Cancer Treatment Centers of America
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Dr. David Phillips
CEO and Founder
Rebuilder Medical

RE: ReBuilder System®

On behalf of the Oncology Rehabilitation team and the Medical Staff at Midwestern Regional Medical Center, I want to personally thank you for inventing and developing the ReBuilder System, a fabulous medical device to help alleviate the symptoms of Peripheral Neuropathy. In the past, we have used traditional physical therapy electrical stimulation devices such as traditional TENS and Interferential Current (IFC), but the ReBuilder System provided our patients with Chemotherapy Induced Peripheral Neuropathy (CIPN) the best and longest lasting pain relief while undergoing chemotherapy treatment.

From 2005 to 2007 we treated 124 cancer patients with CIPN who were actively undergoing chemotherapy treatment at Midwestern Regional Medical Center of which 40% reported a 30% to 50% reduction in their pain scale, 53% reporting 10% to 20% reduction in their Pain scale, and 3% reporting 50% or more reduction in their pain scale, and only 4% reported no change.

With these remarkable results (96% success rate) the ReBuilder System is now being used across all four CTCA sites in Tulsa, Phoenix, Philadelphia and Chicago – touching and helping more oncology patients relieve their CIPN symptoms. This calendar year alone, between all four sites, we have treated over 300 patients successfully.

We believe in your product's ability to alleviate CIPN symptoms for cancer patients receiving chemotherapy. Patients have reduced or stopped taking pain medicine such as Gabapentin and Lyrica for CIPN.

At CTCA…

"It is only… and always will be…..about the patient." Richard Stephenson, Chairman of the Board at of Cancer Treatment Centers of America

Respectfully Yours,

Stanislav Maravilla, PT
Director of Rehabilitation Services
Cancer Treatment Centers of America @
Midwestern Regional Medical Center

Anodyne Therapy

The Anodyne Therapy System both relieves foot and leg pain and improves sensation. Patients also obtain improved balance and often experience less frequent falls after treatment. These conclusions are based on 11 studies published in medical journals that document the results in more than 4,000 patients who were actually treated with the ***The Anodyne Therapy System.*** These published results are further supported by medical textbooks that specifically reference Anodyne therapy.

Published studies also show that patients can obtain relief from chronic knee and back pain through the use the ***Anodyne Therapy System.***

The body of evidence developed using the ***Anodyne Therapy System*** cannot be generalized to support the expectation of positive patient outcomes of other LED products that do not use a substantially similar wavelength of photoenergy (890nm near infrared), power density, treatment areas and clinical protocols. Indeed, the deviation between differing light based technologies and treatment parameters have lead to conflicting clinical results obtained by patients.

Clinical Studies

Statistical Analysis of outcomes in more than 4,000 patients:

- **Substantial reductions in the impairment of foot sensation**
  - 97% of patients reported improved sensation
  - Average sensory improvement- 65%
- **Significant reductions in foot and leg pain**
  - 92% of patients experienced pain reduction
  - Average pain reduction- 60%
- **Major increases in balance and reduced risk of falls**
  - 90% of patients reported reduced fall risk
  - Average balance improvement-85%
- **Ongoing home use of the Anodyne Therapy System provides lasting benefit measured after an 12 months of use**
  - Improved balance
  - Fewer falls
  - Pain reduction
  - Improved activities of daily living
- **Significant reductions in chronic knee pain**
  - 90% of patients experienced pain reduction
  - Average pain reduction - 53%
- **Significant improvements in lower extremity function**
  - 90% of patients experienced improvement in lower extremity function
  - Average improvement-57%
- **Significant reductions in low back pain compared to placebo**
  Significant reductions in disability with physical activity compared to placebo

Study Titles, Journal Name and Outcome Summaries (Actual studies are available upon request)

J Am Podiatr Med Assoc 92(3): 125-130, 2002

Symptomatic Reversal of Peripheral Neuropathy in Patients with Diabetes

Alan B. Kochman, MSPT
Dale H. Carnegie, DPM
Thomas J. Burke, PhD

Patients obtained significant improvement in tactile foot sensation after receiving 12 **Anodyne** treatments over 4 weeks. The authors further reported medically meaningful increases in temperature discrimination.

24 ENDOCRINE PRACTICE Vol 10 No. 1 January/February 2004

IMPROVEMENT OF SENSORY IMPAIRMENT IN PATIENTS WITH PERIPHERAL NEUROPATHY

J. Joseph Prendergast, MD
Galdina Miranda, MA
Manuel Sanchez, MA

The authors documented medically important improvements in nerve function in patients who received 10 **Anodyne** treatments delivered over 2 weeks.

Diabetes Care 27:168–172, 2004

RESTORATION OF SENSATION, REDUCED PAIN, AND IMPROVED BALANCE IN SUBJECTS WITHDIABETIC PERIPHERAL NEUROPATHY

A double-blind, randomized, placebo-controlled study with monochromatic near-infrared treatment

DAVID R. LEONARD, MD, FACE
M. HAMED FAROOQI, MD, FACE
SARA MYERS, RN

Increased foot sensation, decreased foot and leg pain and improved balance were documented in patients who were exposed to 12 **Anodyne** treatments delivered over 4 weeks.

J. Geriatr Phys Ther. 2004; 27(1):16-19

MONOCHROMATIC INFRARED PHOTO ENERGY AND PHYSICAL THERAPY FOR PERIPHERAL NEUROPATHY: INFLUENCE ON SENSATION, BALANCE AND FALLS

Alan B. Kochman, MSPT

The authors reported improved foot sensation, increased balance and fewer actual falls based on pre and post protocol evaluations. Patients received 12 **Anodyne** treatments followed by physical therapy.

Advances in Skin & Wound Care. 2004;17(6):295-300

REVERSAL OF DIABETIC PERIPHERAL NEUROPATHY AND NEW WOUND INCIDENCE: THE ROLE OF MIRE.

Powell MW
Carnegie DE,
Burke TJ

Patients reported improved foot sensation and reduced incidence of diabetic foot wounds after self-treating with **Anodyne** at home for an average of 1 year.

J Am Podiatr Med Assoc 95(2): 143-147, 2005

Improved Sensitivity in Patients with Peripheral Neuropathy Effects of Monochromatic Infrared Photo Energy

Salvatore L. DeLellis, DPM
Dale H. Carnegie, DPM
Thomas J. Burke, PhD

*Patients who received **Anodyne** treatments experienced dramatic improvements in foot sensitivity.*

Journal of Diabetes and Its Complications 20 (2006) 81– 87

IMPROVED FOOT SENSITIVITY AND PAIN REDUCTION IN PATIENTS WITH PERIPHERAL NEUROPATHY AFTER TREATMENT WITH MONOCHROMATIC INFRARED PHOTO ENERGY—MIRE

Lawrence B. Harkless, DPM
Salvatore DeLellis, DPM
Dale H. Carnegie, DPM
Thomas J. Burke, PhD

*Patients treated with Anodyne therapy obtained more than a 66% improvement after in foot sensitivity. Likewise, foot and leg pain decreased 67% after **Anodyne** treatments.*

Physical & Occupational Therapy in Geriatrics, Vol. 24(2) 2006

EFFECTIVENESS OF MONOCHROMATIC INFRARED PHOTO ENERGY AND PHYSICAL THERAPY FOR PERIPHERAL NEUROPATHY:CHANGES IN SENSATION, PAIN, AND BALANCE – A PRELIMINARY, MULTI-CENTER STUDY

Wendy Volkert, MSPT, Ahmed Hassan, PT, MS, Mohamed A. Hassan, PT, MHS, Vicki L. Smock, PT, Justin P. Connor, PT Becky McFee, PT, Shayne K. Ferguson, PT, MHS, PhD, GCS, CWS Thomas J. Burke, PhD

*After receiving an average of 18 **Anodyne** treatments, patients reported medically significant improvements in foot sensation and balance as well as decreased foot and leg pain.*

Age and Ageing 2006; 35: 11–16

REVERSAL OF DIABETIC PERIPHERAL NEUROPATHY WITH PHOTOTHERAPY (MIRE™) DECREASES FALLS AND THE FEAR OF FALLING AND IMPROVES ACTIVITIES OF DAILY LIVING IN SENIORS.

Mark W. Powell
Dale H. Carnegie
Thomas J. Burke

*After an average of 9 months of home use of the **Anodyne Therapy System**, patients reported reduced pain, decreased fear of falling (and number of falls) and improvements in activities of daily living.*

Practical PAIN MANAGEMENT, July/August 2007

Infrared Photo Energy May Reduce Neuropathic Pain-Near infrared light therapy, together with physical therapy, may be able to reduce pain in neuropathy patients and possibly reduce medication dosage levels of those undergoing drug therapy.

Thomas J. Burke, PhD

The authors reported that patients who received Anodyne treatments obtained significant decreases in foot/leg pain and reduced reliance on oral pain medications used for neuropathic pain.

International Scholarly Research Network ISRN Rehabilitation Volume 2012, Article ID 484307, 8 pages

MONOCHROMATIC INFRARED PHOTO ENERGY IN DIABETIC PERIPHERAL NEUROPATHY

Tarek A. Ammar, PT, PhD

*Patients who received 12 physical therapy sessions that included Anodyne treatments reported significant reductions in pain as well as improvements in foot sensation. These results were meaningfully greater than changes in patients who received identical physical therapy but not receive **Anodyne** treatment.*

Bull. Fac. Ph.Th.Cairo Univ., Vol. 15, No. (2) Jul. 2010

MONOCHROMATIC INFRARED PHOTO ENERGY IN PATIENTS WITH KNEE OSTEOARTHRITIS

Tarek A. Anmar, PT, PhD

*Patients who received 12 physical therapy sessions that included Anodyne treatments reported significant reductions in pain as well as improvements in lower extremity function. These results were meaningfully greater than changes in patients who received identical physical therapy but not receive **Anodyne** treatment.*

J. Lasers Med Sci 2014; 5(4):176-82

MONOCHROMATIC INFRARED PHOTO ENERGY VERSUS LOW LEVEL LASER THERAPY IN PATIENTS WITH KNEE OSTEOARTHRITIS

Tarek Anmar, PT, PhD

Patients who received 12 physical therapy sessions that included Anodyne treatments reported significant reductions in pain as well as improvements in lower extremity function. Both the Anodyne and Laser treated patients experienced substantial pain relief and improvement in lower extremity function but patients treated with Anodyne obtained more pain relief and nearly twice the improvement in lower extremity function.

Lasers Med Sci DOI 10.1007/s10103-013-1378-2

Short-term therapeutic effects of 890-nanometer light therapy for chronic low back pain: a double-blind randomized placebo-controlled study

Ru-Lan Hsieh
Wen-Chung Lee

McKinley-Barnard et al. Journal of the International Society of Sports Nutrition (2015) 12:27
DOI 10.1186/s12970-015-0086-7

jissn
journal of the international society of sports nutrition

RESEARCH Open Access

Combined L-citrulline and glutathione supplementation increases the concentration of markers indicative of nitric oxide synthesis

Sarah McKinley-Barnard[1], Tom Andre[1], Masahiko Morita[2] and Darryn S. Willoughby[1*]

Abstract

Background: Nitric oxide (NO) is endogenously synthesized from L-arginine and L-citrulline. Due to its effects on nitric oxide synthase (NOS), reduced glutathione (GSH) may protect against the oxidative reduction of NO. The present study determined the effectiveness of L-citrulline and/or GSH on markers indicative of NO synthesis in *in vivo* conditions with rodents and humans and also in an *in vitro* condition.

Methods: In phase one, human umbilical vein endothelial cells (HUVECs) were treated with either 0.3 mM L-citrulline, 1 mM GSH (Setria®) or a combination of each at 0.3 mM. In phase two, Sprague–Dawley rats (8 weeks old) were randomly assigned to 3 groups and received either purified water, L-citrulline (500 mg/kg/day), or a combination of L-citrulline (500 mg/kg/day) and GSH (50 mg/kg/day) by oral gavage for 3 days. Blood samples were collected and plasma NOx (nitrite + nitrate) assessed. In phase three, resistance-trained males were randomly assigned to orally ingest either cellulose placebo (2.52 g/day), L-citrulline (2 g/day), GSH (1 g/day), or L-citrulline (2 g/day) + GSH (200 mg/day) for 7 days, and then perform a resistance exercise session involving 3 sets of 10-RM involving the elbow flexors. Venous blood was obtained and used to assess plasma cGMP, nitrite, and NOx.

Results: In phase one, nitrite levels in cells treated with L-citrulline and GSH were significantly greater than control ($p < 0.05$). In phase two, plasma NOx with L-citrulline + GSH was significantly greater than control and L-citrulline ($p < 0.05$). In phase three, plasma cGMP was increased, but not significantly ($p > 0.05$). However, nitrite and NOx for L-citrulline + GSH were significantly greater at 30 min post-exercise when compared to placebo ($p < 0.05$).

Conclusions: Combining L-citrulline with GSH augments increases in nitrite and NOx levels during *in vitro* and *in vivo* conditions.

Keywords: Nitric oxide, L-citrulline, L-arginine, Glutathione, Resistance exercise

Introduction

Also known as endothelium-derived relaxing factor (EDRF), nitric oxide (NO) is biosynthesized endogenously from L-arginine and oxygen, by various nitric oxide synthase (NOS) enzymes and by reduction of inorganic nitrate [1]. Cell types containing NOS have been demonstrated to be able to reutilize L-citrulline, the byproduct of NO synthesis, to L-arginine by the arginine-citrulline cycle [2]. Nitric oxide is a gaseous signaling molecule which activates soluble guanylate cyclase (sGC) in smooth muscle cells, thereby catalyzing cyclic guanosine monophosphate (cGMP) synthesis. Intracellular cGMP serves as a cellular messenger and plays a role in a variety of biological processes, and in human blood vessels, results in vasodilation [3]. Cell types containing NOS have been demonstrated to be able to reutilize L-citrulline, the byproduct of NO synthesis, to L-arginine by the arginine-citrulline cycle [2]. An elevation in plasma L-arginine has been shown to improve endothelial function because the vascular endothelium uses NO to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow [4]. During exercise, vasodilation occurs as a result of various intracellular events, including the production and release of NO. However, it has recently been shown that

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seven days of oral L-arginine supplementation at 12 g/day, while effective in elevating plasma L-arginine and NO metabolites nitrite and nitrate (NOx) after exercise, was ineffective at increasing blood flow during exercise [5].

L-citrulline has been indicated to be a second NO donor in the NOS-dependent pathway, since it can be converted to L-arginine [6]. Dietary L-citrulline supplementation has shown conflicting results regarding its effectiveness at improving exercise performance [7, 8]. Moreover, results showing favorable effects in exercise performance [8] did not assess NO status; therefore, this response cannot be related to an improvement in exercise performance. The importance of L-citrulline towards ergogenic support is based on the premise that L-citrulline is not subject to pre-systemic elimination and, consequently, could be a more efficient way to elevate extracellular levels of L-arginine. L-Citrulline can perhaps improve the effects on nitrate elimination during the course of recovery from exhaustive muscular exercise, and also serves as an effective precursor of L-arginine. It has been shown that three grams daily of oral L-citrulline supplementation for seven days elevated plasma L-arginine concentration and augmented NO-dependent signaling [9].

Glutathione is a low molecular weight, water-soluble tripeptide composed of the amino acids cysteine, glutamic acid, and glycine. Glutathione is an important antioxidant and plays a major role in the detoxification of endogenous metabolic products, including lipid peroxides. Intracellular glutathione exists in both the oxidized disulfide form (GSSG) or in reduced (GSH) state; the ratio between GSH and GSSG is held in dynamic balance depending on many factors including the tissue of interest, intracellular demand for conjugation reactions, intracellular demand for reducing power, and extracellular demand for reducing potential. In some cell types, GSH appears to be necessary for NO synthesis and NO has been shown to be correlated with intracellular GSH [10]. GSH stimulates total L-arginine turnover and, in the presence of GSH, NOS activity is increased [11]. This suggests that GSH may play an important role in protection against oxidative reaction of NO, thus contributing to the sustained release of NO. Therefore, combining L-citrulline with GSH may augment the production of NO. However, the effectiveness for oral GSH supplementation in humans, particularly in combination with L-citrulline has not been clearly delineated.

Using *in vitro* (cell culture) and *in vivo* approaches in rodents and humans, the overall purpose of this study was to determine the efficacy of L-citrulline and/or GSH supplementation towards increasing the levels of cGMP, nitrite, and NOx. We hypothesized that the combination of L-citrulline and GSH would preferentially increase the concentrations of cGMP, nitrite, and NOx levels when compared to control conditions.

Methods and procedures

L-citrulline and GSH (Setria®) used in each phase were obtained from KYOWA HAKKO BIO CO., LTD (Tokyo, Japan).

Phase 1 (*in vitro* efficacy study)

Human umbilical vein endothelial cells (HUVECs) were purchased from Clonetics (San Diego, CA, USA) and cultured in EGM-2 Bullet Kit medium (Clonetics) supplemented with 2 % fetal bovine serum (FBS) and complete endothelial growth factors at 37 ° C in humidified 5 % CO_2. The cells were seeded into twenty-four well plates 5000 cells/cm2, and sub-confluent cell monolayers were used for experiments. A subset of sub-confluent HUVECs were used as controls and the remainder were treated with either 0.3 mM L-citrulline, 1 mM GSH, or a combination of each at 0.3 mM, and incubated for 24 h. To measure nitrite production by HUVECs, the culture medium was collected and centrifuged to remove any precipitated materials. Four wells for each condition were used and nitrite concentrations of supernatants from each well were determined by high performance liquid chromatography (HPLC) (ENO-20; Eicom, Kyoto, Japan) using our previous approach [12].

