Title: Effect of antioxidants on stress, work fatigue, and metabolic syndrome in employees of a private corporation in Mexico City

1. Public title	**Effect of antioxidants on stress, work fatigue, and metabolic syndrome in employees of a private corporation in Mexico City**
Scientific title	*Effect of Apple polyphenols, carotenoids, and anthocyanins on stress, work fatigue, and metabolic syndrome in employees of a private corporation in Mexico City*
Acronym	BIOSTRESSMET
Submission number	49139
Overall study status	Ongoing
Recruitment status	Completed
2. Sponsor and Funding **Primary sponsor**	Secretaría de Investigación y Posgrado, Instituto Politécnico Nacional
3. Funders	BioMaussan Biodesarrollos Valmex SA de CV
Study Contacts **Principal Investigator** **Institution** **Email** **Phone**	 PhD Elvia Pérez Soto National School of Medicine and Homeopathy, IPN elvperezs@ipn.mx +52 5557296000 ext. 55571
4. Study Objectives	To evaluate the effect of antioxidant supplements as a preventive and therapeutic strategy against stress, work fatigue, oxidative stress, inflammation and metabolic syndrome in workers of a private corporation subjected to a high workload.
5. Study Design **Type** **Design** **Allocation** **Masking** **Control** **Purpose**	 Interventional Randomized controlled trial Parallel assignment Double-blind (participants and investigators) Placebo Prevention
6. Participants **Population** **Sample size** **Final enrollment**	Full-time employees working on-site at a private corporate office in Mexico City. 120 participants **Eligibility Criteria** **Inclusion** Adults ≥18 years Male or female workers Full-time on-site employees At least 1 year of continuous in-person work Fixed work shift BMI > 20 kg/m² Mentally healthy Signed informed consent **Exclusion** • Neurodegenerative conditions • Pregnancy or lactation

	• Hypothyroidism • Psychiatric disorders • Dyed hair • Use of alternative dietary supplements **Age range:**20–75 years **Sex:** All
7. Interventions	Participants were randomized into four parallel groups, receiving oral supplements for **6 months**, twice daily (5 mL morning, 5 mL evening). **Group 1 – Marine Algae 2.0** • 40 mg astaxanthin • 13.2 mg fucoxanthin • 84 mg apple polyphenols **Group 2 – Marine Algae Premium 2.1** • 166 mg anthocyanins • 194.8 mg total xanthophylls • 125 mg apple polyphenols **Group 3 – Astaxanthin Special Formula** • 500 mg/day astaxanthin from *Haematococcus pluvialis* **Group 4 – Placebo** Purified water, citric acid, sorbic acid, allulose, flavoring.
8. Outcomes	**Primary Outcomes** Measured at T0 (baseline), T1 (mid-intervention), T2 (6 months/end): 1. Work-related stress (IMSS test, Work Stress Questionnaire) 2. Work-related fatigue (Fatigue Severity Scale – Mexican adaptation) 3. Hair cortisol concentration (competitive immunoassay) 4. Determination of sleep quality (SQ). **Secondary Outcomes** Measured at T0, T1, T2: 1. **Biochemical markers:** glucose, lipids, cholesterol profile, urea, creatinine, uric acid, HbA1c 2. **Inflammatory cytokines:** TNF-α, IL-1β, IL-6, IL-10 3. **Oxidative stress biomarkers:** FRAP, lipoperoxidation, nitric oxide, catalase, and SOD activity 4. **Gene expression:** Nrf2/ARE, NF-κB, AP1, HNF4, P38 MAPK1, ACM1/2/3
9. Study Timeline	• **First enrollment:** 16 May 2025 • **Final enrollment:** 30 May 2025 • **Completion date:** 28 November 2025
10. Ethics	Approved on 06 August 2025 by the Bioethics Committee of the National School of Medicine and Homeopathy, IPN (Ref:CBE/002/2025).
11. Data Sharing Plan	*"Anonymized individual participant data will be shared upon reasonable request… for academic purposes only."*

1. General information

Protocolo title	**Effect of antioxidants on stress, work fatigue, and metabolic syndrome in employees of a private corporation in Mexico City**
Protocol identifying number	49139, CBE/002/2025
Protocol identifier	BIOSTRESSMET
Date	19/March/2026
Name and address of the sponsor/funder	1. Secretaría de Investigación y Posgrado, Instituto Politécnico Nacional (IPN). Unidad Profesional "Adolfo López Mateos", Edificio de la Secretaría Académica, 2do. piso. Avenida Luis Enrique Erro s/n, Colonia Zacatenco, Alcaldía Gustavo A. Madero, C.P. 07738, Ciudad de México. Phone: +52 5557296000, Website: https://www.ipn.mx 2. BioMaussan SA de CV. Guillermo González Camarena, 1205, Zedec Santa Fe, Álvaro Obregón, Ciudad de México 01376, México. Phone: +52 55 5555 0055 Inicio BioMaussan - BioMaussan 3. Biodesarrollos Valmex SA de CV. Investigación y Desarrollo, Biodesarrollos Valmex, Circuito Crisantemos 10, Santa Cruz de las Flores, Tlajomulco de Zúñiga, Jalisco 45640, México; BIOVALMEX – Biodesarrollos con moléculas funcionales de alto nivel, +52 331804 2173, atencion@biovalmex.com
Name and title of the investigator(s) who is (are) responsible for conducting the research, and the address and telephone number(s) of the research site(s), including responsibilities of each.	1. Elvia Pérez Soto (Researcher, responsible for the project, training and orientation, protocol implementation, Research collaboration). 2. María del Carmen López García (Researcher, Medical specialist in occupational health, protocol implementation). 3. Montserrat Noemí González Mellado (Physician, data collection and monitoring, capacity building). 4. Eduardo Nateras Molina (Physician, Intervention Delivery, Data collection, and monitoring). Laboratory of Biomedicine and Occupational Health (LABBSO), ENMH-IPN, located at Guillermo Massieu Helguera # 231, Col. La Escalera, Del. Gustavo A. Madero, C.P. 07320, Mexico City, Mexico. Tel 5557296000 ext. 55571
Name(s) and address(es) of the clinical laboratory(ies) and other medical and/or technical department(s) and/or institutions involved in the research	1. Gabriel Lara Hernández, Investigación y Desarrollo, BioMaussan, Guillermo González Camarena, 1205, Zedec Santa Fe, Álvaro Obregón, Ciudad de México 01376, México; gabobmol@gmail.com 2. Ericka Flores-Berrios, Investigación y Desarrollo, Biodesarrollos Valmex, Circuito Crisantemos 10, Santa Cruz de las Flores, Tlajomulco de Zúñiga, Jalisco 45640, México; eflores@biovalmex.com 3. Hamlet Avilés-Arnaut, Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Instituto de Biotecnología, Campus Ciudad Universitaria, Av. S/N, San Nicolás de los Garza 66455, NL, México, hamlet.avilesarn@uanl.edu.mx 4. Virginia Sánchez-Monroy, Escuela Superior de Medicina, Instituto Politécnico Nacional, Casco de Santo Tomas 11340, Ciudad de México, México; vmonroy@ipn.mx 5. Laboratorios Olab diagnósticos médicos. Anzures, Av. Melchor Ocampo No. 253, Col. Verónica, Anzures, C.P. 11300, Miguel Hidalgo, Ciudad de México, México.

