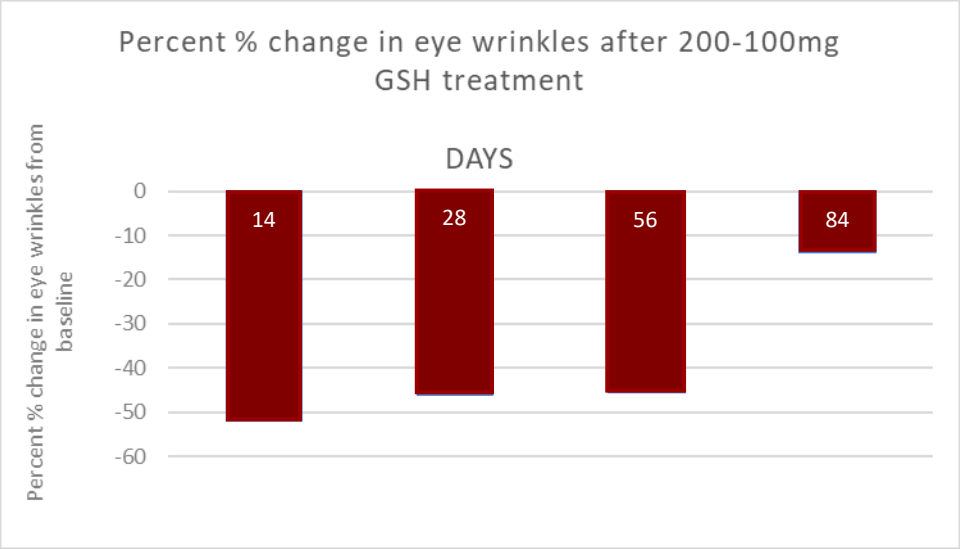


Fig 4. Graph of maximum percent changes from baseline for eye wrinkles for sublingual 200/100 mg treatment group

Skin roughness

SL GSH therapy resulted in a statistically significant reduction in skin roughness compared to baseline (p = 0.04) (see Fig. 5)

Within 14 days, all participants on the 200mg/100mg regime had shown improvements in skin roughness. Within 4 weeks, there was a reduction of up to 64% in skin roughness, which increased to 71% by week 8.

GSH delays skin stiffening and decreases collagen loss from skin injury induced by UV radiation[19]. GSH also assists with skin cell renewal giving the skin a glowing appearance. The aging "dull" skin cells give the skin a rough appearance. GSH assists skin renewal by sloughing away dead cells and giving the new underlying cells a chance to rise to the surface[20]. This study has demonstrated that GSH can improve skin smoothness (or decrease skin roughness) shown by 100% of the participants in the 200/100mg protocol with 14 days of initiation of therapy and maintained till 12 weeks.

The Watanabe et al[21] study showed that GSH significantly increases the moisture content of the stratum corneum plus the suppression of wrinkle formation leading to the improvement in skin smoothness.

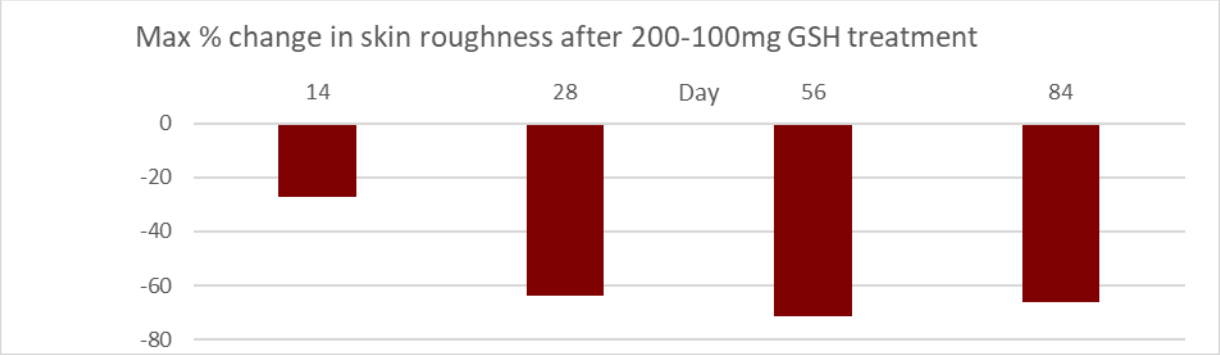


Fig 5. Graph of maximum percent change from baseline for skin roughness for 200/100 sublingual GSH group

Safety and tolerability of the sublingual GSH:

As with previous studies using WaferiX (wafer carrier matrix) there were no abnormal findings of oral cavity tolerability in any subject, with mucosa deemed as normal in all post-dose assessments of oral cavity and sublingual space.[22, 23].