Phase 2 (rodent efficacy study)

This phase of the study was conducted in accordance with the guidelines for the Institutional Animal Care and Use Committee of KYOWA HAKKO BIO CO., LTD. Twenty-three male Sprague–Dawley rats (8 weeks old; Japan SLC, Hamamatsu, Japan) were given free access to standard rat chow (CE-2, CLEA JAPAN Inc., Tokyo, Japan) and tap water in a room with controlled temperature (22 ± 2 ° C), humidity (55 ± 5 %) and a 12-h light/dark cycle. After the rats had been anesthetized with pentobarbital sodium (30 mg/kg, i.p.), a catheter was inserted into the carotid artery. Following 3 days of acclimation, the rats were randomly assigned to 3 groups and received either purified water (CON) (n = 7), L-citrulline (500 mg/kg/day) (n = 8), or a combination of L-citrulline (500 mg/kg/day) plus GSH (50 mg/kg/day) (n = 8) by oral gavage for 3 days. Blood samples were collected from the catheter at baseline and at 0, 0.25, 0.5, 1, 2, and 4 h after the last administration on Day 3. Plasma NOx (nitrite + nitrate) was measured by HPLC (ENO-20; Eicom, Kyoto, Japan) using our previous approach [12].

Phase 3 (human efficacy study)

Participants

Sixty-six apparently healthy, resistance trained [regular, consistent resistance training (i.e., thrice weekly) for at least one year prior to the onset of the study], males between the ages of 18–30 and a body mass index

between 18.5–30 kg/m^2 volunteered to participate in the double-blind, randomized, placebo-controlled, parallel-groups study. Enrollment was open to men of all ethnicities. During the course of the study, six dropped out due to reasons unrelated to the study. As a result, 60 participants completed the study. The age, height, and body mass of participants in each of the four groups can be seen in Table 1. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) and who had not consumed any nutritional supplements (excluding multi-vitamins) one month prior to the study were allowed to participate. All participants provided written informed consent and were cleared for participation by passing a mandatory medical screening. All eligible subjects signed university-approved informed consent documents and approval was granted by the Baylor University Institutional Review Board for the Protection of Human Subjects in Research. Additionally, all experimental procedures involved in the study conformed to the ethical consideration of the Declaration of Helsinki.

Entry and familiarization session (visit 1)

Individuals expressing interest in participating in the study were interviewed on the telephone and/or e-mail to determine whether they appeared to qualify to participate in the study. Participants believed to meet eligibility criteria were then invited to attend an entry/familiarization session (visit 1). Once reporting to the lab, individuals were familiarized to the study protocol via a verbal and written explanation outlining the study design and signed an informed consent document. At this point, participants completed a medical history questionnaire and underwent a general physical examination to determine whether they met eligibility criteria. Participants also performed a muscle strength test of the elbow flexors (biceps), and were then given an appointment time to report to the laboratory for a baseline blood sample (visit 2). At this time, participants were instructed to refrain from exercise for 48 h and fast for 8 h prior to baseline blood sampling (visit 2) and post-supplementation testing at day 7 (visit 3).

Table 1 Age, height, and body mass of participants in each of the four groups

Group	Age (yrs)	Height (cm)	Body mass (kg)
PLC (n = 15)	21.80 ± 0.92	179.52 ± 2.10	83.92 ± 6.65
GSH (n = 15)	22.67 ± 0.97	179.90 ± 1.71	83.42 ± 2.92
CIT (n = 15)	21.07 ± 0.67	177.17 ± 1.55	80.46 ± 3.17
CIT + GSH (n = 15)	21.67 ± 0.56	179.03 ± 2.34	83.06 ± 2.79

Data are expressed as means ± SEM

Assessment of elbow flexor muscle strength (visit 1)

In order to determine maximum muscular strength of the elbow flexors, participants performed a one-repetition maximum (1-RM) test on the same elbow flexor machine used in the resistance exercise session based on our previous study [5]. Participants warmed up by completing 5 to 10 repetitions at approximately 50 % of the estimated 1-RM. The participant rested for 1 min, and then completed 3 to 5 repetitions at approximately 70 % of the estimated 1-RM. The weight was then increased conservatively, and the participant attempted to lift the weight for one repetition. If the lift was successful, the participant rested for 2 min before attempting the next weight increment. This procedure was continued until the participant failed to complete the lift. The 1-RM was recorded as the maximum weight that the participant was able to lift for one repetition.

Resistance exercise protocol (visit 3, day 7)

Based on our previous study [5], on day 7 participants reported to the Exercise and Biochemical Nutrition Lab at approximately 2:00 pm and performed 3 sets of 15 repetitions with as much weight as they could lift per set (typically 70–75 % of 1RM) involving the elbow flexion exercise on a selectorized weight machine (Body Master, Rayne, LA). Rest periods between sets were timed and lasted exactly 10 s. The resistance exercise session was performed under the direct supervision of study personnel.

Venous blood sampling (visit 2, day 0 and visit 3, day 7)

Venous blood samples were obtained from the antecubital vein into 10 ml serum and plasma collection tubes using a standard vacutainer apparatus. Blood samples were allowed to stand at room temperature for 10 min and then centrifuged. The serum and plasma was removed and frozen at −80 °C for later analysis. One baseline blood sample was obtained at visit 2 and 3 samples were obtained at visit 3 (for a total of 4 blood samples). At visit 3 on day 7, the first sample was obtained immediately before ingesting the supplement, the second sample was obtained immediately after resistance exercise, and the third sample 30 min following exercise (Fig. 1).

Supplementation protocol

In a randomized, double-blind fashion participants were randomly assigned to one of four groups (n = 15 per group) involving 7 days of the oral ingestion of four capsules containing a total daily dose of either: cellulose placebo (2.52 g/day), L-citrulline (2 g/day), GSH (1 g/day), or L-citrulline (2 g/day) + GSH (200 mg/day). The total weight of the four capsules for each group was the same. Each participant ingested all four capsules containing their respective daily supplement dose each evening for six consecutive days. At Visit 3 (Day 7), participants

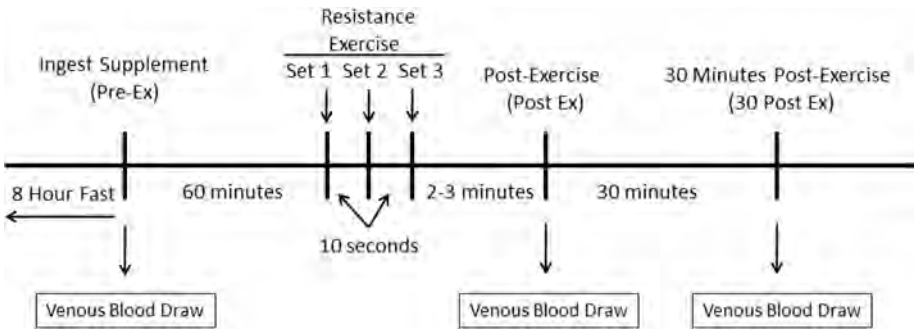

Fig. 1 An illustration of the experimental protocol for the testing session at visit 3, following seven days of L-citrulline and/or GSH supplementation

were provided the final daily dose of their respective supplement ingested one hour prior to performing the resistance exercise. Supplementation compliance was monitored by participants returning empty containers of their supplement on day 7, and also by completing a supplement compliance questionnaire.

Assessment of blood variables (L-citrulline and L-arginine)

To determine plasma L-citrulline and L-arginine concentrations, the plasma was de-proteinated by mixing equal volumes of plasma and trichloroacetic acid (TCA) (6.0 % wt/vol). The samples were vortexed and centrifuged for 15 min at 12,000 × g. Amino acids in the supernatant were analyzed with an amino acid analyzer (L-8900, Hitachi, Japan).

Assessment of plasma cGMP and nitrite

From the blood samples obtained at visit 2 (day 0) and visit 3 (day 7), using commercially-available enzyme-linked immunoabsorbent assay (ELISA) kits (Cayman Chemical, Ann Arbor, MI, USA), plasma cGMP, nitrite, and NOx were determined. Assays were analyzed in duplicate and absorbances for each variable were determined at a wavelength of 450 nm using a microplate reader (iMark, Bio-Rad, Hercules, CA). A set of standards of known concentrations for each variable utilized to construct standard curves and concentrations were determined using data reduction software (Microplate Manager, Bio-Rad, Hercules, CA).

Statistical analysis

For *in vitro* (phase 1), rodent (phase 2), and the human (phase 3) efficacy studies, results were expressed as mean ± SEM. Delta values (differences between the baseline and sequential values) were analyzed using Bonferroni's test following one-way ANOVA. For multiple comparisons to identify the statistical differences among treatments, the Bonferroni correction or Dunnett's multiple test following a comparison of the data by non-repeated ANOVA was employed. Statistical significance was considered as a p-value ≤ 0.05. Statistical analysis was performed using Statcel software for Windows (Version 2, OMS Publishing, Inc., Saitama, Japan) and the Systat 2000 Statistical Program File (Igaku Tosho Shuppan, Tokyo, Japan).

Results

Phase 1 (*in-vitro* cell culture study)

Results demonstrated no significant differences between the control condition and cells treated with L-citrulline and GSH ($p > 0.05$) for nitrite concentration. However, cells treated with L-citrulline and GSH were significantly greater than control-treated cells ($p < 0.05$) (Fig. 2).

Phase 2 (rodent efficacy study)

For plasma NOx delta values, results demonstrated that L-citrulline + GSH was significantly greater than control and L-citrulline at one hr post-supplement infusion ($p < 0.05$) (Fig. 3).

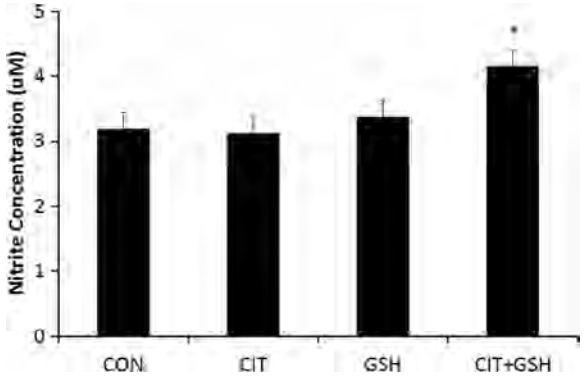

Fig. 2 From phase one, an illustration of nitrite concentration in HUVECs following supplementation with L-citrulline and/or GSH. The symbol * indicates that cells supplemented with a combination of L-citrulline (CIT) and GSH underwent significant increases in nitrite formation compared to cells supplemented with phosphate buffered saline (CON) ($p < 0.05$)

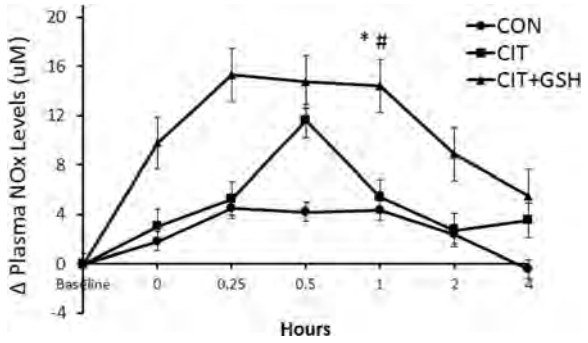

Fig. 3 From phase two, an illustration of plasma NOx levels in rats following supplementation with L-citrulline and/or GSH. The symbols * and # indicate that combined L-citrulline (CIT) and GSH-supplemented rats underwent significant increases in plasma NOx at one hr following supplement infusion compared to animals supplemented with water (CON) and CIT, respectively ($p < 0.05$)

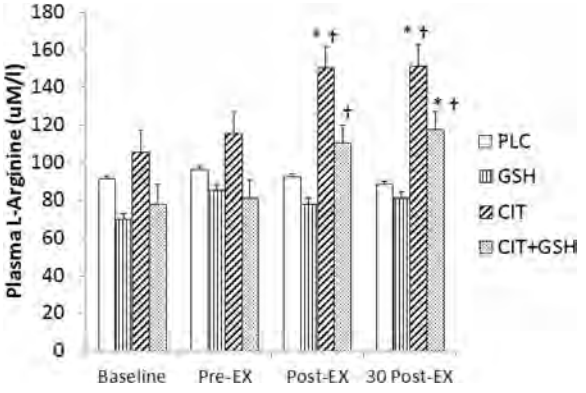

Fig. 4 From phase three, an illustration of plasma arginine levels in humans following seven days of supplementation with L-citrulline and/or GSH. Results indicated that L-citrulline (CIT) and a combination of CIT + GSH produced significant increases in plasma arginine immediately after and 30 min post-exercise compared to groups supplemented with cellulose (PLC) and GSH. The symbol * indicates a significant increase compared to PLC and the symbol † indicates a significant increase compared to GSH ($p < 0.05$)

Phase 3 (human efficacy study)

Plasma L-arginine and L-citrulline

Since no supplementation was involved at the baseline testing session, as expected, no significant differences between groups or time points ($p > 0.05$) for plasma L-citrulline and L-arginine were observed. However, at the follow-up testing session following seven 7 days of supplementation significant increases for plasma L-arginine and L-citrulline were noted. For L-arginine, no significant differences occurred between placebo and GSH at any time points ($p > 0.05$). However, at the immediate post-exercise time point L-citrulline was significantly greater than placebo and GSH, whereas L-citrulline + GSH was greater than GSH ($p < 0.05$). In addition, at 30 min post-exercise L-citrulline and L-citrulline + GSH were both significantly greater than placebo and GSH ($p < 0.05$) (Fig. 4). For plasma L-citrulline, L-citrulline and L-citrulline + GSH were both significantly greater than placebo and GSH immediately post-exercise and at 30 min post-exercise ($p < 0.05$) (Fig. 5).

Plasma cGMP, nitrite, and NOx

The delta values for the plasma levels of cGMP, nitrite, and NOx can be seen in Figs. 6, 7 and 8, respectively. For cGMP (Fig. 6), L-citrulline + GSH was elevated compared to the other three groups, but there were no significant differences between groups and time points observed ($P > 0.05$). For nitrite (Fig. 7) and NOx (Fig. 8), L-citrulline + GSH was significantly greater than placebo at 30 min post-exercise ($P < 0.05$).

Discussion

In the present study, we sought to determine the effectiveness of L-citrulline and/or GSH in increasing NO synthesis during *in vivo* conditions with rodents and humans and also in an *in vitro* condition using HUVEC. Collectively, in phase one and three of the study we observed combining L-citrulline with GSH to be more effective at increasing the concentrations of nitrite and/or NOx than with control/placebo in HUVEC and humans, respectively. In phase two, we observed L-citrulline combined with GSH to be more effective at increasing plasma NOx.

L-citrulline is a ubiquitous amino acid in mammals [13], and in the kidneys, vascular endothelium, and other tissues can be readily converted to L-arginine thus

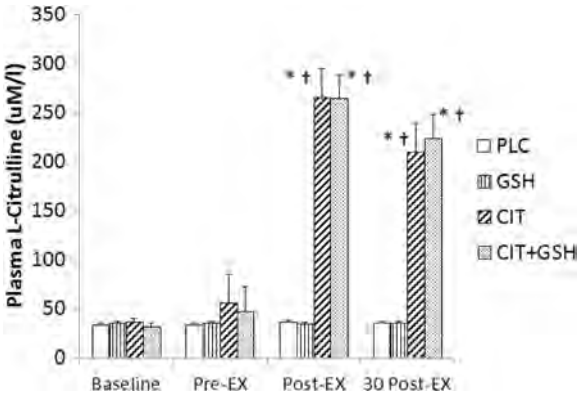

Fig. 5 From phase three, an illustration of plasma citrulline levels in humans following seven days of supplementation with L-citrulline and/or GSH. Results indicated that L-citrulline (CIT) and a combination of CIT + GSH produced significant increases in plasma CIT immediately after and 30 min post-exercise compared to groups supplemented with cellulose (PLC) and GSH. The symbol * indicates a significant increase compared to PLC and the symbol † indicates a significant increase compared to GSH ($p < 0.05$)

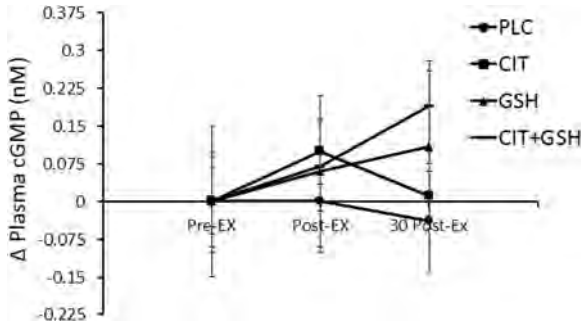

Fig. 6 From phase three, an illustration of plasma cGMP levels in humans following seven days of supplementation with L-citrulline and/or GSH. Results indicated no significant differences between any of the groups at any of the assessed time points ($p > 0.05$)

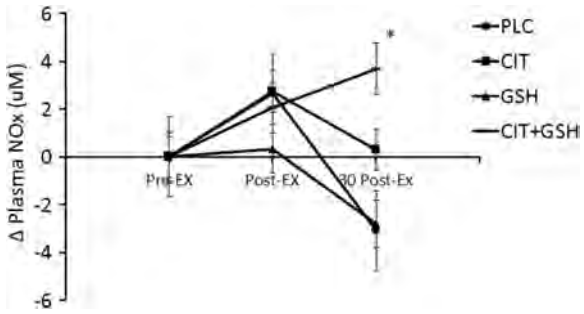

Fig. 8 From phase three, an illustration of plasma NOx levels in humans following seven days of supplementation with L-citrulline and/or GSH. The symbol * indicates that a combination of L-citrulline (CIT) and GSH produced significant increases in plasma NOx at 30 min post-exercise compared to the group supplemented with cellulose placebo (PLC) ($p < 0.05$)

raising plasma and tissue levels of L-arginine which increases NOS synthesis and subsequent NO production [14]. Additionally, L-citrulline has been indicated to be a secondary NO donor in the NOS-dependent pathway, since it can be converted to L-arginine. Nitrate and nitrite are the main substrates to produce NO via the NOS-independent pathway. These anions can be reduced *in vivo* to NO and other bioactive nitrogen oxides.