- **Role of trial sponsor and funders in design, conduct, analysis, and reporting of trial; including any authority over these activities**

The trial sponsor (Instituto Politécnico Nacional) and the funders (BioMaussan, and Biodesarrollos Valmex SA de CV) had no role in the design of the study, data collection, data management, statistical analysis, interpretation of results, or preparation of the final report. They did not participate in decisions regarding study conduct, protocol amendments, or dissemination of findings. The sponsor and funders had no authority over any aspect of the trial and no access to the data at any stage. All scientific, methodological, and reporting decisions were made independently by the research team.

- **Trial oversight committees and operational structure**

This trial did not establish a steering committee, endpoint adjudication committee, or independent data monitoring committee due to the minimal-risk nature of the intervention and the limited sample size. No external oversight groups were involved.

The coordinating site was the National School of Medicine and Homeopathy (IPN). Within the research team, specific operational responsibilities were assigned to designated investigators. Participant recruitment was coordinated by Dr. Monserrat González Mellado, who oversaw screening, eligibility verification, and enrollment procedures. Occupational health–related data were reviewed and validated by PhD María del Carmen López García, ensuring accuracy and consistency of all work-related and clinical assessments. Molecular data, including biomarker and gene expression analyses, were supervised and verified by PhD Virginia Sánchez Monroy and PhD Elvia Pérez Soto, who ensured methodological rigor and quality control in laboratory procedures.

All data management activities were performed internally by the research team, who maintained full responsibility for database creation, data entry, verification, and secure storage.

- **Trial registration** The trial is not yet registered. The intended registry is ISRCTN Registry (International Standard Randomised Controlled Trial Number), ISRCTN Registry. Prospective / late but before publication.

- **Protocol and statistical analysis plan**

The full protocol and statistical analysis plan will be available on the ISRCTN Registry upon registration", https://www.isrctn.com

- **Data sharing**

The anonymized individual data, the data dictionary, and the statistical code used in the analyses will be deposited in the Zenodo repository, an open-access platform widely accepted by journals indexed in the Journal Citation Reports (JCR) and compatible with open data policies promoted by publishers such as MDPI. All material will be publicly available under an open license from the date of publication of the article, ensuring transparency, reproducibility, and compliance with international data sharing standards.

- **Funding and conflicts of interest. Sources of funding and other support**

The study is funded by BioMausan, based on a letter of intent with ENMH-IPN. In addition, non-monetary support was received in the form of dietary supplements and a placebo, provided by BioMaussan and BioValmex, respectively.
None of the funding entities will participate in the study design, data collection, statistical analysis, interpretation of results, or the decision to publish the findings.

- **Financial and other conflicts of interest for principal investigators and steering committee members**

The principal investigators declare that they have no financial or non-financial conflicts of interest related to this study.

- **Dissemination policy**

Once the study is completed, the findings will be shared:

The study is expected to generate rigorous scientific evidence on the efficacy and safety of antioxidant supplementation for reducing work-related fatigue and stress, improving sleep quality, and modulating inflammatory and oxidative stress biomarkers associated with metabolic syndrome. In accordance with SPIRIT guidelines, the dissemination plan ensures transparent, timely, and responsible communication of findings to scientific, clinical, regulatory, and public audiences.

- **Dissemination of Results and Publication Policy**

-Scientific dissemination

Results will be submitted for publication in peer-reviewed international journals and presented at major academic forums, including the Research Forum of the Network of Postgraduate Programs in Occupational Health, the National Congress of Biochemistry, and international meetings on metabolic syndrome and occupational health. Authorship will follow international standards. Manuscripts will be led by the Principal Investigator, with co-authorship granted to contributors meeting authorship criteria. All manuscripts will be reviewed and approved by the study's steering committee before submission.

-Clinical and professional relevance

The evidence generated will support healthcare professionals—nutritionists, occupational physicians, public health specialists, and nurses—in integrating complementary strategies for managing psychosocial and metabolic risk factors associated with metabolic syndrome. Although national guidelines do not currently include recommendations on nutritional supplementation, the findings may inform future updates to institutional clinical practice guidelines.

-Regulatory and policy impact

Study results will be shared with COFEPRIS to contribute to regulatory decisions regarding the safety and responsible use of dietary supplements. At the policy level, the findings may support the strengthening of occupational health strategies within the framework of NOM-035-STPS-2018, promoting healthier work environments and more comprehensive preventive approaches.

-Public and participant dissemination

Outreach activities—including talks, interviews, and educational sessions—will be conducted to communicate results to participants and the public. Dissemination will also occur through Maussan open television in collaboration with the BioMaussan scientific team, who do not have operational authority. Medical interns, medical students, and the Polytechnic Student Medical Association will participate in these activities to promote scientific literacy in occupational and nutritional health. An executive summary and targeted materials will be prepared for occupational health authorities and decision-makers.

-Participant benefits

Participants received a full course of dietary supplements at no cost and individualized nutritional counseling throughout the intervention. This included metabolic assessment, personalized digital meal plans generated through the 2001 Diets clinical platform, and tailored menus and food exchange lists to support adherence and long-term healthy eating habits. The 2001 Diets platform is based on official Mexican regulations and clinical criteria for metabolic conditions.

- **Background**

Work is central to human fulfillment and social identity, yet modern organizational structures—particularly in managerial and corporate roles—often foster unhealthy lifestyles characterized by poor nutrition, sedentary behavior, and chronic stress. These factors contribute to neuroendocrine disturbances that can lead to illness, disability, and premature death. Among the most pressing health consequences is Metabolic Syndrome (MetS), a cluster of cardiovascular risk factors whose prevalence has risen significantly in recent years.

Defined by the National Association of Cardiologists of Mexico as a combination of insulin resistance, endothelial dysfunction, hypercoagulability, and atherosclerosis, MetS represents a complex condition that urgently requires prevention and control strategies. Its magnitude is considerable: MetS doubles the risk of cardiovascular disease, which accounted for approximately 220,000 deaths in Mexico in 2021, including 177,000 from myocardial infarction (IMSS, 2023). The high frequency among adults, combined with the economic burden of obesity and related chronic diseases, underscores the need for innovative interventions.

Stress and fatigue in the workplace further exacerbate these risks, making employees in corporate environments a particularly vulnerable population. Recent studies highlight the potential of antioxidants—such as carotenoids, anthocyanins, and apple polyphenols—as complementary strategies in managing MetS. Carotenoids protect against oxidative damage and modulate immune responses (Terao, 2023); anthocyanins lower blood pressure, improve glycemic control, and reduce adipocyte inflammation (Godyla-Jablonski et al., 2024); and apple polyphenols improve lipid and glucose profiles (Shoji et al., 2024). These compounds act through mechanisms that neutralize free radicals, regulate inflammatory pathways (NF-κB, AP-1, Nrf2/ARE), and enhance cellular protection (Kyazimova & Kornyakova, 2024; Kanner, 2023).