GSH wafers were considered safe with no serious adverse events reported. On the oral symptom questionnaires, there was no sublingual irritation or burning sensation for any subject. This is in sync with several studies where none of the participants demonstrated adverse events during the treatment period of SL GSH.[24, 25]

GSH is a tripeptide consists of cysteine, glycine and glutamate. These three amino acids are bonded together to form a tripeptide called GSH. Cysteine is a sulphur-containing amino acid and some people may taste and/or smell the sulphur which may not be a likable for some people. Our wafer contained taste masking agent incorporated into the formulation to minimise any sulphur taste pertaining to the cysteine residue.

All participants were given questionnaires to rate on acceptability of smell, taste, after-taste as well as the sublingual disintegration rate of the GSH wafers.

70.7% of participants reported that the smell of the GSH wafers was good to excellent. 64.7% of participants reported that the taste of the GSH wafers was good to excellent. Overall tolerability of the GSH wafers was reported as good to excellent in 74.2% of participants.

CONCLUSION

This study showed that GSH sublingual wafers are an effective therapeutic skin health supplement. Statistically significant positive results were observed for skin gloss and luminosity, skin elasticity, eye wrinkles, and skin roughness. All these parameters showed positive therapeutic results within 14 days of therapy with high positive responder rates.

The safety and tolerability of the sublingual GSH after 84 days were good to excellence as rated by the participants.

The ideal initiation dose of GSH is 200mg daily for 28 days (4 weeks), followed by a maintenance dose of 100mg daily thereafter.

REFERENCES

1. Alyssa Abdelnour, Taylor Adlam, Geoffrey A. Potts. Effects of Plant-Derived Dietary Supplements on Skin Health: A Review. Cureus. 5(6): e40892. DOI 10.7759/cureus.40892.
2. Duperray J, Sergheraert R, Chalothorn K, Tachalerdmanee P, Perin F. The effects of the oral supplementation of L-cystine associated with reduced L-glutathione-GSH on human skin pigmentation: a randomized, double-blinded, benchmark-and placebo-controlled clinical trial. J Cosmet Dermatol. 2022, 21:802-13. 10.1111/jocd.14137.
3. Ewelina Rostkowska, Ewa Poleszak, Katarzyna Wojciechowska and Katarzyna Dos Santos Szewczyk. Dermatological Management of Aged Skin. Cosmetics 2023, 10, 55.
4. Zihe Shan, David T, Joseph Satriano, Sharon Silbiger, and Detlef Schlondorff. Intracellular glutathione influences collagen generation by mesangial cells. Kidney International, VoL 46 (1994), pp. 388—395.
5. Arthur J.L. Cooper and Bruce S. Kristal. Multiple Roles of Glutathione in the Central Nervous System. Biol. Chem., Vol. 378, pp. 793-802, August 1997.
6. Witoo Dilokthornsakul, Teerapon Dhippayom, Piyameth Dilokthornsakul. The clinical effect of glutathione on skin color and other related skin conditions: A systematic review Cosmet. J Dermatol. 2019; 18:728–737.
7. Lynna J. Yang, Justin Knoll, Roopal V. Kundu. Consumer attitudes toward aging skin during the COVID-19 pandemic. International Journal of Women's Dermatology (2023) 9:e095.
8. Daniela Buonocore, Matteo Grosini, Silvana Giardina, Angela Michelotti, Mariaelena Carrabetta, Antonio Seneci, Manuela Verri, Maurizia Dossena, and Fulvio Marzatico. Bioavailability Study of an Innovative Orobuccal Formulation of Glutathione. Oxidative Medicine and Cellular Longevity 2016; 1-7.
9. Bernard Schmitt, Morgane Vicenzi, Catherine Garrel, Frédéric Denis. Effects of N-acetylcysteine, oral glutathione (GSH) and a novel sublingual form of GSH on oxidatives tress markers: A comparative cross over study. Redox Biology, 2015:6, 198–205.
10. S. Rahrovan,, F. Fanian, P.Mehryan, P. Humbert, A. Firooz. Male versus female skin: What dermatologists and cosmeticians should know. International Journal of Women's Dermatology. 4 (2018) 122–130;