Previous studies have reported that L-citrulline could increase plasma L-arginine concentration by the L-citrulline-NO cycle [15]. Fu et al. [16] showed that pre-treatment with L-citrulline in rodents for seven days at doses of 300, 600, and 900 mg/kg increased the NO content. Since L-citrulline can be readily converted to L-arginine, it provides a recycling pathway for the conversion of L-citrulline to NO via L-arginine [14, 17]. In phase three of the present study, we observed seven days of L-citrulline supplementation, with and without GSH, to result in significant increases in the levels of plasma citrulline and arginine. Our present data support

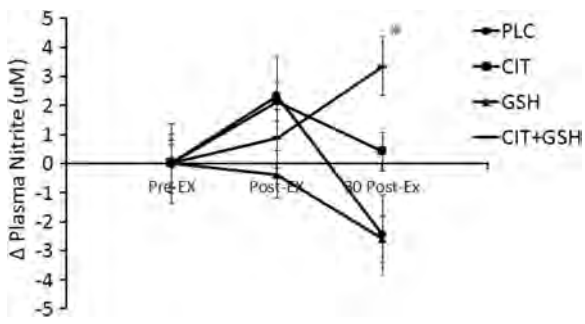

Fig. 7 From phase three, an illustration of plasma nitrite levels in humans following seven days of supplementation with L-citrulline and/or GSH. The symbol * indicates that a combination of L-citrulline (CIT) and GSH produced significant increases in plasma nitrite at 30 min post-exercise compared to the group supplemented with cellulose placebo (PLC) ($p < 0.05$)

previous results [18] showing that a 10-g oral bolus of L-citrulline significantly enhanced plasma citrulline and arginine levels compared with placebo. Therefore, our present observations indicate that L-citrulline is indeed a precursor to L-arginine formation which subsequently increases circulating levels of NOx, and that recycling of L-citrulline to L-arginine may maintain substrate concentration in favor of NO synthesis [19].

It has been shown in some mammalian cell types, that GSH and NO activity are linked [20]. Furthermore, results suggest that GSH is necessary in HUVEC for NO synthesis rather than for the NO-related effect on guanylate cyclase, because when cells were depleted of GSH, citrulline synthesis and cGMP production were inhibited in a concentration-dependent manner [21]. This may be explained based on the premise that the synthesis of NO, detected as L-citrulline production, in HUVEC and murine endothelial cells has been shown to be correlated with intracellular GSH [10]. A previous study suggested that in some cell types, the activity of NO is influenced by the endogenous antioxidant GSH [22]. It is conceivable that GSH activity may be augmented by L-citrulline as it has been shown that pre-treatment with L-citrulline in rodents for seven consecutive days lead to an elevation in the level of GSH [23].

Furthermore, in phase one of the present study, we showed that combining L-citrulline and GSH effectively increased nitrite concentration in HUVEC cells compared to control; although, both L-citrulline and GSH alone had no effect on nitrite. However, in phase two, the combined L-citrulline and GSH provided to rodents resulted in a significant increase in plasma NOx one hr following ingestion compared to control and L-citrulline. Moreover, we observed a similar response in phase three compared to phase one, where combining L-citrulline and GSH effectively increased plasma nitrite and NOx concentration in humans compared to placebo.

Oral supplementation with L-arginine can increase plasma L-arginine levels; although, oral supplementation with L-citrulline, a precursor for arginine biosynthesis, has been shown to be more efficient than oral L-arginine in increasing plasma L-arginine [9], due to splanchnic catabolism of ingested L-Arginine [24]. NO synthesis is primarily dependent upon intracellular arginine availability and is affected by: 1) the transport of extracellular arginine; 2) intracellular synthesis of arginine from citrulline, which is dependent on citrulline availability; and 3) the activity of arginase [17]. Moreover, this latter point can be further supported based on the data demonstrating increased arginine availability in cultured cell model or by supplementation *in vivo* was able to overcome the effects of arginase and to enhance NO synthesis [25]. Based on results from all three phases of the present study, it is evident that L-citrulline supplementation impacted extracellular arginine concentration and the subsequent intracellular arginine synthesis based on the responses we observed in nitrite and NOx concentrations.

In phase 3 of the present study, we were also interested to determine if increased plasma arginine availability and subsequent NO synthesis due to oral L-citrulline and/or GSH supplementation was effected by resistance exercise. Interestingly, we observed increases in plasma NOx in all four groups immediately following resistance exercise, which indicates this response in plasma NOx to be particularly due to the stimulus of resistance exercise. These results are similar to our previous study where resistance exercise increased plasma NOx, independent of increased plasma arginine, due to seven days of L-arginine supplementation [5]. In the same way as NOx, plasma cGMP levels were increased by the combination of L-citrulline and GSH; however, this increase was not significantly different. Nevertheless, this suggests a possible synergistic effect from GSH that may be partially mediated by the formation of the NO-GSH complex. However, in the present study, significantly different increases in NOx occurred 30 min following resistance exercise, and only for the L-citrulline + GSH group. This suggests that a resistance exercise-related mechanism of inducing plasma NO, perhaps due to increased shear stress that triggered an up-regulation in NO-cGMP signaling, is a conceivable candidate for this response.

Consequently, there are possible physiological benefits of having high NO levels at 30 min post-exercise relative to its impact on muscle protein metabolism and possible muscle performance in response to resistance exercise training. It has been shown that NOS activity is necessary for calcium-induced activation of the Akt pathway (involved in translation initiation and thus muscle protein synthesis), and that NO is sufficient to elevate Akt activity in primary myotubes. Nitric oxide appears to influence Akt signaling though a cGMP/PI3K-dependent pathway [26], which is the primary pathway for up-regulating translation initiation and protein synthesis in skeletal muscle. Additionally, nitrite has been shown to enhance the proliferation and mTOR activity of myoblasts [27]. Similarly, NO seems to influence skeletal muscle function through effects on excitation-contraction coupling, myofibrillar function, perfusion, and metabolism. Another study showed that by using an agent to inhibit phosphodiesterase-5, that the augmentation of NO-cGMP signaling increased protein synthesis and reduced fatigue in human skeletal muscle [28]. In the present study, L-citrulline + GSH showed an improvement in cGMP activity suggesting that this outcome could likely play a role in muscle protein synthesis and muscle performance when combined with resistance training.

Our present data suggest that the oral supplementation of L-citrulline combined with GSH provides an augmenting effect on plasma NOx. Based on results from recent studies, this may be explained based on the premise that in some cell types, the activity of NO is influenced by the endogenous antioxidant, GSH [10]. Therefore, GSH may play an important role in protection against oxidative reaction of NO, thus contributing to the sustained release of NO.

Conclusions

Herein, we have presented *in vitro* and *in vivo* data demonstrating the efficacy of combining L-citrulline and GSH and the subsequent effects on NO synthesis and, collectively, we conclude that the combination of L-citrulline and GSH increases the levels of cGMP, nitrite, and NOx.

Competing interests

Masahiko Morita is an employee of KYOWA HAKKO BIO CO., LTD. The other co-authors declare no conflicts of interest.

Authors' contributions

SM served as the study coordinator and was involved in participant recruitment, testing, laboratory analyses, and assisted in manuscript preparation. TA was involved in testing and laboratory analyses. MM was involved in conducting the phase 1 and 2 portions of this study, in performing the plasma L-citrulline and L-arginine analyses in phase 3, and was involved in manuscript preparation. DSW was the principal investigator and, was responsible for securing grant funding and developing the experimental design. He was also involved in training and mentoring for laboratory analyses, provided primary oversight during the course of the study, and supervised manuscript preparation. All authors read and approved the final manuscript.

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White Paper:
L-Citrulline

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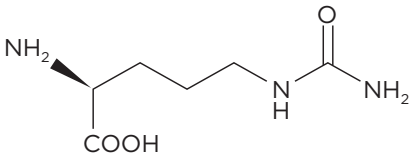
L-Citrulline is a non-essential amino acid that previously was thought of as just an intermediate metabolite. However, over the past decade research on L-Citrulline has dramatically increased due to the understanding and importance of L-Citrulline's metabolism. L-Citrulline is converted to L-Arginine in the body, leading to increases in both L-Arginine and nitric oxide. Increased production of nitric oxide (NO) promotes vascular dilation, which helps support normal oxygen and blood circulation throughout the body.[3] Since L-Citrulline is not a component of proteins unlike most other amino acids, dietary proteins cannot be a direct source of L-Citrulline to the body. As a result, interest in this nutrient is emerging in various applications for improving health and wellness. In this paper, we will specifically discuss L-Citrulline's role in nitric oxide production, vascular health, muscle protein synthesis, ammonia elimination, and immune function.

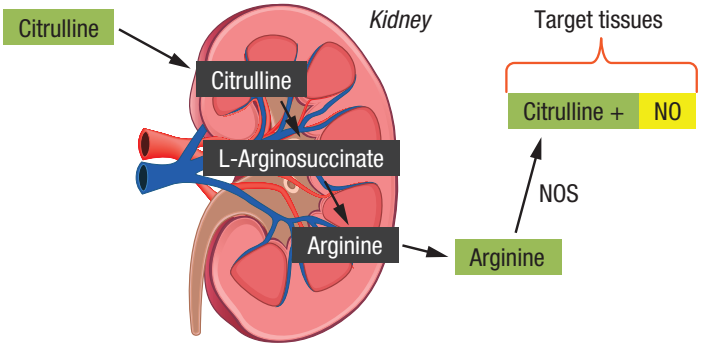
Properties of L-Citrulline

Table 1. Physical properties of L-Citrulline[11]

Synonym	L-2-amino-5-ureidovaleric acid
CAS No.	372-75-8
Structural formula	
Molecular formula	$C_6H_{13}N_3O_3$
MW	175.19
Solubility	Freely soluble in water (200 g/L)
Hygroscopicity	None (0.02% at 75% relative humidity for 7 days)
Taste	Slightly bitter taste
Odor	Unique slight odor
Appearance	White powder

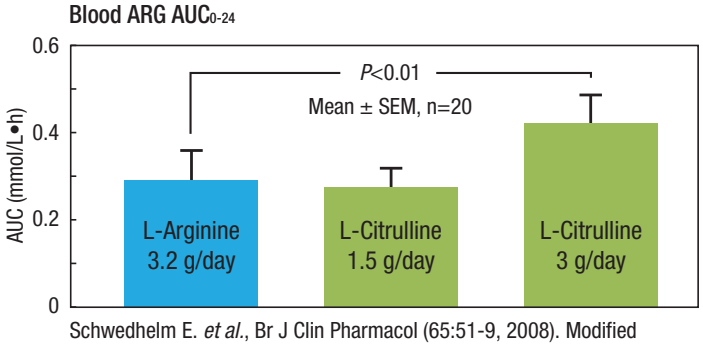
Biosynthesis, metabolism and pharmacokinetics of L-Citrulline

L-Citrulline is made from L-Ornithine and carbamoyl phosphate, is a component of the urea cycle in the liver. L-Citrulline is also synthesized from L-Arginine and L-Glutamine in enterocytes. The majority of L-Citrulline is converted to L-Arginine in the kidney.[3] **Once in circulation, L-Arginine is readily converted into L-Citrulline and nitric oxide, which in turn serves as an L-Arginine precursor (see figure below).**[10]



Pharmacokinetic advantages

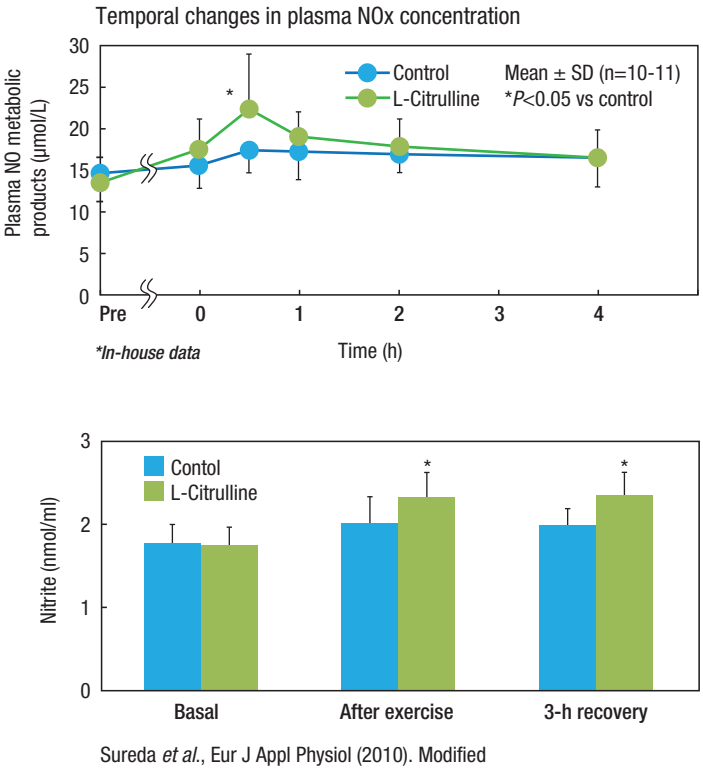
The pharmacokinetic advantage of L-Citrulline is that it is not subject to any elimination by the liver prior to entering the bloodstream. Conversely, L-Arginine is subject to extensive elimination by the gut wall and liver.[26] The main organ responsible for L-Citrulline conversion is the kidney. In fact, it is metabolized solely by the kidneys into L-Arginine. The kidneys will release L-Arginine in response to the uptake of L-Citrulline. Thus, the release of L-Arginine is a factor of the level of L-Citrulline in the blood. Oswoska et al (2004) showed that oral L-Citrulline administration was more potent at raising plasma L-Arginine levels in rats with a massive intestinal resection and was able to restore nitrogen balance over that of L-Arginine supplementation. **Schwedhelm et al (2008) confirmed in humans that oral supplementation of L-Citrulline was able to raise plasma levels of L-Arginine more effectively than L-Arginine itself and in a dose-dependent manner.**



Schwedhelm E. *et al.*, Br J Clin Pharmacol (65:51-9, 2008). Modified

Nitric oxide effects

As the natural precursor of L-Arginine, L-Citrulline plays an important role in the metabolism and regulation of nitric oxide. Nitric oxide is synthesized from L-Arginine as mentioned previously. An in-house study demonstrated that L-Citrulline oral administration as compared to a control was able to effectively increase nitric oxide in SD rats.[11]



Sureda *et al.*, Eur J Appl Physiol (2010). Modified

In a human study, male cyclists were supplemented with L-Citrulline vs. placebo and NO production was measured as nitrite plasma concentration. Nitrates in the plasma were significantly increased after the cycling stage in the supplemented group and maintained high during recovery.[25] **A more recent study by Ochiai et al., confirmed that oral L-Citrulline significantly increased serum nitric oxide and NO metabolic products as compared to placebo. Altogether, this evidence helps to confirm that L-Citrulline plays a critical role in NO production.**

Ammonia detoxification

L-Citrulline is involved in ammonia detoxification as an L-Ornithine cycle amino acid. L-Citrulline, along with L-Arginine and L-Ornithine, has various functions as an amino acid in the L-Ornithine cycle. The effect of supplemental L-Citrulline on young animals fed arginine-deficient diets is similar to that of supplemental L-Arginine. In rat experiments, L-Citrulline was shown not only to promote the metabolism of ammonia accumulated in muscle during exercise, but also to elevate the survival rate of rats intraperitoneally injected with a lethal dose of ammonia.[18]

Effects on muscle protein metabolism

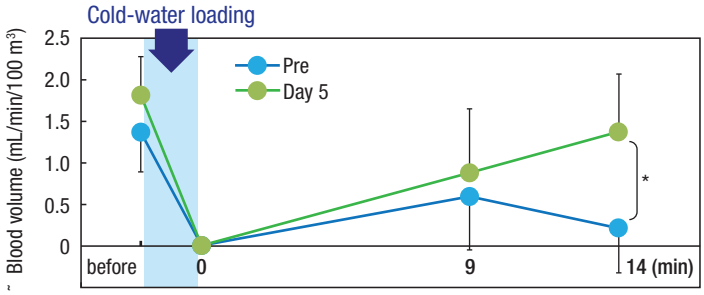
A series of studies have proven that L-Citrulline can increase muscle protein content and protein synthesis in animal models. Osowska et. al., found in malnourished aged rats that were fed a L-Citrulline-supplemented diet, there was an increase in protein synthesis and protein content in the muscle as compared to the standard diet fed rats. It was also found that L-Citrulline improved nitrogen balance in rats with short bowel syndrome. In addition, L-Citrulline stimulates muscle protein synthesis in fasted adult rats.[13] Most recently, Faure et. al., found that L-Citrulline supplementation increases expression of the main myofibrillar proteins and seems to induce a switch in muscle energy metabolism, from aerobia toward anaerobia.

It has been confirmed in a human clinical study that L-Citrulline was effective at stimulating muscle protein synthesis without affecting whole body protein turn over.[9] Further studies in humans are warranted.