- **Rationale**

Work-related stress (WS) and fatigue are highly prevalent occupational health problems, with a significant impact on physical and psychological well-being. Evidence from European and Latin American populations shows high rates of stress among workers, leading to affective disorders, chronic fatigue, sedentary lifestyles, poor dietary habits, and increased risk of substance use (Ippoliti et al., 2017). Physiologically, stress is mediated by cortisol, a glucocorticoid linked to obesity and MetS. Elevated cortisol and insulin levels, combined with poor nutrition and inactivity, contribute to visceral adiposity, dyslipidemia, hypertension, and insulin resistance.

Fatigue, often arising from poor sleep hygiene and occupational overexertion, is reliably measured using validated instruments such as the Fatigue Severity Scale (Duarte-Ayala et al., 2019). Stress biomarkers, including cortisol in hair, have been validated as effective tools for monitoring occupational stress (Iglesias et al., 2015).

Oxidative stress (OS) and inflammation are central mechanisms linking stress, fatigue, and MetS. Antioxidants (AO) such as carotenoids (astaxanthin, lutein, zeaxanthin), anthocyanins, and vitamins E and C have demonstrated benefits in reducing OS markers, improving insulin sensitivity, lowering cortisol, and enhancing mental health outcomes (Li et al., 2011; Hongo, 2016; Darabi et al., 2023). Systematic reviews highlight mixed but promising evidence for anthocyanins in reducing inflammatory markers and regulating carbohydrate metabolism (Godyla-Jablonski et al., 2024). The brain, due to its high oxygen demand and lipid-rich environment, is particularly vulnerable to OS, linking stress with anxiety, depression, and neurodegeneration (Queck, 2022; Queen et al., 2024).

Why this research is needed:

- WS and fatigue are widespread, with high prevalence in corporate and industrial settings.
- Current interventions are limited, and there is a need for preventive strategies targeting OS and inflammation.
- Antioxidant supplementation has shown promise but requires further evaluation in occupational populations.
- **Justification**

Globally, it is estimated that 12 billion workdays are lost annually due to depression, anxiety, and work-related stress. This represents an estimated cost of one hundred billion dollars in lost productivity for businesses (World Health Organization and ILO, 2022, 2024). In Mexico, according to 2024 data from the Mexican Social Security Institute (IMSS), at least 75% of workers suffer from work-related stress. This figure surpasses that of workers in China and the United States, where the rates are 73% and 74%, respectively. Stress is among the top five causes of illness in the Mexican population, with work being the primary cause. From an economic perspective, obesity increases the risk of work-related injuries by approximately 25% due to weight-related complications such as sleep apnea, work fatigue, and osteoarthritis, which contribute to physical injuries or biomechanical alterations leading to postural imbalances (Spanish Association of Occupational Medicine, 2021).

Overweight and obesity are non-communicable diseases that represent a significant public health challenge worldwide and in Mexico, with a prevalence of 75.2%, higher than the average of other Latin American countries (62.5%). In 2016, Mexican health authorities declared it a public health emergency.

In Mexican adults, abdominal obesity is associated with chronic diseases such as diabetes, hypertension, dyslipidemia, and cardiovascular diseases (Campos-Nonato et al., 2023).

Regarding metabolic syndrome in Mexican adults, the National Health and Nutrition Survey (ENSANUT) has reported rates between 40% and 50%, depending on the diagnostic criteria used. This represents a public health expenditure of 5% to 10% of the gross domestic product (Ministry of Health, 2024), making it one of the main public health challenges in Mexico.

Iza Safany et al. (2024) establish a connection between psychological stress and increased oxidative stress, which impacts metabolic alterations. Few treatments or supplements prevent oxidative damage and inflammation. One option is dietary supplements, which possess independent antioxidant, anti-fatigue, and anti-stress properties. This motivates the present research, which anticipates that administering dietary supplements (SA, 2.0 Seaweed, 2.1 Seaweed Premium, and 3.0 Astaxanthin) containing carotenoids, anthocyanins, and apple polyphenols will have beneficial effects on workers' health by reducing work-related stress, fatigue, and sleep quality, as well as the prevalence of metabolic syndrome. This is due to the regulation of biochemical parameters related to glucose and lipid metabolism and cardiovascular risk, as well as the modulation of cytokines involved in inflammation and the regulation of oxidative stress markers in workers of a private corporation in Mexico City, thus improving their quality of life.

- **Explanation for choice of comparator**

The study includes three antioxidant dietary supplements as experimental interventions and a placebo group as a comparator. The placebo was selected as appropriate control for several reasons. First, the placebo contains the same excipients as the active formulations—the same carrier base, flavorings, colorings, or sweeteners—except for the active ingredients. This ensures adequate blinding of participants, researchers, and outcome assessors, strengthens internal validity, and allows for a rigorous comparison of efficacy and safety among the three intervention groups. Second, the use of the placebo group allows for the isolation of the true psychological, immunological, and oxidative stress effects of the antioxidant dietary supplements by minimizing expectation bias and controlling for nonspecific effects arising from the clinical trial.

Third, there is no standard treatment to reduce psychosocial risks, such as work-related stress, work fatigue, and metabolic syndrome; therefore, the use of the placebo is ethically and scientifically justifiable. Finally, the inclusion of a placebo group allows evaluation of the specific effects of each formulation and distinguishes them from nonspecific responses, thereby enabling determination of the relative and absolute clinical impact of the supplements studied.

- **Ethical Justification for Placebo**

The use of placebo is ethically justified because:

- There is no established standard treatment for occupational stress, fatigue, or metabolic syndrome in corporate settings.
- The placebo contains the same excipients as the active formulations, ensuring blinding and minimizing expectation bias.
- All participants receive additional benefits: personalized nutritional counseling, access to the 2001 Diets platform, and free medical follow-up.

- **Relevance**

This study will assess whether dietary antioxidant supplements (marine algae extracts and astaxanthin, containing carotenoids, anthocyanins, and apple polyphenols) can reduce stress, fatigue, poor sleep quality, and MetS prevalence in workers of a private corporation in Mexico City. The findings will contribute to occupational health strategies, with potential implications for improving quality of life and reducing cardiovascular and metabolic risk in diverse working populations.

- **Study goals and objectives**

-General Objective

To evaluate the effect of three antioxidant dietary supplements on stress, work-related fatigue, and metabolic syndrome in employees of a private corporation in Mexico City.

-Specific Objectives

1. To identify employees with metabolic syndrome at the private corporation.
2. To identify the presence of stress and work-related fatigue in the employees of the corporation under study.
3. To quantify biochemical parameters (glucose, triglycerides, total cholesterol) in serum at the beginning, during, and at the end of the intervention for the employees in the study.

4. To determine the levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and anti-inflammatory cytokines (IL-10) in the employees' serum at the beginning, during, and at the end of the intervention.
5. To quantify oxidative stress markers (FRAP, SOD, NO, LPO) in the employees' serum at the beginning, during, and at the end of the intervention.
6. Detect the genetic expression of transcription factors involved in weight loss, fat reduction, and inflammation (Nrf2/ARE, NFκB, AP1, HNF4, P38 MAPK, ACM1, 2, 3) using real-time PCR.
7. Analyze the impact of antioxidants (carotenoids, anthocyanins, and apple polyphenols) on stress, work-related fatigue, and sleep quality at the beginning, during, and at the end of the intervention for the workers in the study.
8. Propose workplace intervention strategies based on the results obtained.