11. S. Luebberding, N. Krueger and M. Kerscher. Mechanical properties of human skin in vivo: a comparative evaluation in 300 men and women Skin Research and Technology 2014; 20: 127–135
12. Volodymyr I. Lushchak. Maintaining GSH homeostasis is of utmost importance for proper cells function. Glutathione Homeostasis and Functions: Potential Targets for Medical Interventions. Journal of Amino Acids Volume 2012, 1-26.
13. P. Diaz Vivancos, T. Wolff, J. Markovic, F. V. Pallard, and C.H. Foyer. A nuclear glutathione cycle within the cell cycle, Biochemical Journal, vol. 431, no. 2, pp. 169–178, 2010.
14. Sonthalia S, Daulatabad D, Sarkar R. Glutathione as a skin whitening agent: Facts, myths, evidence, and controversies. Indian J Dermatol Venereol Leprol. 2016 May-Jun;82(3):262-72.
15. Weschawalit S, Thongthip S, Phutrakool P, et al. Glutathione and its antiaging and antimelanogenic effects. Clin Cosmet Investig Dermatol. 2017; 10: 147‑153.
16. Kandola, K.; Bowman, A.; Birch-Machin, M.A. Oxidative stress—A key emerging impact factor in health, ageing, lifestyle and aesthetics. Int. J. Cosmet. Sci. 2015, 37 (Suppl. 2), 1–8.
17. Reilly, D.M.; Lozano, J. Skin collagen through the lifestages: Importance for skin health and beauty. Plast. Aesthet. Res. 2021, 8, 2.
18. Kyung-A Byun, Seyeon Oh, Sosorburam Batsukh, Min Jeong Kim, Je Hyuk Lee, Hyun Jun Park, Moon Suk Chung, Kuk Hui Son and Kyunghee Byun. The Extracellular Matrix Vitalizer RATM Increased Skin Elasticity by Modulating Mitochondrial Function in Aged Animal Skin. Antioxidants 2023, 12, 694.
19. Watanabe F, Hashizume E, Chan GP, Kamimura A. Skin-whitening and skin-condition-improving effects of topical oxidized glutathione: a double-blind and placebo-controlled clinical trial in healthy women. Clinical, cosmetic and investigational dermatology. 2014;7:267-274.
20. Dickinson DA, Forman HJ. Glutathione in defense and signaling: Lessons from a small thiol. Ann N Y Acad Sci 2002; 973:488-504.
21. Fumiko Watanabe, Erika Hashizume, Gertrude P Chan, Ayako Kamimura. Skin-whitening and skin-condition-improving effects of topical oxidized glutathione: a double-blind and placebo-controlled clinical trial in healthy women. Clinical, Cosmetic and Investigational Dermatology, 2014:7 267–274.
22. Paul Rolan, Stephen Lim, Vivian Sunderland, Yandi Liu & Valeria Molnar. The absolute bioavailability of racemic ketamine from a novel sublingual formulation. Br J Clin Pharmacol. 77:6. 1011–1016 / 1011-1016.
23. Stephen Lim, Stephan Schug, and Janakan Krishnarajah. The Pharmacokinetics and Local Tolerability of a Novel Sublingual Formulation of Buprenorphine. Pain Medicine 2019; 20: 143–152. (23)
24. Jonica Campolo et al. Medium-term effect of sublingual L-glutathione supplementation on flow-mediated dilation in subjects with cardiovascular risk factors. Nutrition 38 (2017) 41–47.
25. Bernard Schmitt, Morgane Vicenzi, Catherine Garrel, Frédéric Denis. Effects of N-acetylcysteine, oral glutathione (GSH) and a novel sublingual form of GSH on oxidative stress markers: A comparative crossover study. Redox Biology 6 (2015) 198–205.