Vascular health

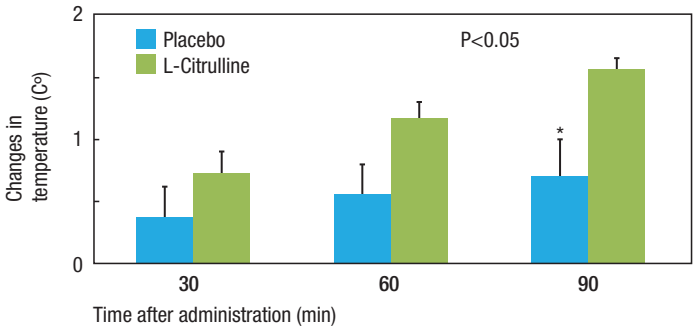
Because L-Citrulline is an effective precursor of L-Arginine for NO synthesis, it has been implicated to help support vascular health. More recently, many studies have been published exploring its use in various vascular disorders. It has not yet been found effective at controlling blood pressure. More studies are necessary in this area. However, Ochiai et al., observed the effects of L-Citrulline on aterial stiffness. Brachial-ankle pulse wave velocity (baPWV) was measured in 15 healthy male subjects. Compared to the placebo group, baPWV was significantly reduced in the L-Citrulline group. The serum nitrogen oxide and NO metabolic products were significantly increased in the L-Citrulline group as well. Most recently, Morita et. al. observed 21 subjects with diagnosed vasospastic angina. They were treated with 800 mg/day of L-Citrulline for 8 weeks. Flow-mediated dilation (FMD) of the brachial artery and oxidized LDL was measured. Compared with baseline values, FMD percent was significantly increased at 4 and 8 weeks as well as 4 weeks supplementation ended. At the same time, a reduction in serum oxidized LDL was reported. It was concluded that L-Citrulline supplementation improves endothelial dysfunction, probably due to potentiating NO-dependent reactions and decreasing the state of lipoprotein oxidation in humans.

In an in-house human clinical study, L-Citrulline was administered to women with poor blood flow. Peripheral blood flow was monitored using the back of the hands using laser imaging. **Compared to placebo, there was an improvement of peripheral blood flow after cold water emersion in the L-Citrulline group (see chart below).** Another in-house study observed, L-Citrulline was administered to healthy adults and body surface temperature was measured using thermography. The study showed that L-Citrulline was effective in raising body surface temperature at the neck, shoulders, and right palm.



**In-house data* Mean ± SE (n=14), **P*<0.05 vs Pre

Changes in surface temperature of neck/shoulder/palm area after L-Citrulline administration



Another area of interest is for supporting normal erectile function. A most recent pre-clinical study, Shiota et. al. found that oral L-Citrulline supplementation improves erectile function in rats with acute arteriogenic erectile dysfunction. Cormeo et al. explored the role of L-Citrulline in humans for supporting normal erectile function. Compared to placebo, the L-Citrulline group reported mild improvement in erection hardness and an increase in sexual activity.

Immune function

L-Citrulline may be a safe means of immunomodulation that preserves the anti-inflammatory mediator response. Asgeirsson et al., observed the efficacy of L-Citrulline supplementation on systemic response mediators and cytokines in Wistar rats induced with sepsis. **They concluded that L-Citrulline may decrease the proinflammatory response (IL-6 and resistin) without impairing the secretion of anti-inflammatory mediators (IL-10 and adiponectin).**

Watermelon studies

Watermelon is known to have high concentrations of L-Citrulline and consuming watermelon is known to elevate both plasma L-Citrulline and L-Arginine.[7] Watermelon extract displays similar effects as L-Citrulline on vascular health. Figueroa et. al. observed significant reductions in ankle and brachial blood pressure in middle aged adults supplemented with watermelon extract. **A more recent study demonstrated that watermelon juice containing 1.17 g of L-Citrulline was capable of reducing the recovery heart rate and muscle soreness after 24 hours in athletes as compared to placebo.**[27]

Recommended intake of L-Citrulline

L-Citrulline dosing varies greatly among in the literature and depends on the intended use. The general range is 1-6 grams per day. A reasonable median is 2-3 grams depending on the intended use. More specific doses will be found in the studies pertaining to certain physiological effects.

Safety information about L-Citrulline

Because it occurs naturally in the living bodies of animals and humans where it plays an important role, L-Citrulline is regarded as a highly safe ingredient. Studies in humans have demonstrated that L-Citrulline is substantially innocuous. In an experiment in patients with lysinuric protein intolerance, 19 subjects were given 2.0-2.2 g (depending on body weight) of L-Citrulline each day for two years. Blood hemoglobin values, and plasma albumin and L-valine concentrations were increased, suggesting an improvement in protein turnover. No side effects were identified.[21]

An acute toxicity test in mice indicated the LD_{50} value for oral administration of L-Citrulline is 5,000 mg/kg (B.W.) or more.[11]

Conclusion

L-Citrulline is a substance present in the body and is used as a highly safe ingredient. Recent research has helped in understanding the importance of L-Citrulline's metabolism and role in the body. As mentioned throughout this paper, L-Citrulline is converted to L-Arginine in the body, enhancing the production of nitrogen monoxide (NO). Additionally, current research is helping our understanding of its positive role in vascular health, muscle protein synthesis, ammonia elimination, and immune function. There is still more to learn and discover about this important amino acid. Kyowa Hakko U.S.A., Inc. provides pure, high-quality L-Citrulline.

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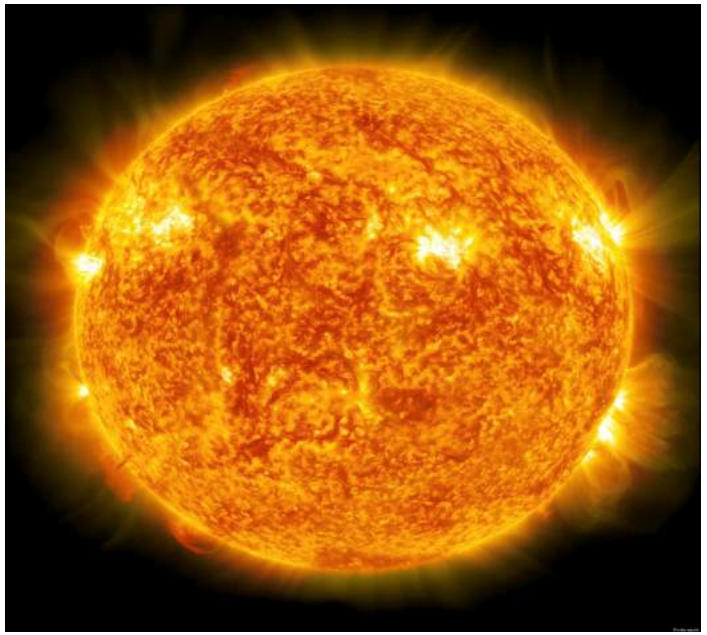
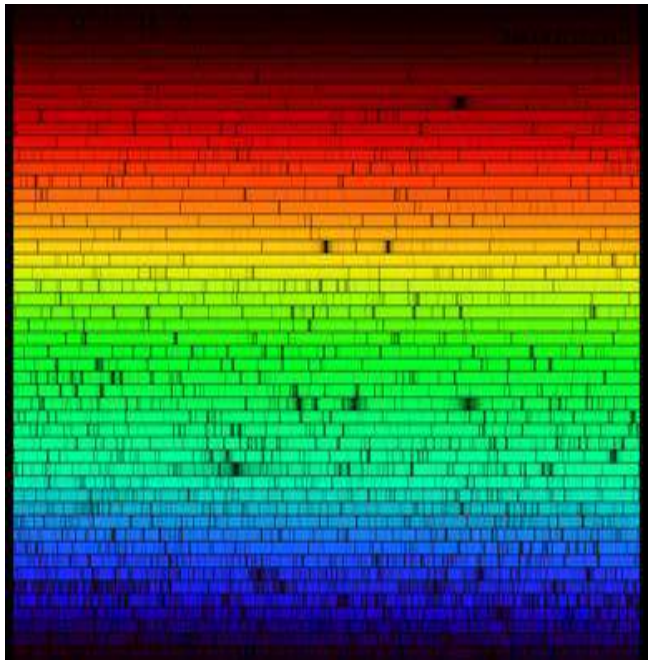
Synergistic Effects of Light Therapy and Nutrition

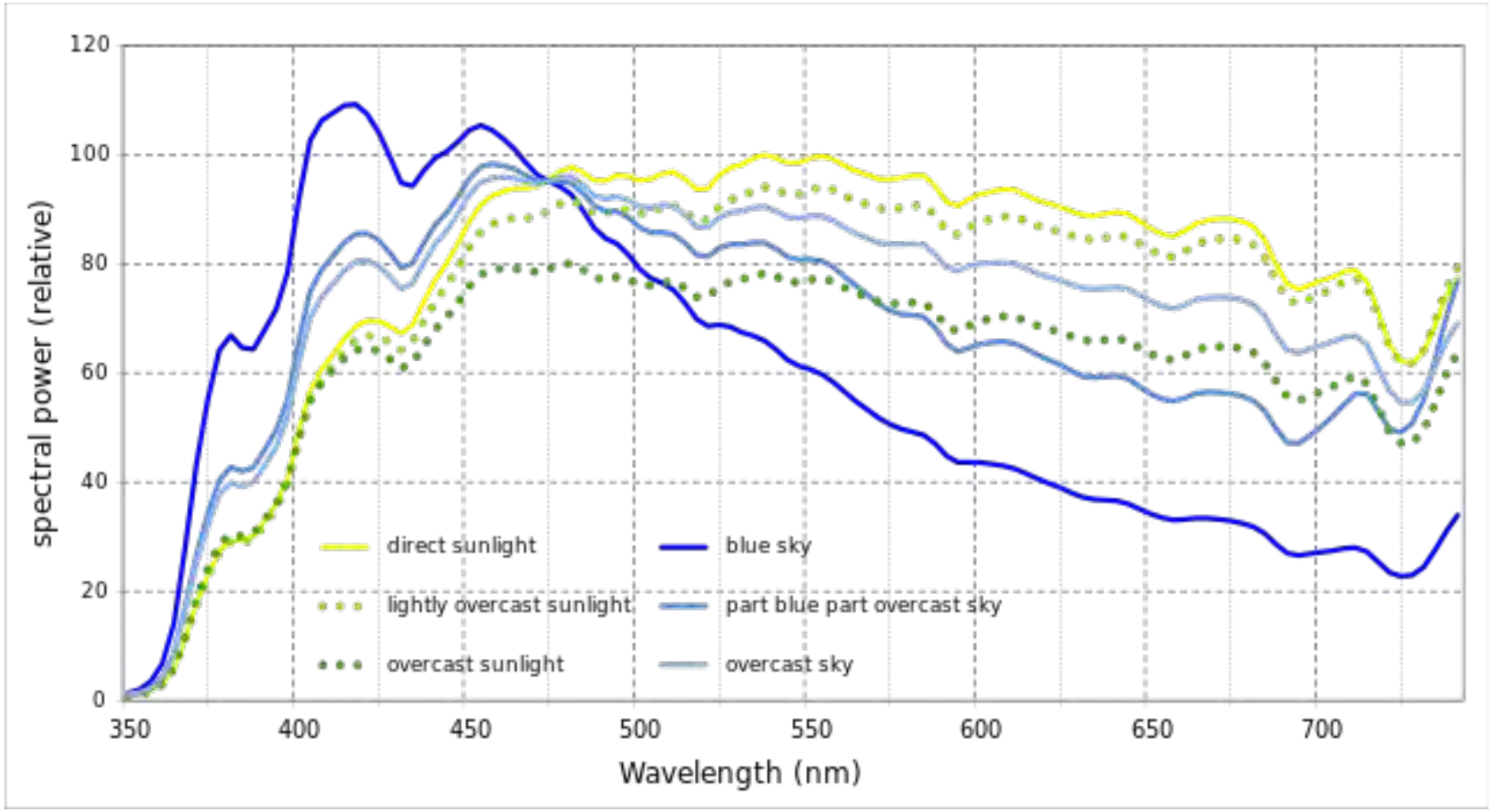
Kyl Smith, DC

Sunlight is a portion of the electromagnetic radiation given off by the *Sun*





Synergistic Effects of Light Therapy and Nutrition



Yellow line = the spectrum of direct illumination under optimal conditions

Synergistic Effects of Light Therapy and Nutrition

- **Although cells in vitro are responsive to a variety of wavelengths in the electromagnetic spectrum, *beneficial responses in vivo are observed within a narrow wavelength range*.**
- Lower wavelengths such as violet and ultraviolet penetrate less, whereas ***those in the red and infrared range have higher penetration*.**
- Energy at wavelengths shorter than 600nm are generally scattered in biological tissues in vivo and are absorbed by melanin, whereas water significantly absorbs energy at wavelengths higher than 1150nm.
- **For clinical purposes = *the in vivo therapeutic "optical window" strongly corresponds to red and near-infrared wavelengths*.**

Synergistic Effects of Light Therapy and Nutrition

Low-Level-Laser (Light) Therapy (LLLT) involves exposing cells or tissue to low levels of red and near infrared (NIR) light, and is referred to as "low level" because of its use of light at energy densities that are low compared to other forms of laser therapy that are used for ablation, cutting, and thermally coagulating tissue.

LLLT is also known as "cold laser" therapy as the power densities used are lower than those needed to produce heating of tissue. It was originally believed that LLLT or **photobiomodulation** required the use of coherent laser light, but more recently, **light emitting diodes (LEDs)** have been proposed as a cheaper alternative.

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The Nuts and Bolts of Low-level Laser (Light) Therapy

Hoon Chung,[1,2] Tianhong Dai,[1,2] Sulbha K. Sharma,[1] Ying-Ying Huang,[1,2,3] James D. Carroll,[4] and Michael R. Hamblin[1,2,5]

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Abstract

Soon after the discovery of lasers in the 1960s it was realized that laser therapy had the potential to improve wound healing and reduce pain, inflammation and swelling. In recent years the field sometimes known as photobiomodulation has broadened to include light-emitting diodes and other light sources, and the range of wavelengths used now includes many in the red and near infrared. The term "low level laser therapy" or LLLT has become widely recognized and implies the existence of the biphasic dose response or the Arndt-Schulz curve. This review will cover the mechanisms of action of LLLT at a cellular and at a tissular level and will summarize the various light sources and principles of dosimetry that are employed in clinical practice. The range of diseases, injuries, and conditions that can be benefited by LLLT will be summarized with an emphasis on those that have reported randomized controlled clinical trials. Serious life-threatening diseases such as stroke, heart attack, spinal cord injury, and traumatic brain injury may soon be amenable to LLLT therapy.

Keywords: Low level laser therapy, Photobiomodulation, Mitochondria, Tissue optics, Wound healing,

Synergistic Effects of Light Therapy and Nutrition

Light Therapy has now developed into a therapeutic procedure that is science-based, well-substantiated, and utilized in three main ways:

1. ***to reduce inflammation, edema, and chronic joint disorders;***
2. ***to promote healing of wounds, deeper tissues, and nerves;***
3. ***and to treat neurological disorders and pain.***

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The Nuts and Bolts of Low-level Laser (Light) Therapy

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Soon after the discovery of lasers in the 1960s it was realized that laser therapy had the potential to improve wound healing and reduce pain, inflammation and swelling. In recent years the field sometimes known as photobiomodulation has broadened to include light-emitting diodes and other light sources, and the range of wavelengths used now includes many in the red and near infrared. The term "low level laser therapy" or LLLT has become widely recognized and implies the existence of the biphasic dose response or the Arndt-Schulz curve. This review will cover the mechanisms of action of LLLT at a cellular and at a tissular level and will summarize the various light sources and principles of dosimetry that are employed in clinical practice. The range of diseases, injuries, and conditions that can be benefited by LLLT will be summarized with an emphasis on those that have reported randomized controlled clinical trials. Serious life-threatening diseases such as stroke, heart attack, spinal cord injury, and traumatic brain injury may soon be amenable to LLLT therapy.