- **Specific objectives related to benefits and harms**

-Benefits: To determine whether antioxidant supplementation reduces work-related fatigue and stress, improves sleep quality, and favorably modifies inflammatory and oxidative stress biomarkers associated with metabolic syndrome.
-Harms: To evaluate the safety and tolerability of the supplements by monitoring adverse events, gastrointestinal symptoms, metabolic alterations, and any unexpected reactions throughout the intervention period.

- **Patient and Public Involvement**

-Details of, or plans for, patient or public involvement in the design, conduct, and reporting of the trial

Through voluntary participation, some study subjects took part in interviews before, during, and after supplementation to communicate their results, providing a non-technical perspective that allowed the findings to be contextualized in terms of their practical relevance and potential impact on occupational and metabolic health. Volunteers also participated in the public communication of the findings, collaborating on the development of clear language outreach materials and community outreach activities.

- **Trial design**

-Description of trial design, including type of trial (e.g., parallel group, crossover), allocation ratio, and framework (e.g., superiority, equivalence, non-inferiority, exploratory)

An experimental, controlled, double-blind trial will be conducted over a period of 6 months at a private Information Technology corporation in Mexico City. Participants who agree to participate in the study and meet the eligibility criteria will sign an informed consent form (See Appendix 1). It is important to note that the private corporation has authorized the implementation of this protocol.

- **Trial setting**

-Settings (e.g., community, hospital) and locations (e.g., countries, sites) where the trial will be conducted

The trial will be conducted in an occupational health setting within a private corporation located in Mexico City, Mexico. All study procedures, including participant recruitment, intervention delivery, and outcome assessments, will take place at the corporate site and affiliated certified laboratories, including LABBSO.

- **Study Population, Study Groups, and Supplementation Regimen.**

-Study population and study design

The study will be conducted in a private corporation with a total workforce of 206 employees. A representative sample of 120 workers will be recruited, corresponding to a 95% confidence level with a margin of error of ±5%.

Eligible participants will be employees aged 18 years or older, of either sex, with full-time, on-site work schedules, fixed work shifts, a body mass index (BMI) > 20 kg/m², and who will provide written informed consent. Exclusion criteria will include the presence of neurodegenerative diseases, pregnancy or lactation, hypothyroidism, psychiatric disorders, dyed hair, or the use of alternative dietary supplements.

The study will be designed as a randomized, parallel-group, four-arm clinical trial with an allocation ratio of 1:1:1:1. Participants will be randomly assigned to one of four groups (n = 30 per group): three intervention groups receiving different dietary supplements (2.0 Seaweed, 2.1 Seaweed Premium, and a special 3.0 astaxanthin formulation) and one placebo group. The dietary supplements will be donated by BioMaussan®, and the study will be conducted as a collaboration between the National School of Medicine and Homeopathy (ENMH) and BioMaussan®.

-Supplement administration and blinding

The dietary supplements and placebo will be administered orally at a dose of 5 mL twice daily (morning and afternoon, after meals) for a period of six months. All participants will receive the same dosage throughout the study, with no changes in formulation or supplier. In cases of taste intolerance, the supplement may be diluted in plain water to facilitate adherence.

Supplement distribution will take place in person at the corporate site. Adherence will be monitored daily via videoconference, and administration will be supervised by the study physician.

Intervention fidelity will be assessed by verifying the absence of remaining supplement at the end of the study period.

The study will be conducted under double-blind conditions. Neither participants nor investigators will be aware of group allocation. The dietary supplements and placebo will be provided in identical presentations to ensure blinding. Only the company responsible for supplement development (BioDesarrollos Valmex) will have access to the intervention codes.

-Intervention groups

Participants assigned to the intervention groups will receive the following formulations:

- 2.0 Seaweed: 40 mg astaxanthin, 13.2 mg fucoxanthin, and 84 mg apple polyphenols per day.
- 2.1 Seaweed Premium: 166 mg anthocyanins, 194.8 mg total xanthophylls, and 125 mg apple polyphenols per day.
- 3.0 Astaxanthin (special formulation): 500 mg/day of astaxanthin derived from *Haematococcus pluvialis*.
- The placebo group will receive a formulation consisting of purified water, citric acid, sorbic acid, allulose, and flavoring (See Appendix 1).

Although optimal therapeutic doses for fatigue, chronic stress, and metabolic syndrome have not yet been established, the selected doses are expected to provide antioxidant and anti-inflammatory effects based on previous evidence.

It is important to mention that the optimal therapeutic doses for relieving fatigue, chronic stress, and metabolic syndrome in humans have not yet been established, although several studies suggest beneficial effects at the concentrations evaluated in the present study, although independently, due to their antioxidant and anti-inflammatory properties, improving physical performance and mental well-being, as is the case with apple polyphenols and astaxanthin (Cásedas et al., 2020; Hurst et al., 2020; Stringham et al., 2017).

- **Safety considerations**

In controlled clinical trials (RCTs), expected adverse effects have been reported in study subjects at doses ranging from 0.5 mg to 20 mg, for periods of 1 to 4 months. These include slight reddening of the stool, increased bowel movements, dyspepsia, and itching (1 of 170 participants) in less than 1% of participants, in addition to diarrhea and muscle pain only in the first few days of supplementation (Trimarco et al., 2017; Choi et al., 2011). Coombes et al., in 2016, administered 12 mg of AXT daily to study subjects for 12 months, and no adverse effects were reported, as was Ito et al., in 2018, who administered 6 mg of AXT daily for 3 months in their RCT. It is concluded that, despite the dosage and administration time of AXT, few adverse effects are observed, and these are minor, without harming physical health.

In the case of polyphenols (including anthocyanins and apple polyphenols, used in this research and validated as polyphenols http://phenol-explorer.eu/compounds), it has been shown that harmful effects can occur under certain conditions. These include reported inhibition of iron absorption, digestive enzyme activity, and interactions with medications at high doses of polyphenols, which can lead to adverse health outcomes in susceptible patients (low iron levels, anemia) (Duda-Choodak and Tarko, 2023). In conclusion, at the doses evaluated in this research, no adverse effects were observed, and our study population did not present the susceptibility referred to as anemia.

In this clinical study, adverse effects of dietary supplements will be monitored as an essential component of safety and scientific validity. Expected, unexpected, and serious adverse effects will be monitored and recorded in a standardized adverse event reporting form for each study subject in the supplemented group. Continuous follow-up will be conducted through periodic evaluations to collect clinical and biochemical data, ensuring daily monitoring of adherence and reported symptoms via videoconference. Action procedures will be followed according to the severity of the adverse effects, and if necessary, the intervention will be immediately suspended for that participant, and the institutional ethics committee and COFEPRIS, the regulatory authority, will be notified.

The final report will include all analyses of the results, reporting the incidence of adverse effects by study group.

- **Strategies for achieving adequate participant enrollment**

To reach the target sample size, a multi-channel recruitment strategy will be implemented. Participants will be recruited through outreach activities within the "Lose Weight, Gain Life" wellness program in the workplace. Company Teams and in-person information sessions will be used to explain the study and answer questions. In addition, logistical barriers will be minimized through flexible scheduling, personalized reminders, and simplified procedures.
Dr. Mellado and the LABBSO team will monitor recruitment progress weekly, and if the enrollment rate is lower than expected, additional recruitment sites will be activated, and outreach efforts will be intensified. These strategies are designed to ensure that the study reaches the required sample size within the planned timeframe.