Keywords: Low level laser therapy, Photobiomodulation, Mitochondria, Tissue optics, Wound healing,

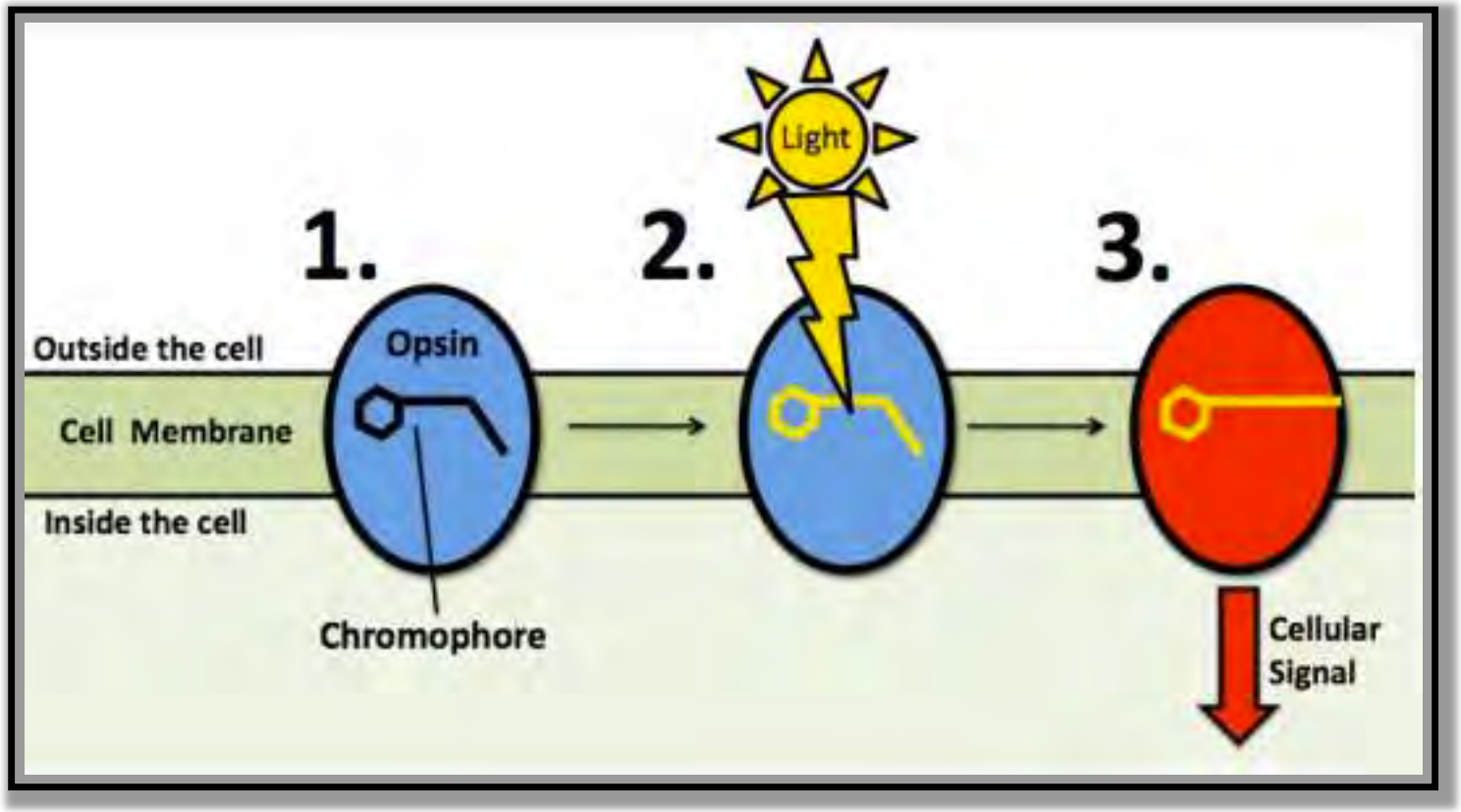
Synergistic Effects of Light Therapy and Nutrition

Photochemical reaction = Biostimulation = Photobiomodulation

A **chromophore** is the part of a molecule responsible for its color.[1]

The color arises when a molecule absorbs certain wavelengths of visible light and transmits or reflects others.

Visible light that hits the chromophore can thus be absorbed by exciting an electron from its ground state into an excited state.



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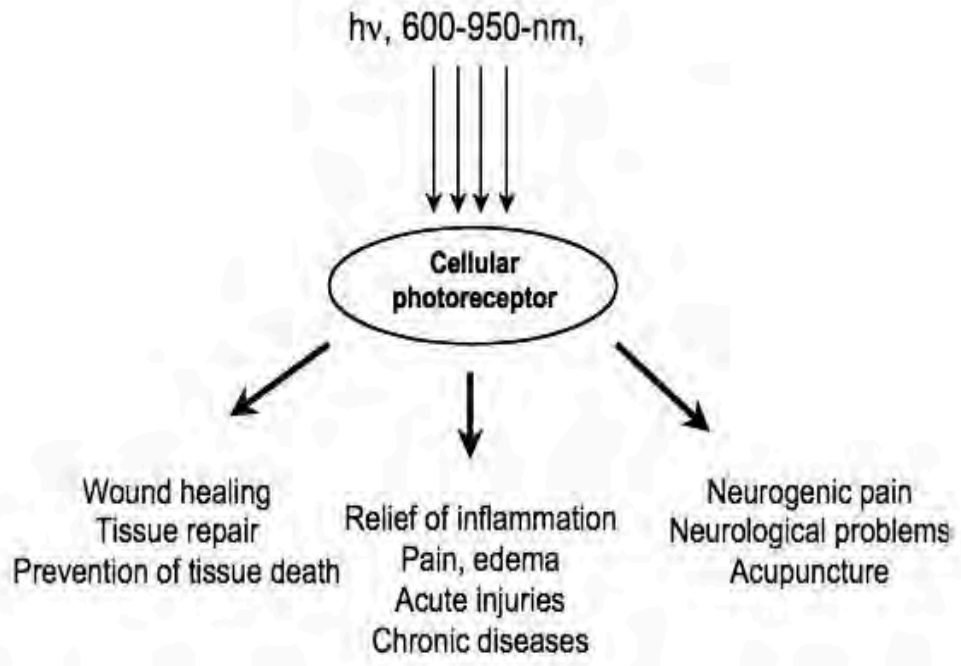
Chromophore within Mitochondria = Initial Target of LLLT



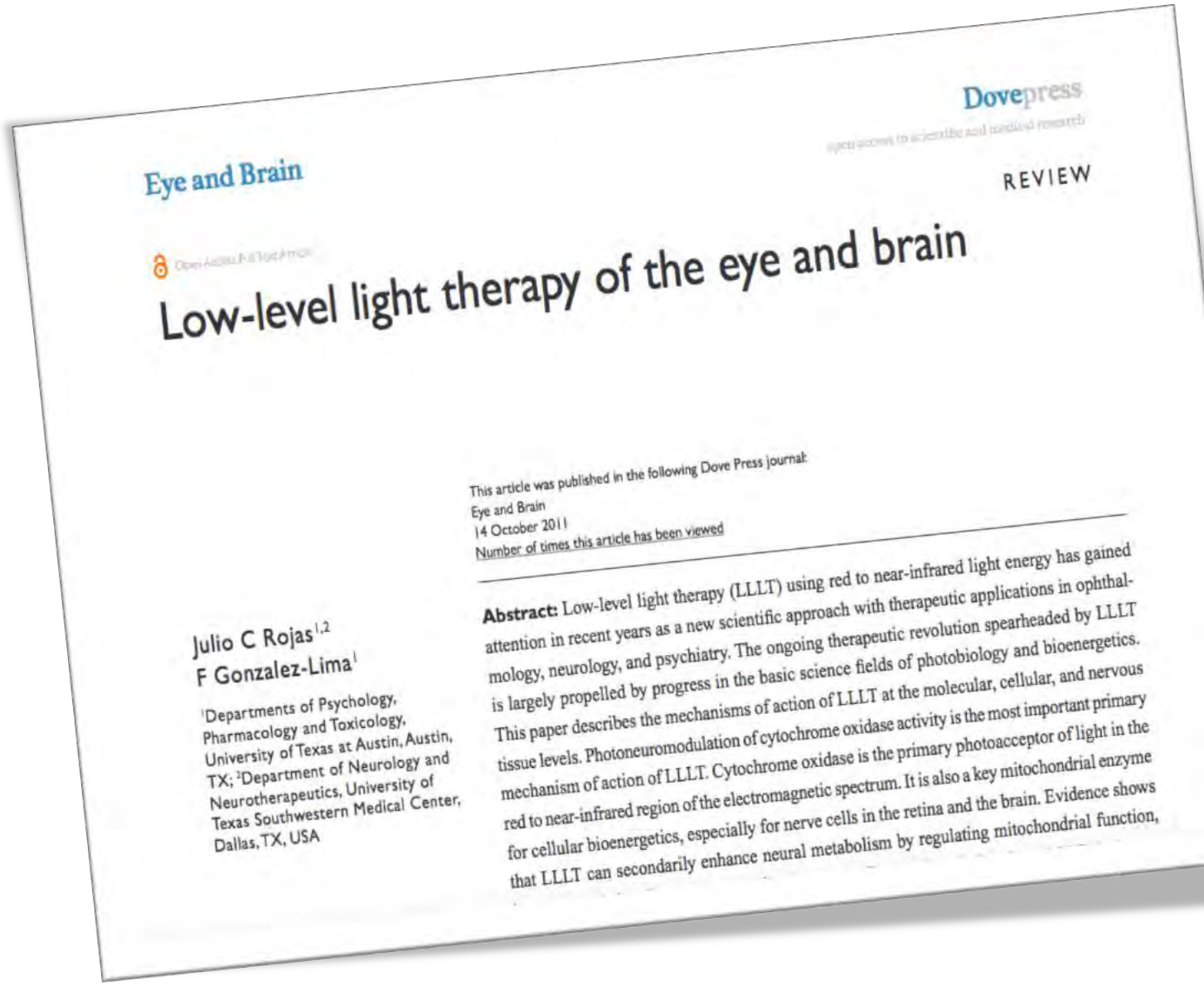
Mitochondria are stimulated, leading to increased ATP production, modulation of reactive oxygen species, and induction of transcription factors.

Patient Benefits Include:

- **Increased healing of chronic wounds**
- **Improvements in sports injuries and carpal tunnel syndrome**
- **Pain reduction in arthritis and neuropathies**
- **Amelioration of damage after heart attacks, stroke, and nerve injury**

Synergistic Effects of Light Therapy and Nutrition

"Photoneuromodulation of cytochrome oxidase activity is the most important primary mechanism of action of LLLT. Cytochrome oxidase is the primary photoacceptor of light in the red to near-infrared region of the electromagnetic spectrum. It is also a key mitochondrial enzyme for cellular bioenergetics, especially for nerve cells in the retina and the brain. Evidence shows that LLLT can secondarily enhance neural metabolism by regulating mitochondrial function, intraneuronal signaling systems, and redox states."



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Eye and Brain

REVIEW

Low-level light therapy of the eye and brain

This article was published in the following Dove Press journal:
Eye and Brain
14 October 2011
Number of times this article has been viewed

Julio C Rojas[1,2]
F Gonzalez-Lima[1]

[1]Departments of Psychology, Pharmacology and Toxicology, University of Texas at Austin, Austin, TX; [2]Department of Neurology and Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, TX, USA

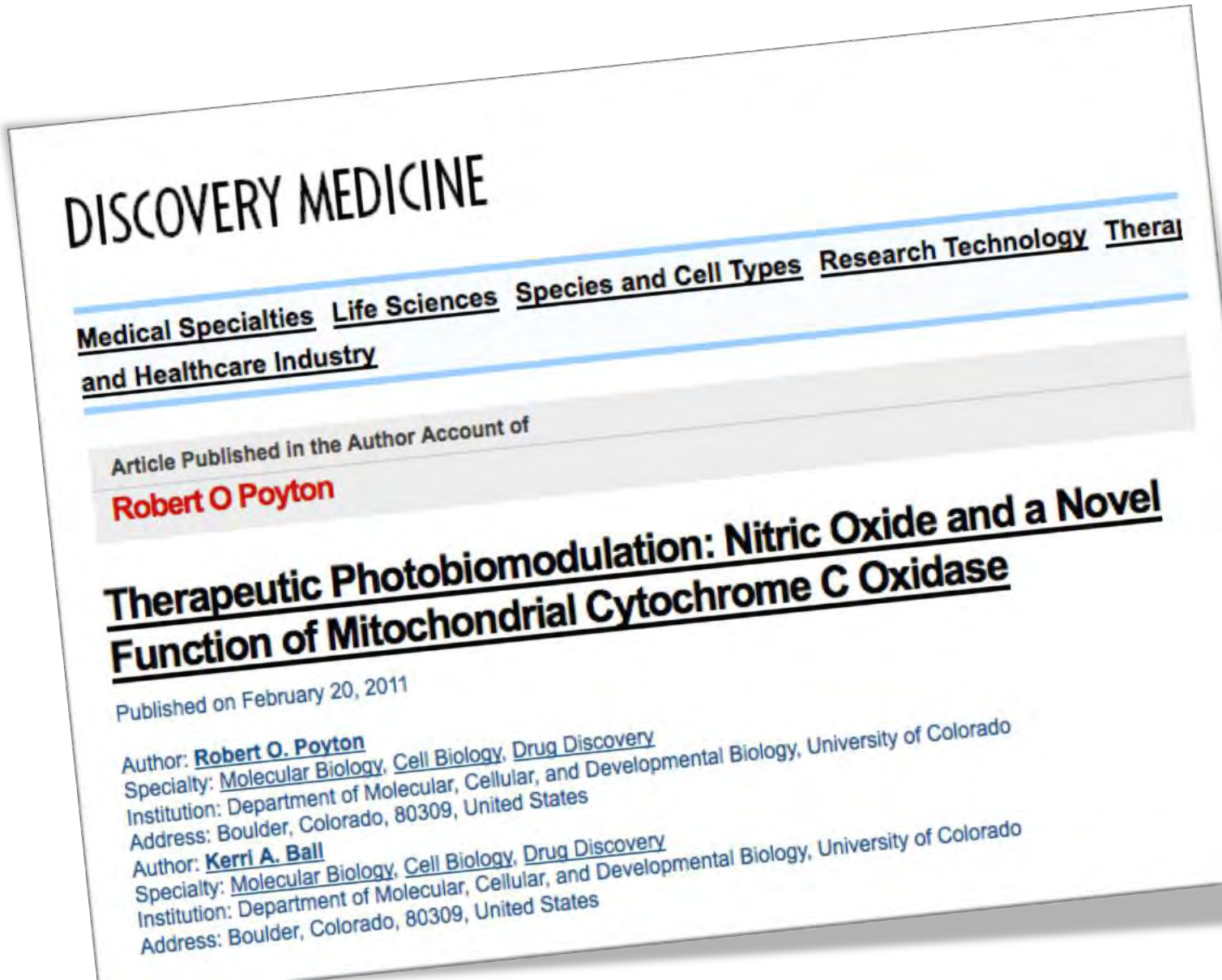
Abstract: Low-level light therapy (LLLT) using red to near-infrared light energy has gained attention in recent years as a new scientific approach with therapeutic applications in ophthalmology, neurology, and psychiatry. The ongoing therapeutic revolution spearheaded by LLLT is largely propelled by progress in the basic science fields of photobiology and bioenergetics. This paper describes the mechanisms of action of LLLT at the molecular, cellular, and nervous tissue levels. Photoneuromodulation of cytochrome oxidase activity is the most important primary mechanism of action of LLLT. Cytochrome oxidase is the primary photoacceptor of light in the red to near-infrared region of the electromagnetic spectrum. It is also a key mitochondrial enzyme for cellular bioenergetics, especially for nerve cells in the retina and the brain. Evidence shows that LLLT can secondarily enhance neural metabolism by regulating mitochondrial function,

Synergistic Effects of Light Therapy and Nutrition

Cytochrome c oxidase has been shown to have a new enzymatic activity----*the reduction of nitrite to nitric oxide*.

Low intensity light enhances nitric oxide synthesis by cytochrome c oxidase without altering its ability to reduce oxygen.

From these findings, we propose that cytochrome c oxidase functions in photobiomodulation by producing nitric oxide, a signaling molecule which can then function in both intra- and extracellular signaling pathways.



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Robert O Poyton

Therapeutic Photobiomodulation: Nitric Oxide and a Novel Function of Mitochondrial Cytochrome C Oxidase

Published on February 20, 2011

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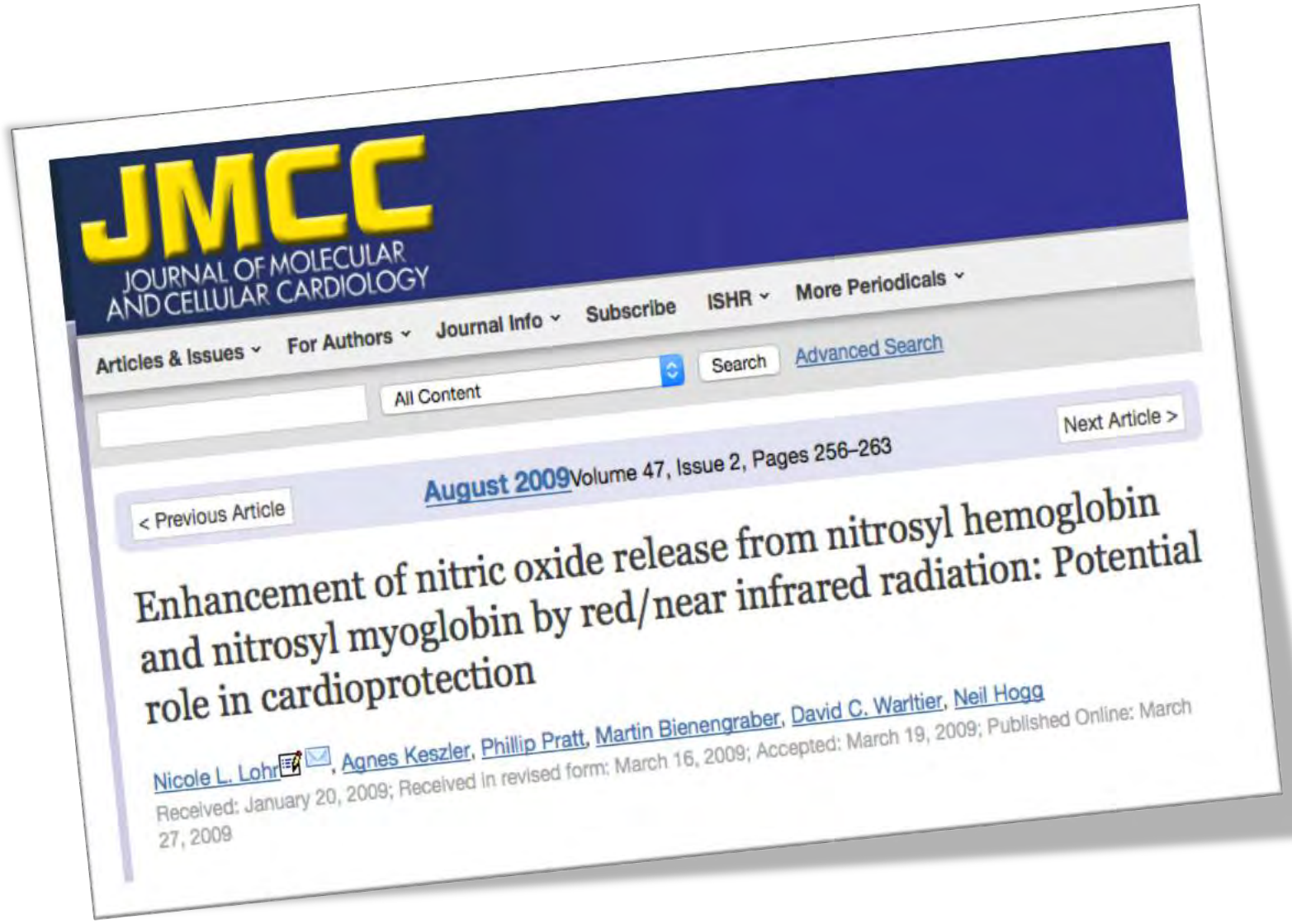
Synergistic Effects of Light Therapy and Nutrition