- **Concomitant care that is permitted or prohibited during the trial**

Participants may continue their usual medical care, including prescribed medications that do not interfere with the study outcomes. They may maintain their usual diet and level of physical activity.
The use of additional antioxidant supplements, vitamins, or minerals in doses exceeding the recommended daily intake is prohibited, as is the use of any nutraceutical product that may modify the inflammatory, oxidative stress, or metabolic biomarkers being assessed.
If a participant requires initiating a prohibited treatment for medical reasons, the reason will be documented, and the need to discontinue the assigned intervention will be evaluated, maintaining follow-up for safety analysis when possible.

- **Outcomes**

Primary outcomes will include:
1. Work-related stress, measured using the IMSS Work Stress Questionnaire. The primary analysis metric will be the change in stress score from baseline to 6 months. Results will be summarized as mean ± standard deviation and compared between groups. Measurements will be obtained at baseline, 3 months, and 6 months.
2. Work-related fatigue, measured using the Fatigue Severity Scale. The analysis metric will be the change from baseline to 6 months. Data will be summarized using mean ± standard deviation. Assessments will be conducted at baseline, 3 months, and 6 months.
3. Hair cortisol concentration (pg/mg), measured by competitive immunoassay. The analysis metric will be the change from baseline to 6 months. Results will be summarized as mean ± standard deviation. Measurements will be obtained at baseline, 3 months, and 6 months.

Secondary outcomes will include:
1. Biochemical markers of metabolic syndrome (fasting glucose, lipid profile, HbA1c), analyzed as changes from baseline to 6 months and summarized as mean ± standard deviation.
2. Inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-10), analyzed as changes from baseline to 6 months and summarized as mean ± standard deviation.
3. Oxidative stress markers (FRAP, nitric oxide, SOD activity), analyzed as changes from baseline to 6 months and summarized as mean ± standard deviation.
4. Gene expression levels of Nrf2/ARE, NFκB, AP-1, HNF4, and P38 MAPK, analyzed as relative expression changes using the 2-ΔΔCt method at baseline, 3 months, and 6 months.

- **Harms**

Harms will be defined as any unfavorable or unintended sign, symptom, or medical condition occurring during the study period, whether considered related to the intervention. Adverse events will be assessed systematically throughout the trial. Participants will be actively asked about the occurrence of adverse events during scheduled visits and daily monitoring, and all reported events will be recorded by the study physician, including their severity and potential relationship to the intervention.

- **Safety Monitoring and Adverse Event Reporting**

Although the study is considered **minimal risk**, a formal safety monitoring system will be implemented:

- **Definition of adverse event (AE)**: any unexpected medical sign, symptom, or condition occurring during the intervention, regardless of causal relationship.
- **Classification of AE**: mild, moderate, severe. Severe events include hospitalization, significant disability, or life-threatening conditions.
- **Reporting procedure**: Participants will report AEs directly to the study physician. The physician will document the AE using a standardized form and notify the IPN Bioethics Committee within 48 hours for severe events. All AEs will be recorded in a secure electronic database and followed until resolution.
- **Safety measures**: in case of severe AE, supplementation will be discontinued for the affected participant, and the research team will evaluate whether to modify or halt the study.

- **Participant timeline**

Participants will be recruited from a workforce of 206 employees at the study site. Eligibility screening and informed consent will be completed prior to enrolment. A total of 120 eligible participants will be enrolled and randomized at baseline (month 0) into four parallel groups (30 participants per group). Following randomization, participants will receive the allocated dietary supplement or placebo continuously for six months. Study assessments will be conducted at baseline, during follow-up, and at the end of the intervention period. Adherence and adverse events will be monitored throughout the study. The trial will conclude with a final assessment at month 6 (Table 1).

- **Sample size**

As this is a pilot randomized clinical trial, no formal sample size calculation based on statistical power will be performed. The sample size will be determined based on feasibility considerations and the size of the accessible population. The total number of employees at the study site is 206; therefore, a sample of 120 participants will be considered feasible and sufficient to assess recruitment, adherence, safety, and variability of outcomes, and to inform the design of a future definitive trial. Participants will be allocated equally into four study groups (30 participants per group).

- **Recruitment**

Participants will be recruited from a private IT corporation located in Mexico City, specifically targeting employees who work 12-hour shifts from Monday to Friday on-site. The recruitment process will be managed by the corporate Medical Service, which will issue invitations through occupational health campaigns distributed via corporate email. Participation will be encouraged by several key factors, including the provision of dietary supplements, continuous medical follow-up, and the convenience of attending consultations at the in-site clinic during working hours. Should the recruitment rate be slower than anticipated, the strategy will be reinforced by re-launching the email campaigns to ensure the enrollment goals are met.

Table 1. Participant timeline: Schedule of enrollment, interventions, and assessments

Study period	Pre-enrolment	Baseline (Month 0)	Month 3	Month 6 (Close-out)
TIMEPOINT	$-t_i$ to 0	0	t1	t2
ENROLMENT				
Eligibility screening	X			
Informed consent	X			
Randomization		X		
INTERVENTION				
Supplement/placebo administration		→	→	→
Distribution of supplements		X	X	X
ASSESSMENTS				
Clinical and anthropometric assessment		X		X
Biological sampling		X	X	X
Application of study instruments		X	X	X
Adherence monitoring		→	→	→
Adverse event monitoring		→	→	→
CLOSE-OUT				
End of supplementation				X

→ = continuous activity

- **Follow up**

Participants will be followed for six months during supplementation and an additional two-month post-intervention. Follow-up will include daily videoconference check-ins to monitor adherence and adverse events, monthly in-person visits for clinical evaluation, and quarterly laboratory tests to assess safety biomarkers. All adverse events will be documented in standardized case report forms, classified by severity and relationship to the supplement, and managed according to protocol guidelines. Serious adverse events will be immediately reported to the Ethics Committee and regulatory authorities. Post-intervention follow-up will ensure detection of late-onset adverse events and confirm the long-term safety of the antioxidant supplementation.

- **Methods: Assignment of interventions. Sequence generation**

The random allocation sequence will be generated by an independent researcher not involved in participant recruitment, intervention delivery, or outcome assessment. A computer-generated randomization list will be created using Microsoft Excel. Simple randomization with a 1:1:1:1 allocation ratio will be applied to assign participants to one of four groups (three supplement groups and one placebo group).

- **Allocation concealment mechanism**

The allocation sequence will be concealed using a secure electronic allocation list managed exclusively by the independent researcher. Group assignments will be disclosed only after a participant has been enrolled in the study, ensuring concealment of the allocation sequence until interventions are assigned.

- **Implementation**

The independent researcher will be responsible for generating the random allocation sequence and assigning participants to the intervention groups. Study personnel involved in participant enrollment, intervention administration, outcome assessment, and data analysis will not have access to the random allocation sequence.

- **Blinding**

This will be a blinded clinical study. Participants, care providers, outcome assessors, and data analysts will remain blinded to group allocation throughout the study.

- **Unblinding**

Unblinding will be permissible only in the event of a serious adverse event where knowledge of the allocated intervention is necessary for clinical management. In such cases, unblinding will be performed by the independent researcher. No other study personnel will have access to allocation codes.

- **Methods: Data collection, management, and analysis**

Data quality will be ensured using trained study personnel, certified laboratories, standardized procedures, and validated instruments. All assessments will be performed by personnel trained prior to study initiation and will follow standardized protocols. Laboratory analyses will be conducted in laboratories certified by the National System for Certification of Healthcare Establishments (SINaCEAM), using standardized and quality-controlled methods. Validated questionnaires and laboratory assays with established reliability and validity will be used to ensure accuracy and consistency of data collection.

-Identification of Metabolic Syndrome, Work-Related Stress, and Work-Related Fatigue in Workers

For the identification of MetS in workers, the diagnostic criteria of the Mexican Consensus on the Comprehensive Treatment of MetS (2002) carried out by the Mexican Association of Cardiologists will be used.

According to the consensus, the presence of abdominal obesity and at least one of the following criteria are required: Dyslipidemia: hypertriglyceridemia, hypercholesterolemia, or the patient is taking lipid-lowering drugs; altered blood pressure: systolic blood pressure ≥120 mmHg, diastolic blood pressure ≥80 mmHg, or the patient is taking antihypertensive drugs; altered blood glucose: fasting glucose ≥100 mg/dl, random glucose ≥140 mg/dl, glycated hemoglobin ≥5.7%, or the patient is taking hypoglycemic drugs; and, in addition, that the workers present abdominal obesity, which is evaluated with abdominal circumference: Women ≥80 cm and for men ≥90 cm and body mass index ≥25 kg/m^2.

The identification of work-related stress will be carried out through the application of the following instrument: the "Work Stress Test" of the Mexican Social Security Institute's Psychosomatic Problems Questionnaire, culturally adapted to the Mexican population, which uses a Likert-type scale ranging from "Never" (1 point) to "Very Frequent" (6 points). It consists of 12 questions that assess workers' physical and behavioral aspects related to work-related stress during the past three months. The scale yields results ranging from "No Stress" to "Severe Stress." This instrument has a Cronbach's alpha internal consistency of 0.90.

The International Labour Organization's "Work Stress Questionnaire," validated and approved by the World Health Organization, consists of 25 questions that evaluate work-related stressors based on organizational climate, organizational structure, organizational territory, technology, leader influence, lack of cohesion, and group support. It is a Likert-type scale ranging from “Never” (1 point) to “Always” (7 points). This questionnaire has a validity of 64%, 43.55% of variance explained, and a Cronbach's alpha reliability of 0.92, making it a reliable and useful instrument in this research. For the identification of work-related fatigue, the following questionnaire will be used: the “Work Fatigue Severity Scale” by Krupp et al. (1989), culturally adapted to the Mexican population and translated into the appropriate language in 2017 by Velasco-Rojano et al. It has 6 items, a Cronbach's alpha reliability of 0.93, and a good invariance fit [$\chi^2 = 12.56$, $p = .183$; CF1 = .99; RMSEA = .05], making it a reliable and valid measure for this research.

In addition, the Pittsburgh Sleep Quality Index (PSQI), consisting of 19 items, will be administered, assessing sleep quality, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction. This instrument has a Cronbach's alpha of 0.83 (Jiménez-Genchi et al., 2008).

-Determination of Chemistry Blood

For glucose, triglyceride, and cholesterol testing, workers who have fasted for 12 hours will have 8 mL of whole blood drawn via aseptic brachial venipuncture. The blood sample will be processed using a Mindray bs200® wet clinical chemistry analyzer with two-point spectrophotometry to obtain glucose, total cholesterol, and triglyceride levels.

This testing will be performed in private laboratories certified by the National System for Certification of Healthcare Establishments (SINaCEAM) of the General Health Council, which guarantees the quality of services. This is made possible through a corporate agreement with the private study group.

-Detection of cortisol in hair as a biomarker of chronic stress.

The methodology used to determine the concentration of capillary cortisol will consist of extraction and quantification, according to the supplier, using the competitive immunoassay technique (ALPCO, catalog number 11-CORHU). E01). Samples will be read at 450 nm using a microplate reader (Bioteck, USA). Cortisol levels will be calculated from a standard curve and expressed in pg/mg of hair.

-Quantification of cytokines involved in inflammation

Cytokines will be quantified in serum samples. Samples will be collected, frozen, and stored at -70°C. TNF-α, IL-1β, IL-6, and IL-10 concentrations will be quantified using human-specific ELISA kits (900-K25K, 900-M95, 900-M16, and 900-K21K, respectively), along with the ABTS ELISA Buffer kit (900-K00) (PeproTech Inc., Rocky Hill, NJ, USA) according to the manufacturer's instructions. Serum cytokines will be expressed as pg/mL.

-Determination of oxidative stress markers.

Total antioxidant capacity in workers' serum will be quantified using the FRAP method, following the technique described by Benzie & Devaki (2018) with some modifications. Nitric oxide (NO) concentrations will be determined by quantifying nitrates/nitrites (ADI-917-020, ENZO, Farmingdale, NY, USA), and sodium dendritic acid (SOD) concentrations will be determined (ADI-900-157, ENZO, Farmingdale, NY, USA), strictly following the manufacturer's instructions.

Plasma NO concentrations are represented as NO2- + NO3- nmol/mL. SOD concentrations will be quantified using an enzyme-linked immunosorbent assay (ELISA) (ADI-900-157, ENZO, Farmingdale, NY, USA), following the instructions.

-Gene expression was assessed using real-time PCR.

The expression levels of the genes Nrf2/ARE, NFκB, AP1, HNF4, P38 MAPK, and ACM1,2,3 were quantitatively analyzed by real-time reverse transcription PCR (qRT-PCR). Total RNA was isolated from serum samples using Trizol reagent, followed by DNase I treatment and cDNA synthesis using the SuperScript First-Strand Kit, with β-actin or GADPH as an internal control. qRT-PCR assays were performed on a Stratagene Mx3005 platform, using specific primers designed with Primer Express software, and relative expression was calculated using the 2-ΔΔCT method.

Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols

Participant retention will be promoted through regular contact and on-site follow-up by trained personnel. Participants who discontinue the intervention will be encouraged to continue outcome and safety assessments using standardized procedures, validated instruments, and certified laboratories to ensure complete follow-up and data quality.

- **Data management**

All participant data will be coded using a unique identification number to protect confidentiality. Personal identifiers will be stored separately from study data. Initial data entry will be performed by trained study personnel using an electronic database (Microsoft Excel®), following standardized procedures. The database will subsequently be exported to statistical software (SPSS v.27 for Windows®) for analysis.

Data quality will be ensured through continuous monitoring, including database checks, consistency reviews, and periodic audits conducted by the research team. Access to electronic records will be restricted to authorized personnel and protected by password-controlled systems. Data will be stored on secure institutional computers with regular backups. Detailed data management procedures are described in the study protocol and corresponding appendices.

- **Statistical methods**

Descriptive statistics will be used to summarize participant characteristics and study variables. Data distribution will be assessed using the Shapiro-Wilk test. Comparisons between the intervention and placebo groups will be performed using ANOVA for parametric data, or the Kruskal-Wallis test if the data do not meet normality criteria. Any difference with a p-value ≤ 0.05 will be considered significant.