"We show both in purified systems and in myocardium that R/NIR light can decay nitrosyl hemes and release NO, and that this released NO may enhance the cardioprotective effects of nitrite. ***Thus, the photodissociation to NO and its synergistic effect with sodium nitrite may represent a noninvasive and site-specific means for increasing NO bioavailability.***"



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Enhancement of nitric oxide release from nitrosyl hemoglobin and nitrosyl myoglobin by red/near infrared radiation: Potential role in cardioprotection

Nicole L. Lohr, Agnes Keszler, Phillip Pratt, Martin Bienengraber, David C. Warltier, Neil Hogg

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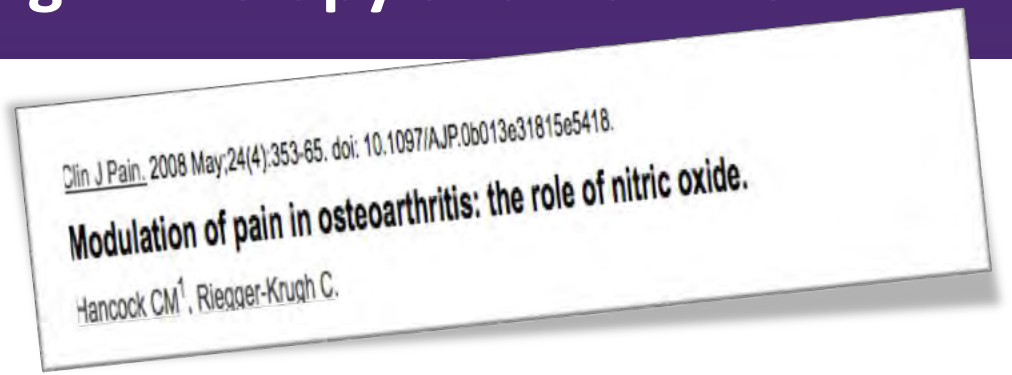


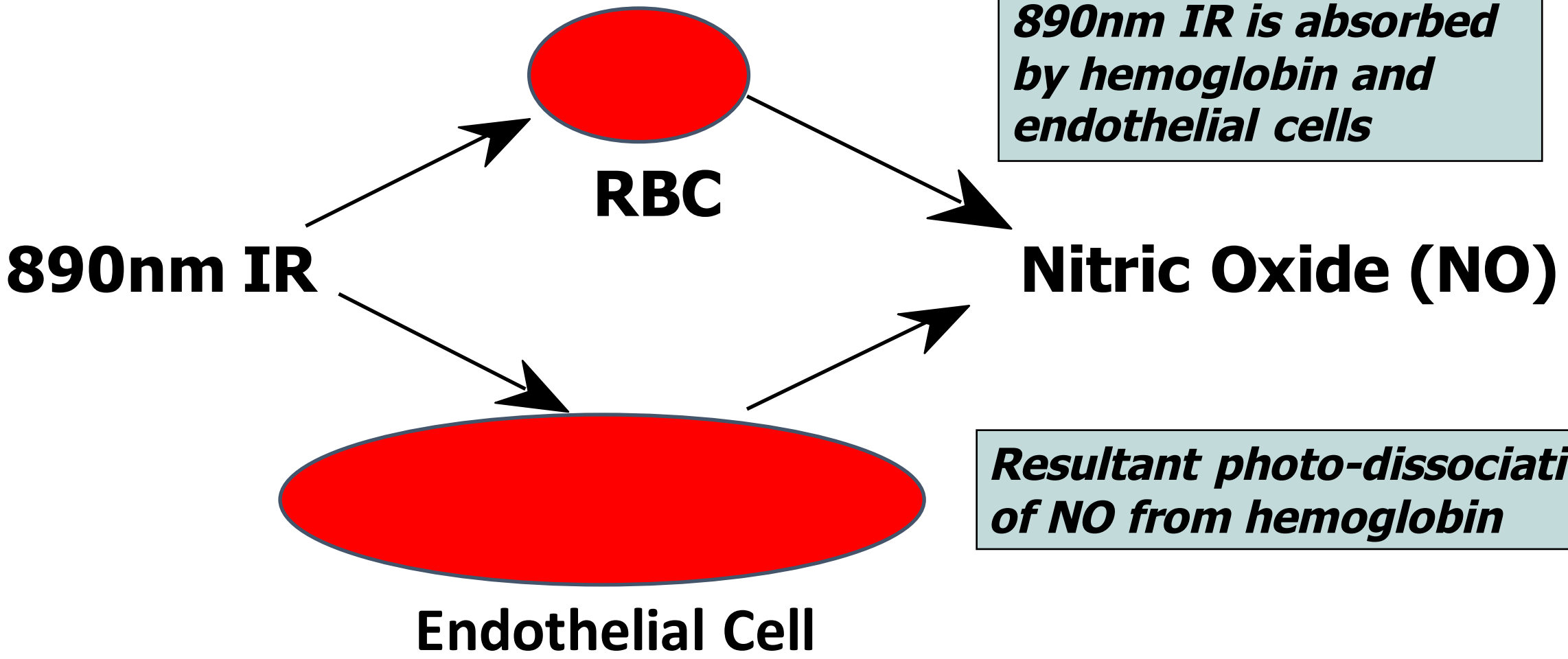
(1) NO via the beneficial cNOS pathway is decreased in joint structures exposed to chronic load-induced stresses and biochemical change-induced stresses,

(2) Monochromatic infrared light energy at an 890 nm wavelength, applied at the skin surface, is absorbed into blood vessels and stimulates production of NO in joints by the beneficial cNOS pathway,

(3) NO from the cNOS pathway may help decrease the detrimental effects of NO induced by iNOS and produced in OA pathology, and

(4) NO-based intervention may produce substantial pain relief without undesirable side effects by increasing circulation, decreasing nerve irritation, and decreasing inflammation in joints.

Synergistic Effects of Light Therapy and Nutrition

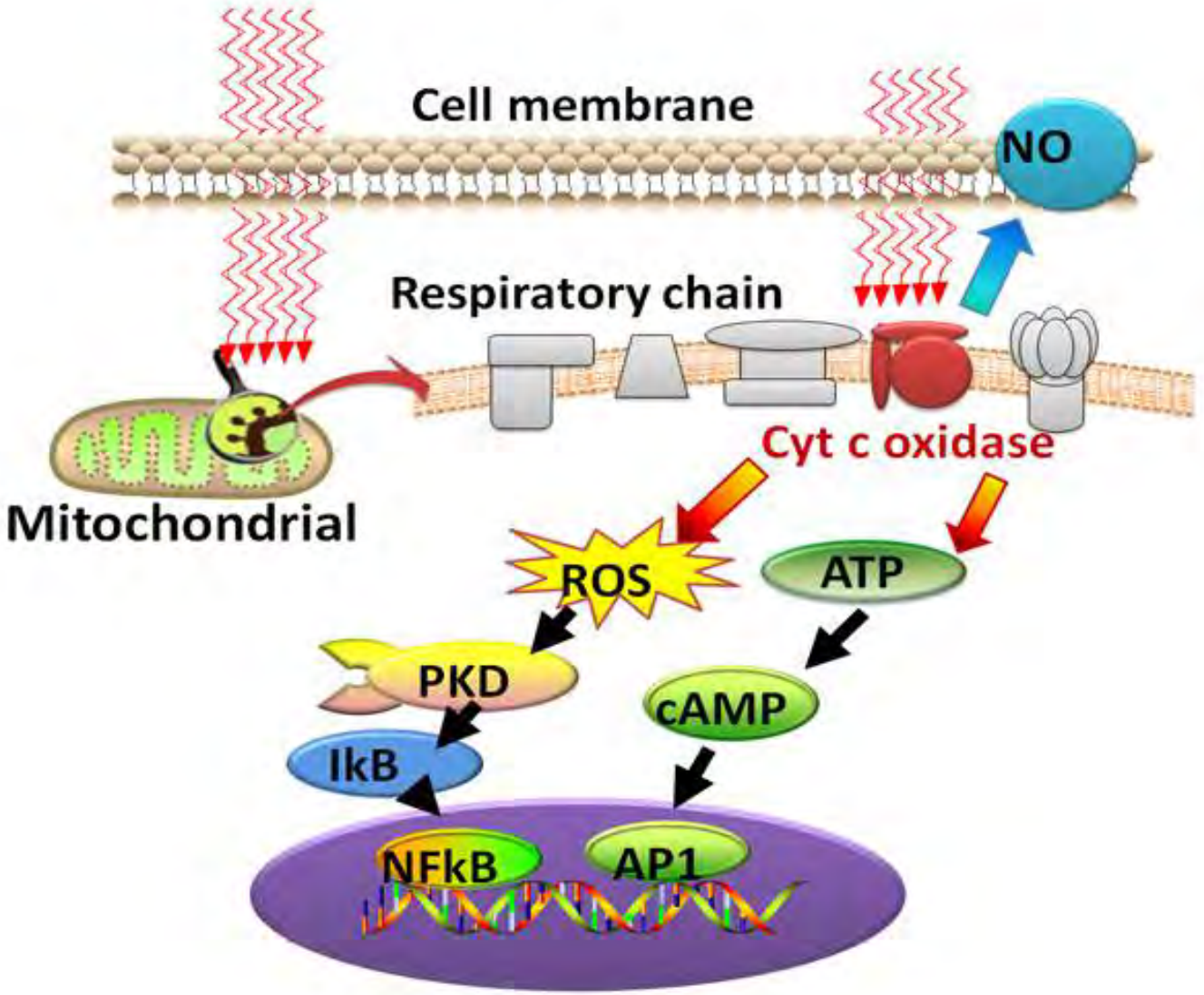


890nm (IR) causes the photo-dissociation of NO from hemoglobin in the red blood cells (and possibly from the endothelial cells as well) allowing NO to be free (locally) to do its work.

Synergistic Effects of Light Therapy and Nutrition

NO Release From Tissue or Blood:

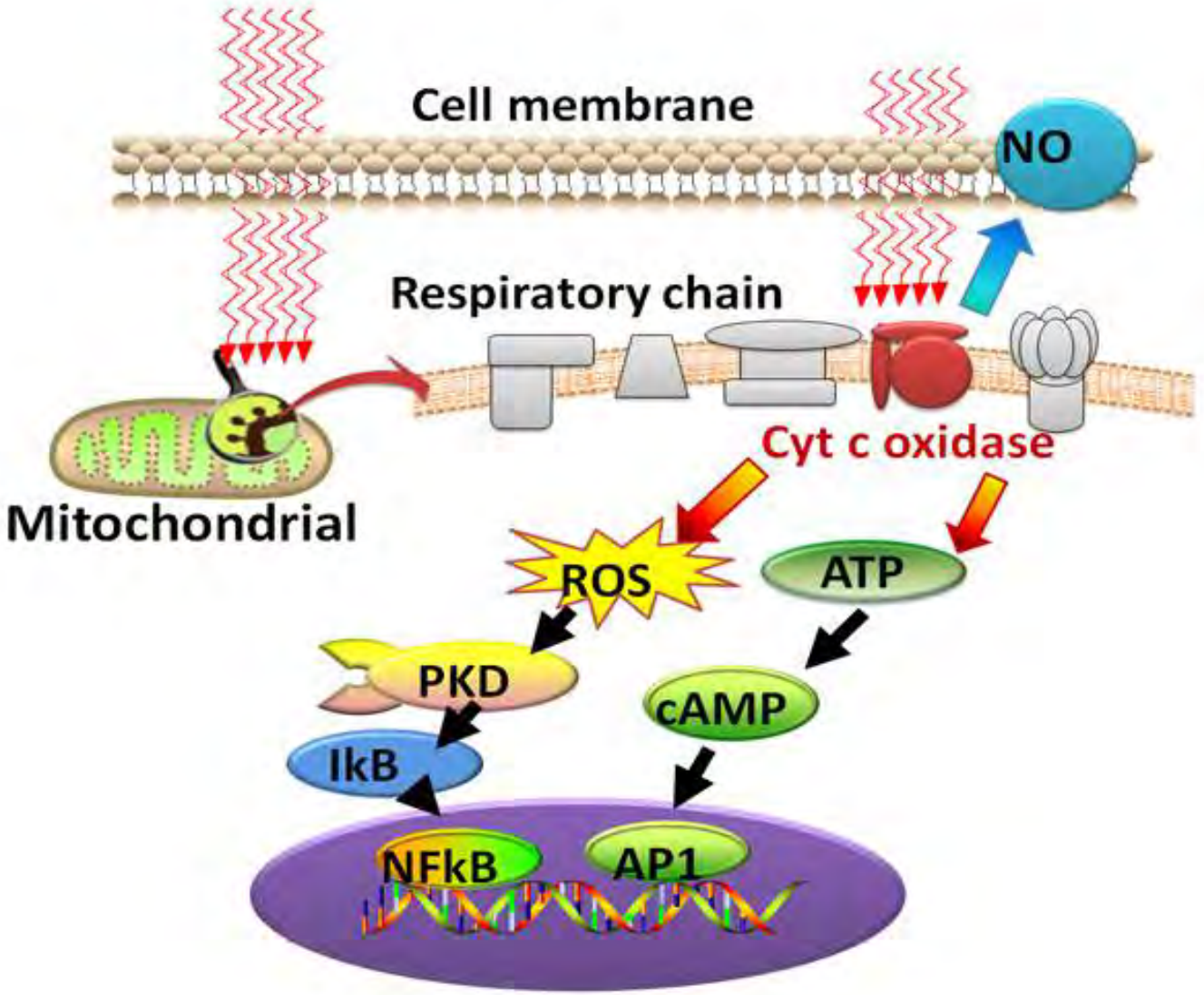
- **Significantly Improves circulation**
  (via vasodilation)
- **Reduces inflammation**
- **Decreases pain**
- **Increases angiogenesis**
  - Builds new vessels
- **Increases lymphatic activity**
  - Decreases swelling



Synergistic Effects of Light Therapy and Nutrition

NO Release From Tissue or Blood:

- **Increases cell regeneration** (wound healing)
  - Stimulates tissue granulation & connective tissue
- **Increases bone mineralization**
  - Reduces osteoporosis
- **Increases phagocytosis** (immune response)
- **Increases RNA-DNA synthesis** (cell building)



Synergistic Effects of Light Therapy and Nutrition

- **Certain vegetables possess a high nitrate content representing a potential source of vasoprotective nitric oxide via bioactivation.**
- In healthy volunteers, approximately 3 hours after ingestion of a dietary nitrate load (beetroot juice 500 mL), BP was substantially reduced (max 10.4/8 mm Hg); an effect that correlated with peak increases in plasma nitrite concentration.

CME Available

Nitric Oxide, Oxidative Stress

Acute Blood Pressure Lowering, Vasoprotective, and Antiplatelet Properties of Dietary Nitrate via Bioconversion to Nitrite

Andrew J. Webb, Nakul Patel, Stavros Loukogeorgakis, Mike Okorie, Zainab Aboud, Shivani Misra, Rahim Rashid, Philip Miall, John Deanfield, Nigel Benjamin, Raymond MacAllister, Adrian J. Hobbs, Amrita Ahluwalia

Abstract—Diets rich in fruits and vegetables reduce blood pressure (BP) and the risk of adverse cardiovascular events. However, the mechanisms of this effect have not been elucidated. Certain vegetables possess a high nitrate content, and we hypothesized that this might represent a source of vasoprotective nitric oxide via bioactivation. In healthy volunteers, approximately 3 hours after ingestion of a dietary nitrate load (beetroot juice 500 mL), BP was substantially reduced (Δ_{max} −10.4/8 mm Hg); an effect that correlated with peak increases in plasma nitrite concentration. The dietary nitrate load also prevented endothelial dysfunction induced by an acute ischemic insult in the human forearm and significantly attenuated ex vivo platelet aggregation in response to collagen and ADP. Interruption of the enterosalivary conversion of nitrate to nitrite (facilitated by bacterial anaerobes situated on the surface of the tongue) prevented the rise in plasma nitrite, blocked the decrease in BP, and abolished the inhibitory effects on platelet aggregation, confirming that these vasoprotective effects were attributable to the activity of nitrite converted from the ingested nitrate. These findings suggest that dietary nitrate underlies the beneficial effects of a vegetable-rich diet and highlights the potential of a "natural" low cost approach for the treatment of cardiovascular disease. (***Hypertension***. 2008;51:784-790.)