As this is a pilot randomized clinical trial, no formal sample size calculation based on statistical power is planned. The sample size is based on feasibility considerations and the size of the accessible population. Statistical analyses will be conducted according to the intention-to-treat principle. Missing data will be handled using appropriate methods, including multiple imputation when applicable.

- **Data monitoring committee**

A Data Monitoring Committee will not be established due to the pilot, single-center, low-risk nature of the study. Safety will be monitored continuously by the study team.

No formal interim analysis is planned. Study continuation or termination decisions will be based on safety considerations and made by the principal investigator in consultation with the research team and ethics committee.

Trial conduct will be monitored on an ongoing basis by the research team to ensure protocol adherence, data completeness, and participant safety.

- **Internal Monitoring Plan**

Given the sample size and low-risk nature of the intervention, no external independent committee will be established. Instead, an internal monitoring structure will be implemented:

- Monitoring team: composed of three principal investigators (Elvia Pérez Soto, María del Carmen López García, and Virginia Sánchez-Monroy).
- Responsibilities: Monthly review of safety and adherence data, validation of AE records and protocol compliance and oversight of laboratory and questionnaire data quality.
- Safety meetings: held every six weeks to evaluate study progress and determine corrective actions if needed.
- Final safety report: prepared at study completion and included in the main publication.

Ethics

- **Research ethics approval**

The study protocol, informed consent form, and related materials were submitted for review and approval to the Bioethics Committee of the National School of Medicine and Homeopathy, registration number CBE/002/2025, before participant recruitment began. The study was conducted in accordance with ethical principles for research involving human subjects, and no study procedure was initiated without ethical approval.

- **Protocol amendments**

Any important protocol modifications will be submitted for review and approval to the Research Ethics Committee/Institutional Review Board prior to implementation. Approved amendments will be communicated to the study sponsor, investigators, and relevant study personnel. Participants will be informed of any protocol changes that may affect their participation, and updated informed consent will be obtained when required. Relevant trial registries will be updated accordingly.

- **Consent or assent**

Written informed consent will be obtained from all potential participants prior to any study procedures. The consent process will be conducted by a trained member of the research team, who will provide detailed verbal and written information about the study objectives, procedures, potential risks, and benefits. Participants will be given sufficient time to ask questions and to decide whether to participate. Consent will be documented by the participant’s signature on the informed consent form.

No ancillary studies are planned. Participant data and biological specimens will be used exclusively for the objectives described in this protocol. No additional consent for future use of data or biological samples will be required.

- **Consent, Safety, and Monitoring**

1. Informed Consent and Data Protection

All participants will receive clear and comprehensive information about the study's objectives, procedures, potential benefits, and possible risks. The consent process will include:

- Information sheet written in accessible language, explaining the study nature, interventions, duration, and potential side effects.
- Individual orientation session with a trained investigator, who will answer questions and confirm participant understanding.
- Signed informed consent obtained in the presence of an independent witness, ensuring voluntary participation.
- Data protection**:** personal information will be coded using alphanumeric identifiers, stored securely on IPN servers, and accessible only to authorized research staff.
- Right to withdraw**:** participants may leave the study at any time without consequences for their employment or medical care.

This process complies with the Mexican Federal Law on Protection of Personal Data and the principles of the Declaration of Helsinki.

- **Confidentiality**

Personal information from potential and enrolled participants will be collected only as necessary for eligibility assessment and study conduct. All participants will be assigned a unique identification code, and identifiable information will be stored separately from study data. Access to personal information will be restricted to authorized members of the research team.

Data will be stored in secure electronic databases with password-protected access. Participant information will not be shared outside the research team except as required by ethics or regulatory authorities. After study completion, data will be retained in accordance with institutional and ethical guidelines, and confidentiality will be maintained at all stages of the trial.

- **Ancillary and post-trial care**

Given the low risk of the dietary supplements used in this pilot study, only one kit of dietary supplements is provided as compensation for participation. Participants who experience adverse events related to the study will receive appropriate medical evaluation and care from the study physician and, if necessary, will be referred to appropriate healthcare services. No post-study care beyond standard clinical referral is provided.

- **Duration of the Project**

2025

Stages/Time	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Ago**	**Sep**	**Oct**	**Nov**	**Dec**
Definition of the research protocol		**X**									
Protocol writing for ethics committee evaluation	**X**										
Ethical and regulatory approval: submit to the ethics committee, prepare informed consent		**X**									
Logistical preparation: Formal request for permission to carry out the research, establish supplementation schedule.			**X**								
Validation of techniques, instruments, and diagnosis of metabolic syndrome. Randomization and blinding: Generation of allocation list, preparation of supplements/placebo.			**X**								
Participant recruitment: dissemination, eligibility assessment, signing of informed consent				**X**							
Implementation of safety procedures: continuous monitoring of adverse events.				**X**							
First distribution of supplements for participants. Supplement supply sufficient until July 31th.				**X**							
First biological sampling for analysis. First application of study variable instruments. Official start of consumption of supplements. All groups.					**X**						
Second distribution of supplements for participants. Supplement supply sufficient until September 29th.						**X**					
Second biological sampling for analysis. Second application of study variable instruments.							**X**				
Third, distribution of supplements for participants. Supplement supply sufficient until November 29th.								**X**			
Conclusion of supplementation phase										**X**	
Last biological sampling for analysis. Last application of study variable instruments.										**X**	
Interpretation of measuring instruments											**X**

- **Problems anticipated**

Stages/time	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Ago**	**Sep**	**Oct**	**Nov**	**Dec**
Interpretation of laboratory studies and diagnosis of metabolic syndrome	**X**	**X**										
Determination of hair cortisol concentrations			**X**									
Determination of oxidative stress biomarkers				**X**	**X**							
Determination of inflammatory cytokines					**X**	**X**						
Statistical analysis						**X**	**X**					
Interpretation of results							**X**	**X**				
Dissemination and outreach of research									**X**			

Challenges affecting adherence to the activity schedule and budget have been considered. The anticipated problems and mitigation strategies to ensure the continuity of the study analyses are described below:

1. Low adherence to supplementation. Participants may forget or discontinue their daily intake of the dietary supplement due to a prolonged intervention period and workload. Therefore, they will be reminded through the WhatsApp group, with daily follow-up by the BioMaussan nutrition team, who do not have operational authority, and the LABBSO team. Attendance will be taken from the daily log at the same time as the supplement administration, twice a day.

Monitoring will be carried out via WhatsApp and records.

2. Delays or technical limitations in the laboratory. Late processing of biochemical and molecular samples due to high sample volume at the three time points or limited kit capacity. Therefore, certified alternative laboratories will be considered, analyses will be scheduled in advance, and validated alternative techniques will be considered.

3. Variability in stress and fatigue measurements. Questionnaire scores may fluctuate due to external factors and individual variability. This can be mitigated by standardizing assessment times for study groups and using instruments validated in the Mexican population to reduce variability. For some techniques (immunoassay for cortisol and FRAP assay), these can be repeated at least twice on the same study samples.