Key Words: diet ■ nitric oxide ■ blood pressure ■ hypertension ■ ischemia/reperfusion ■ platelets ■ endothelium

Perhaps the largest public health initiative in the Western world has focused on improvement of diet, particularly in those with a high risk of cardiovascular disease. Trials have shown that diets rich in fruits and vegetables reduce blood pressure (BP; Dietary Approaches to Stop Hypertension; DASH, Vegetarian Diet and BP)[1,2] and adverse cardiovascular events.[3–7] These protective effects have previously been attributed to the high antioxidant vitamin content, yet large clinical trials have failed to provide evidence in support of this thesis.[8,9] The greatest protection against coronary heart disease afforded by a change in diet is that associated with the consumption of green leafy vegetables (eg, spinach, lettuce).[6] Such vegetables, also including beetroot, commonly have a high inorganic nitrate (NO_3^-) content.[10,11] In humans, after absorption through the stomach wall, ≈25% of consumed nitrate enters the enterosalivary circulation where it is reduced to nitrite (NO_2^-) by bacterial nitrate reductases from facultative anaerobes on the dorsal surface of the tongue.[12–14] This nitrite is swallowed and in the acidic environment of the stomach is reduced to nitric oxide (NO) or re-enters the circulation as nitrite. Indeed, it has been hypothesized that dietary nitrate represents an intravascular source of the pleiotropic, vasoprotective molecule NO, which supplements conventional NO generation by NO synthases (NOS).[15]

Endothelium-derived NO is a potent dilator, governs systemic BP, and retards atherogenesis (NO inhibits inflammatory cell recruitment and platelet aggregation).[16] Consequently, numerous cardiovascular pathologies (including preh ypertension,[17] hypertension,[18] atherosclerosis,[19] and stroke[20]) are associated with endothelial dysfunction and diminished NO bioactivity. Recently, studies have demonstrated that nitrite confers marked protection against ischemia/reperfusion (I/R) injury in the myocardial, hepatic, renal, pulmonary, and cerebral vasculature.[21,22] This cytoprotective effect has been attributed to reduction of nitrite to NO during ischemia or hypoxemia (conditions that inactivate endothelial NOS, the

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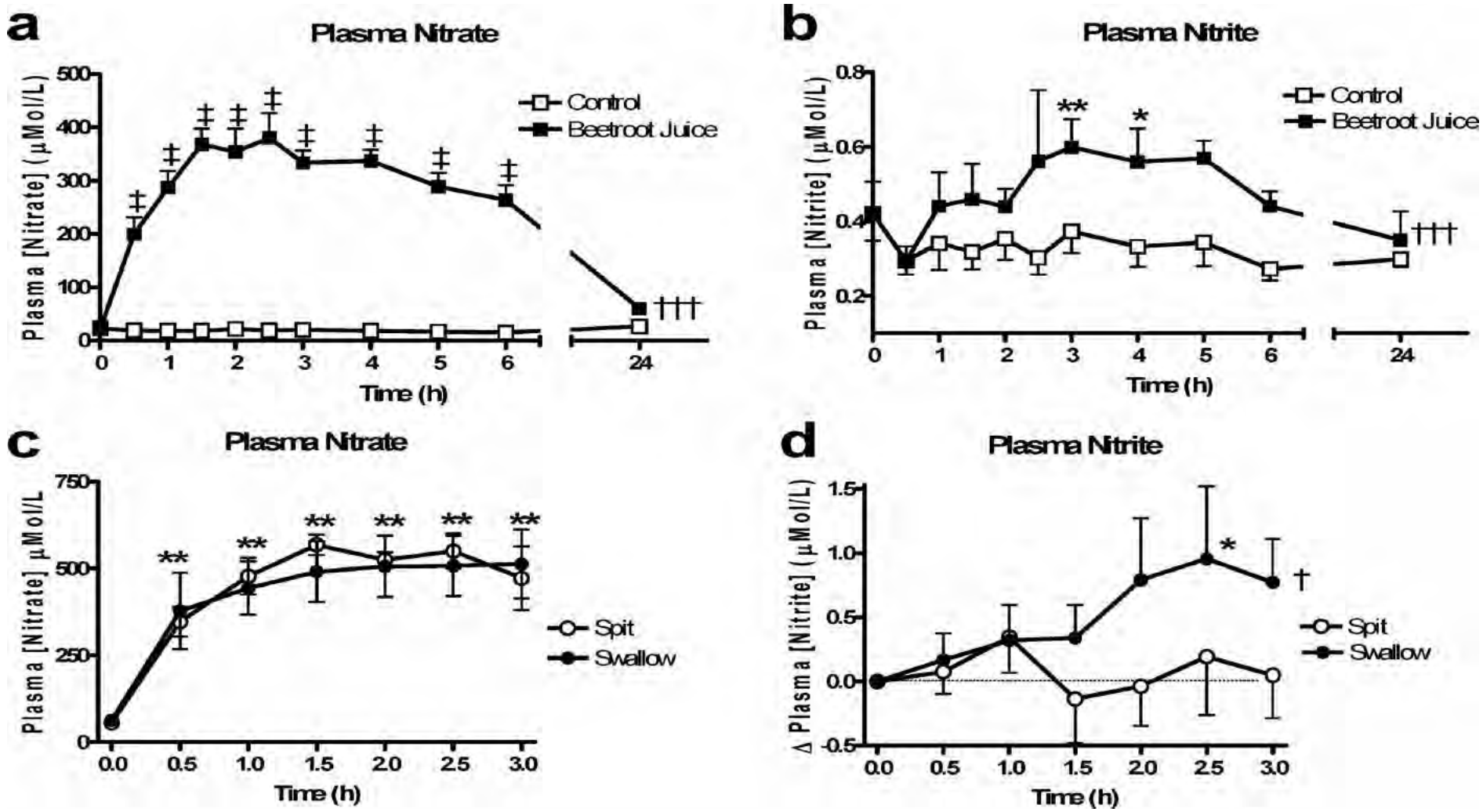
Correspondence to Professor Amrita Ahluwalia, Clinical Pharmacology, William Harvey Research Institute, Barts & the London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK. E-mail a.ahluwalia@qmul.ac.uk

Hypertension **is available at http://hyper.ahajournals.org** **DOI: 10.1161/HYPERTENSIONAHA.107.103523**

Figure 1. The effect of beetroot juice on the plasma concentrations of (a) nitrate and (b) nitrite and the effects of spitting vs swallowing of saliva on plasma concentrations of (c) nitrate and (d) nitrite.



Andrew J. Webb et al. Hypertension. 2008;51:784-790

Synergistic Effects of Light Therapy and Nutrition

Classification of vegetables according to nitrate content[1]	
Nitrate content (mg/100 g fresh weight)	Vegetable varieties
Very low, <20	Artichoke, asparagus, broad bean, eggplant, garlic, onion, green bean, mushroom, pea, pepper, potato, summer squash, sweet potato, tomato, watermelon
Low, 20 to <50	Broccoli, carrot, cauliflower, cucumber, pumpkin, chicory
Middle, 50 to <100	Cabbage, dill, turnip, savoy cabbage
High, 100 to <250	Celeriac, Chinese cabbage, endive, fennel, kohlrabi, leek, parsley
Very high, >250	Celery, cress, chervil, lettuce, red beetroot, spinach, rocket (rucola)

Synergistic Effects of Light Therapy and Nutrition

➔ Nitrite levels in cells treated with L-citrulline and GSH were significantly greater than control ($p < 0.05$).

➔ Plasma NOx with L-citrulline + GSH was significantly greater than control and L-citrulline ($p < 0.05$).

➔ Nitrite and NOx for L-citrulline + GSH were significantly greater at 30 min post-exercise when compared to placebo ($p < 0.05$).

Conclusion:

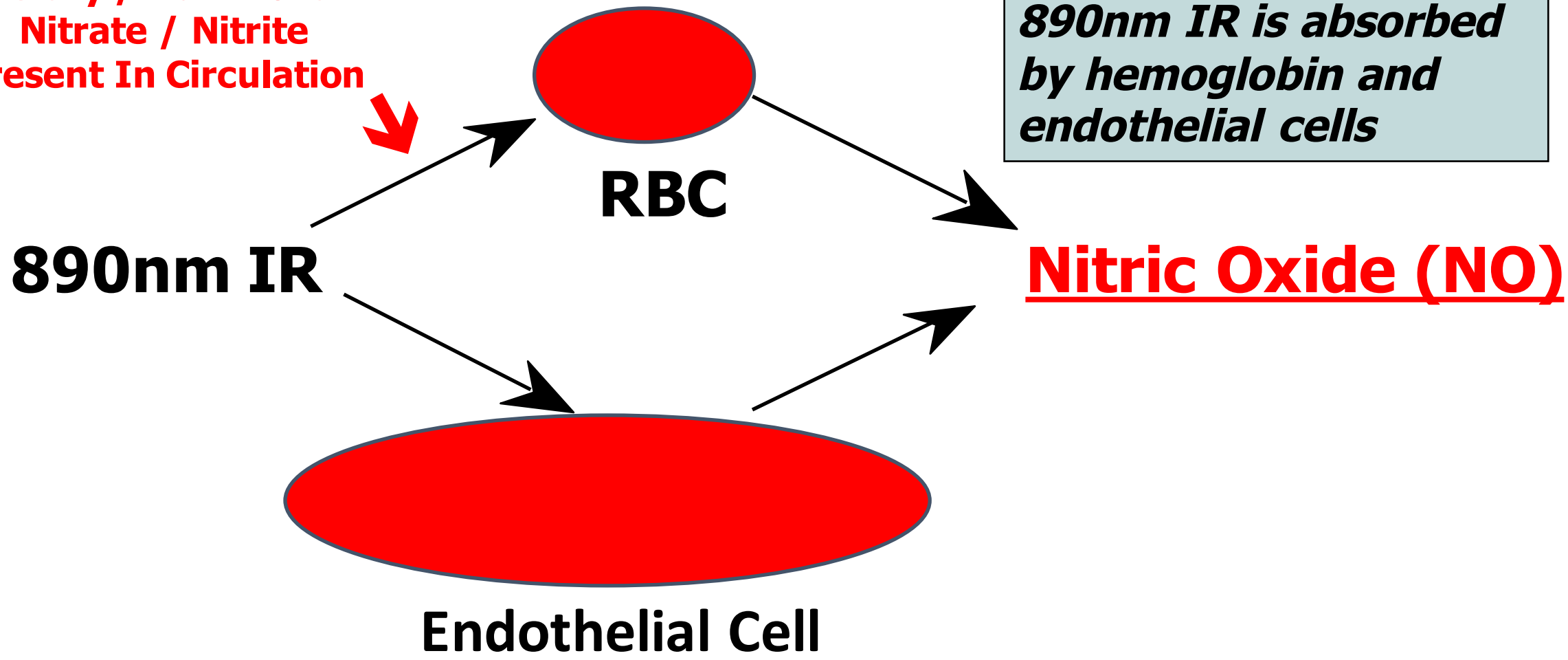
Combining L-citrulline with GSH augments increases in nitrite and NOx levels during *in vitro* and *in vivo* conditions.

McKinley S, Andrew T, Morita M, Willoughby D. *Journal of the International Society of Sports Nutrition*. 2015, 12:27

Synergistic Effects of Light Therapy and Nutrition

Dietary / Nutritional Nitrate / Nitrite Present In Circulation

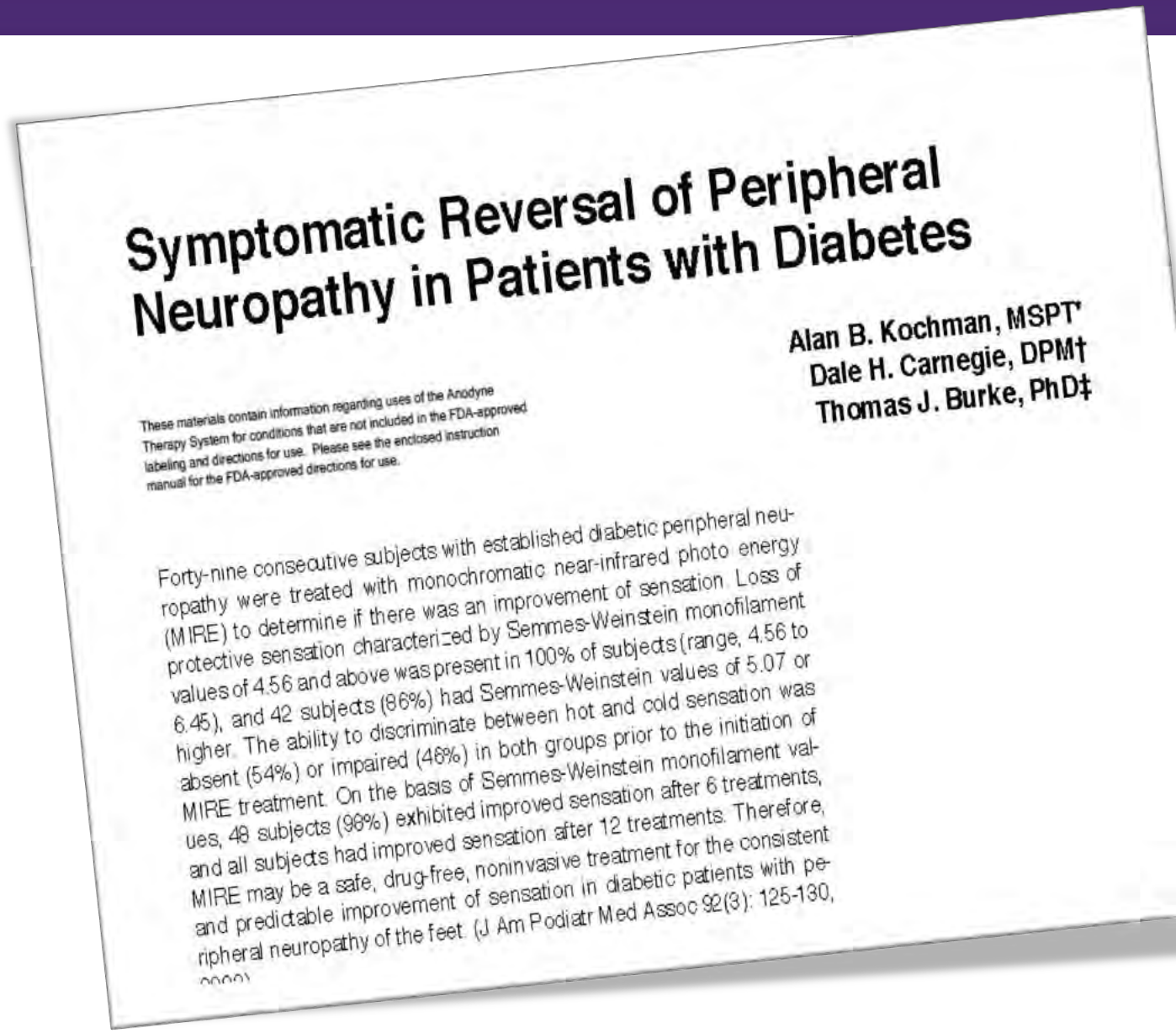
890nm IR is absorbed by hemoglobin and endothelial cells



Synergistic Effects of Light Therapy and Nutrition

Forty-nine subjects with established diabetic peripheral neuropathy were treated with monochromatic near-infrared photo energy (MIRE) to determine if there was an improvement of sensation.

Loss of protective sensation characterized by Semmes-Weinstein monofilament values of 4.56 and above was present in 100% of subjects (range, 4.56 to 6.45), and 42 subjects (86%) had Semmes-Weinstein values of 5.07 or higher.



Symptomatic Reversal of Peripheral Neuropathy in Patients with Diabetes

Alan B. Kochman, MSPT*
Dale H. Carnegie, DPM†
Thomas J. Burke, PhD‡

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Forty-nine consecutive subjects with established diabetic peripheral neuropathy were treated with monochromatic near-infrared photo energy (MIRE) to determine if there was an improvement of sensation. Loss of protective sensation characterized by Semmes-Weinstein monofilament values of 4.56 and above was present in 100% of subjects (range, 4.56 to 6.45), and 42 subjects (86%) had Semmes-Weinstein values of 5.07 or higher. The ability to discriminate between hot and cold sensation was absent (54%) or impaired (46%) in both groups prior to the initiation of MIRE treatment. On the basis of Semmes-Weinstein monofilament values, 48 subjects (96%) exhibited improved sensation after 6 treatments, and all subjects had improved sensation after 12 treatments. Therefore, MIRE may be a safe, drug-free, noninvasive treatment for the consistent and predictable improvement of sensation in diabetic patients with peripheral neuropathy of the feet. (J Am Podiatr Med Assoc 92(3): 125-130,

Synergistic Effects of Light Therapy and Nutrition

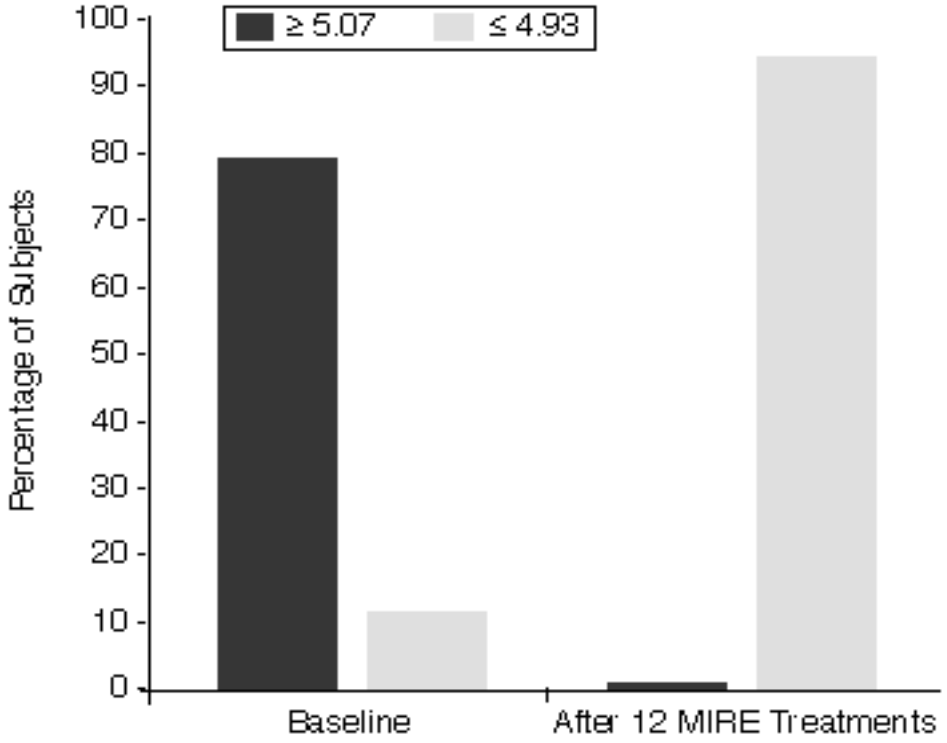

Figure 1. Percentage distribution of patients with type 1 diabetes (N = 25) with Semmes-Weinstein monofilament values ≥ 5.07 and ≤ 4.93 before (left) and after (right) 12 MIRE treatments.

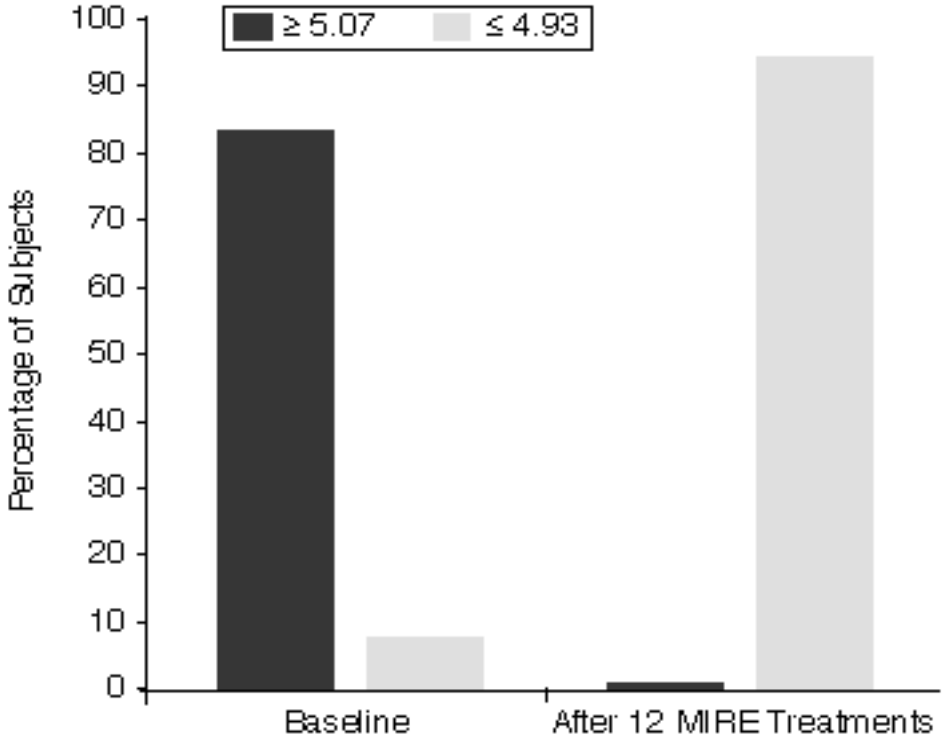

Figure 2. Percentage distribution of patients with type 2 diabetes (N = 24) with Semmes-Weinstein monofilament values ≥ 5.07 and ≤ 4.93 before (left) and after (right) 12 MIRE treatments.

Synergistic Effects of Light Therapy and Nutrition

The ability to discriminate between hot and cold sensation was absent (54%) or impaired (46%) in both groups prior to the initiation of MIRE treatment.

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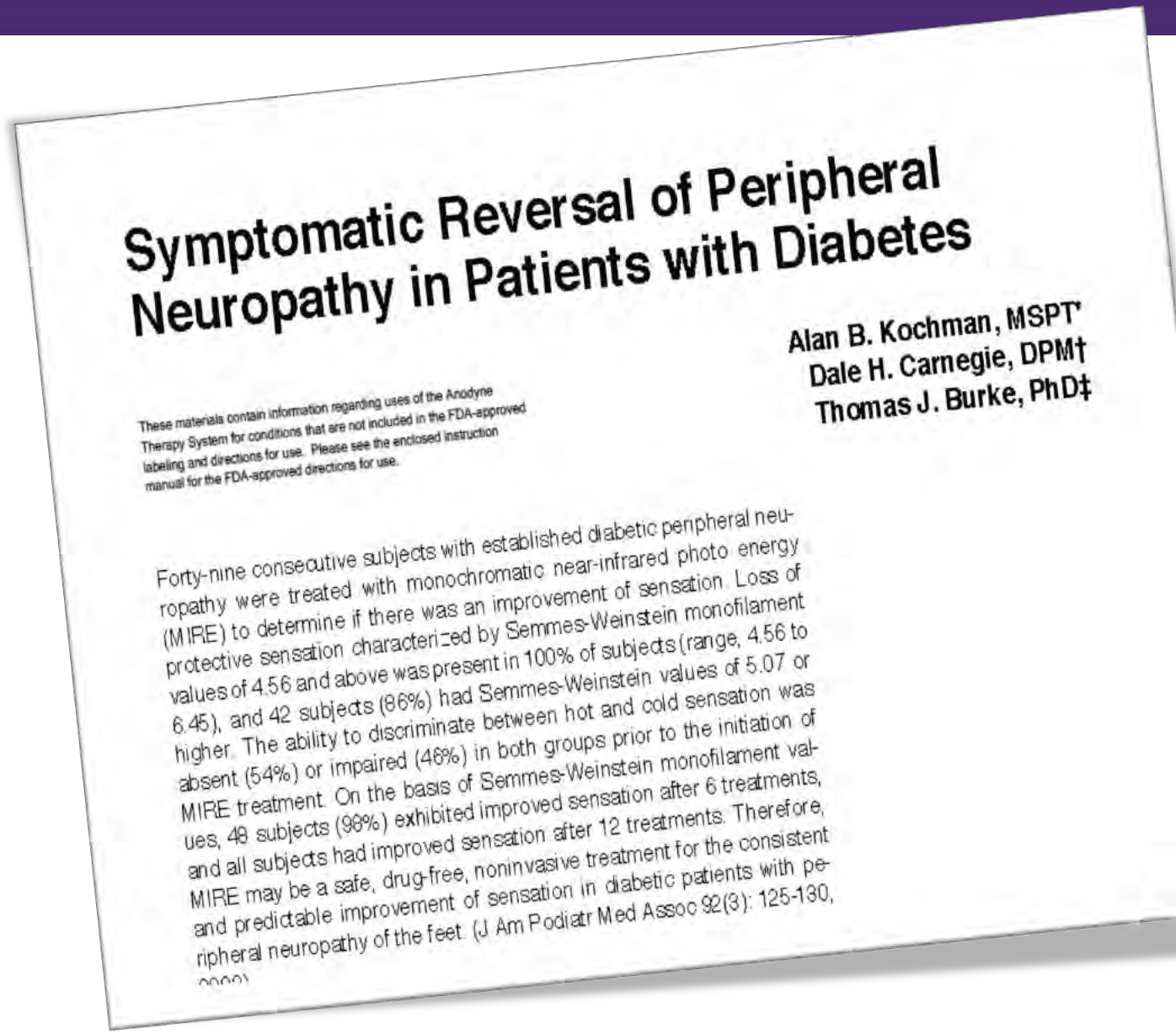
Therefore, MIRE may be a safe, drug-free, noninvasive treatment for the consistent and predictable improvement of sensation in diabetic patients with peripheral neuropathy of the feet. (*J Am Podiatr Med Assoc* 92(3): 125-130, 2002)



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Synergistic Effects of Light Therapy and Nutrition

27 patients with peripheral neuropathy received treatment with monochromatic near-infrared photoenergy (890 nm).

Methods: All enrolled patients exhibited abnormal sensory perception (either hyperesthesia or hypoesthesia) based on a qualifying examination with the Neurometer CPT (current perception threshold) (baseline CPT). The patients received 10 treatments (each lasting 40 minutes) during a 2-week period and then underwent CPT retesting to determine the extent of improvement of sensory impairment in myelinated and unmyelinated sensory fibers of the peroneal nerve.

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Original Article

IMPROVEMENT OF SENSORY IMPAIRMENT IN PATIENTS WITH PERIPHERAL NEUROPATHY

J. Joseph Prendergast, MD, Galdina Miranda, MA, and Manuel Sanchez, MA

ABSTRACT

Objective: To report the findings in 27 patients with peripheral neuropathy (21 with lower extremity sensory impairment associated with diabetic peripheral neuropathy and 6 with other causes), who received treatment with monochromatic near-infrared photoenergy (890 nm) delivered by the Anodyne Therapy System (ATS).

Methods: All enrolled patients exhibited abnormal sensory perception (either hyperesthesia or hypoesthesia) based on a qualifying examination with the Neurometer CPT (current perception threshold) (baseline CPT). The patients received 10 ATS treatments (each lasting 40 minutes) during a 2-week period and then underwent CPT retesting to determine the extent of improvement of sensory impairment in myelinated and unmyelinated sensory fibers of the peroneal nerve.

Results: All patients obtained improvement in sensory impairment in comparison with baseline CPT measures, and 16 of the 27 patients achieved normal sensory

INTRODUCTION

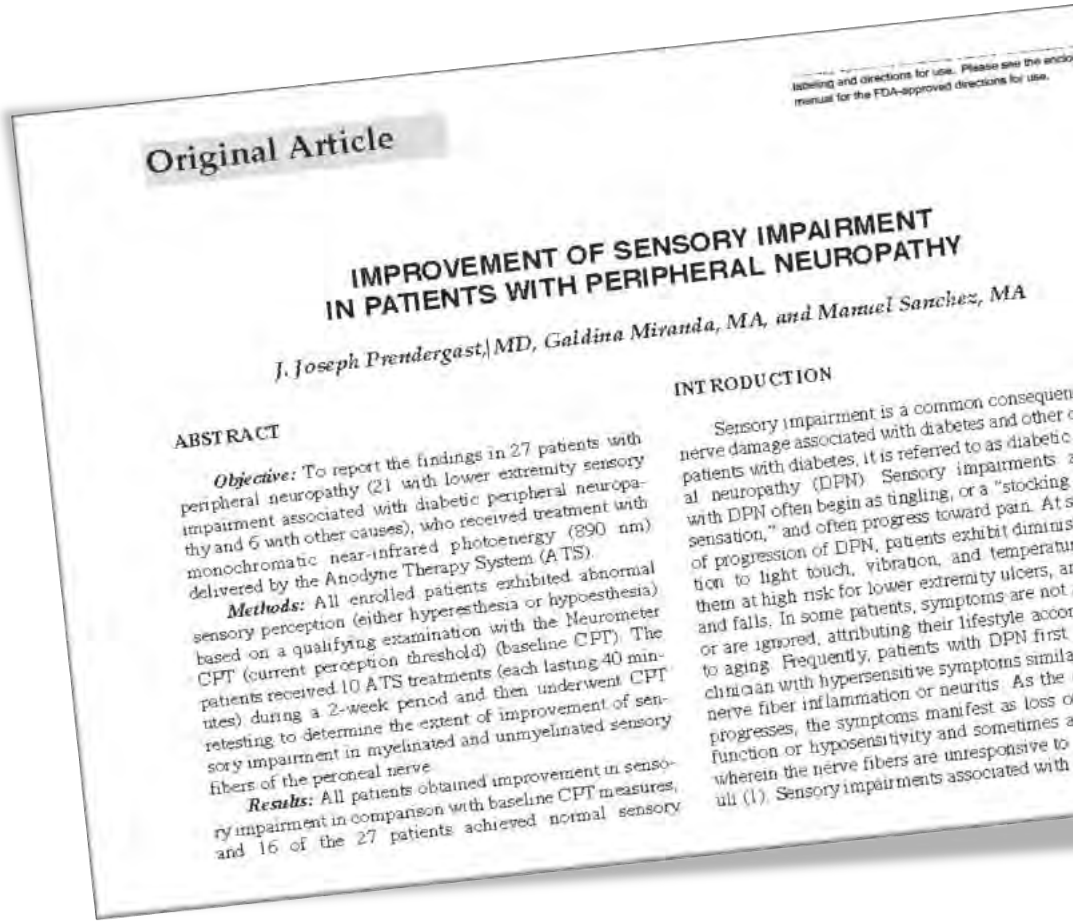
Sensory impairment is a common consequence of the nerve damage associated with diabetes and other causes. In patients with diabetes, it is referred to as diabetic peripheral neuropathy (DPN). Sensory impairments associated with DPN often begin as tingling, or a "stocking and glove sensation," and often progress toward pain. At some stage of progression of DPN, patients exhibit diminished sensation to light touch, vibration, and temperature, placing them at high risk for lower extremity ulcers, amputations, and falls. In some patients, symptoms are not appreciated or are ignored, attributing their lifestyle accommodations to aging. Frequently, patients with DPN first present to a clinician with hypersensitive symptoms similar to those in nerve fiber inflammation or neuritis. As the disease state progresses, the symptoms manifest as loss of nerve fiber function or hyposensitivity and sometimes as anesthesia, wherein the nerve fibers are unresponsive to various stimuli (1). Sensory impairments associated with diabetes have

Synergistic Effects of Light Therapy and Nutrition

Results: All patients obtained improvement in sensory impairment in comparison with baseline CPT measures, and 16 of the 27 patients achieved normal sensory responses in all nerve fiber subpopulations.

Ten patients had been tested previously (initial CPT) and did not exhibit spontaneous improvement in sensory impairment during a mean period of 27 months before baseline CPT. After receiving the ATS treatments, however, this group of patients showed improvement in comparison with both initial CPT results and baseline CPT.

Conclusion: On the basis of the data from this study, the ATS seems to be a safe and effective treatment to improve sensory impairment associated with peripheral neuropathy due to diabetes and other causes. (*Endocr Pract*. 2004;10:24-30)



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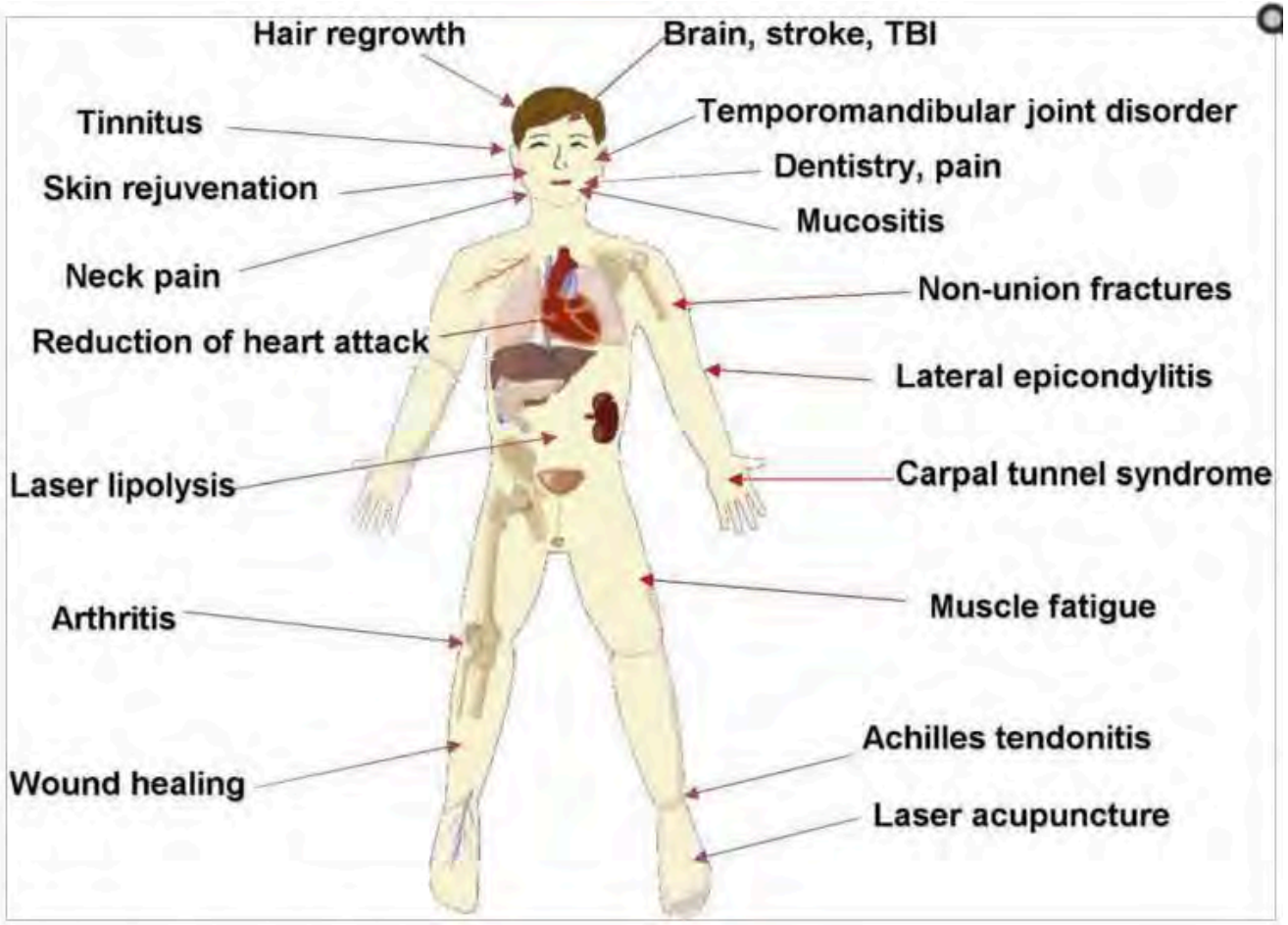

Diagram of the various medical applications of low-level light therapy.

THANK YOU!