4. Increased reagent costs due to market fluctuations. This can be mitigated through fixed-price agreements with suppliers and by prioritizing essential analyses.

- **Project management**

To ensure the clinical study's success, a structured operational framework will be implemented to guarantee regulatory compliance, methodological integrity, and timely study execution. The principal investigator (PhD Elvia Pérez Soto) will oversee protocol implementation and ethical compliance. Co-investigators will contribute clinical, biochemical, immunological, and molecular expertise (PhD María del Carmen López García, PhD Hamlet Avilés-Arnaut, and PhD Virginia Sánchez-Monroy).
A project manager will coordinate the timeline, budget, documentation, and internal communication (Ms. Monserrat Noemí Mellado Gutiérrez and Dr. Gabriel Lara Hernández). The head of the LABBSO will oversee the implementation of laboratory techniques, sample processing, and quality control for all experiments (PhD Elvia Pérez Soto and the LABBSO team). Safety oversight will be the responsibility of the school's ethics committee, to which any adverse effects of the supplements will be reported.

- **Ethics**

In accordance with the Regulations of the General Health Law on Health Research, Art. 17, II, the risk of this research was considered as minimal, due to the taking of blood samples, as well as hair. As indicated by the guidelines of the Nuremberg Code (1947) and the Declaration of Helsinki (1964), privacy and confidentiality of the data will be maintained. Before the sample collection procedure, all participants must provide their consent by signing the informed consent form (Appendix 1).
Furthermore, according to the Regulations of the Health Law of the Federal District, Chapter XVIII, Health Research, Article 232: This refers to the support and incentives directly provided for the development of specific programs aimed at health research, particularly in the areas of education, environmental effects, nutrition, eating disorders, among others, for the dissemination and application of their results and discoveries. Article 234: Each health research program aims to leverage the city's scientific and technological activities to generate knowledge and its application through products, goods, and services useful in the prevention, care, and health of Mexico City residents, and the economic growth of Mexico City. And Article 235: The Healthy City program, It is composed of the following subprograms:
I. Obesity, Diabetes, and Cardiovascular Diseases
II. Mental Health and Addictive Behaviors or
III. Emerging and Re-emerging Diseases.
Risk level of the research for measuring biochemical parameters in blood and hair: Research with no risk to humans. This research comprises a development activity to understand the relationship between MetS and work-related stress, using hair cortisol concentration and blood stress biomarkers.

All risks will be minimized through standardized procedures, trained personnel, and continuous safety monitoring.

Risk level of the research for antioxidant supplementation: Research with minimal risk to humans. This research involves data collection through common procedures in diagnostic and treatment physical examinations, considering three blood sample collections, moderate physical exercise in volunteers, and research with commonly used dietary supplements, which are authorized for sale, using established indications, dosages, and routes of administration. The COFEPRIS authorizes these dietary supplements. In compliance with Article 215, Section V of the General Health Law, which defines dietary supplements as:

"products based on herbs, plant extracts, traditional foods, dehydrated or concentrated fruits, with or without added vitamins or minerals, which may be presented in pharmaceutical form and whose purpose is to increase total dietary intake, complement or supply any of the components," as well as Title Nineteen of the Regulations for Sanitary Control of Products and Services, regarding dietary supplements: "Dietary supplements may consist of carbohydrates, proteins, amino acids, fatty acids, metabolic compounds, plants, herbs, algae, dehydrated traditional foods, or others established by the Ministry, presented either in isolation or in combination, with or without added vitamins or minerals, and their consumption must not represent a health risk."

- **Informed consent forms**

Informed consent was obtained before any study procedure. Participants received clear verbal and written information from the occupational health physician, including details about the study objectives, procedures, risks, benefits, confidentiality, and their right to withdraw at any time without consequence. Consent will be documented in writing on paper. The participant will sign the physical form, as will the researcher as a witness, and it will be kept in the clinical study record. Confidentiality will be ensured through secure storage, restricted access, and pseudonymization of data. Biological samples will be used according to approved protocols (Appendix 2, 3).

- **References**

- Benzie, I. & Devaki, M. (2018). The Ferric Reducing/Antioxidant Power (FRAP) Assay for Non-Enzymatic Antioxidant Capacity: Concepts, Procedures, Limitations and Applications.
- Centro Nacional de Vigilancia Epidemiológica y Control de Enfermedades, Subsecretaría de Prevención y Promoción de la Salud, Secretaría de Salud y el Grupo Mexicano para el estudio de síndrome metabólico y resistencia a la insulina. (2016). Posición de consenso: recomendaciones para el diagnóstico, estratificación del riesgo cardiovascular, prevención y tratamiento del síndrome metabólico. Revista Mexicana de Cardiología, 17 (1), 3-61
- Choi, H. D., Youn, Y. K., & Shin, W. G. (2011). Positive effects of astaxanthin on lipid profiles and oxidative stress in overweight sub- jects. *Plant Foods for Human Nutrition*, *66*(4), 363–369. https://doi.org/ 10.1007/s11130-011-0258-9
- Coombes, J. S., Sharman, J. E., & Fassett, R. G. (2016). Astaxanthin has no effect on arterial stiffness, oxidative stress, or inflammation in renal transplant recipients: A randomized controlled trial (the XANTHIN trial). *The American Journal of Clinical Nutrition*, *103*(1), 283–289. https://doi.org/10.3945/ajcn.115.115477
- Godyla-Jablonski M. (2024) Effects of Anthocyanins on Components of Metabolic Syndrome. Nutrients, 16 (1103).
- Darabi, F.; Keshavarzi, M.; Khanjani, N.; Yousefinejad, S.; Zamanian, Z. Effect of Antioxidants on Oxidative Damage, and Hematological and Psychological Parameters in Cement-Exposed Workers. Hyg. Environ. Health Adv. 2023, 6, 100060, doi:10.1016/j.heha.2023.100060.
- Duda-Chodak, A., & Tarko, T. (2023). Possible Side Effects of Polyphenols and Their Interactions with Medicines. Molecules, 28(6), 2536. https://doi.org/10.3390/molecules28062536
- Grupo de Trabajo de la Asociación Española de Especialistas en Medicina del Trabajo. (2021). La obesidad en salud laboral: una visión desde la medicina del trabajo de la persona trabajadora que vive con obesidad. Asociación Española de Especialistas en Medicina del Trabajo, 9-42.
- Instituto Mexicano del Seguro Social. (2018). Guía de práctica clínica para diagnóstico y tratamiento del sobrepeso y obesidad exógena. Catálogo maestro de guías de práctica clínica, GPC-IMSS-046-18, 9-18.
- Ippoliti, P., Carbosiero, N., Cantiano, F., Massoni, M.R., Ricciardi, L., Ricci, T.& Archer, S. (2017)., Work-related Stress, over-nutrition and cognitive disability. Clínica Terapéutica, 168 (1), 42-47. https://pubmed.ncbi.nlm.nih.gov/28240762
- Iza Safany Che Hanafi, Chandrarohini Saravanan, Rabiatul Basria S. M. N. Mydin. (2024). Oxidative Stress: Insights into Nutrition, Psychological Stress, Environmental Exposure, and Antioxidants Roles, Journal of Angiotherapy, 8(3), 1-6, 9452
- Kanner, J. (2023). Food Polyphenols as Preventive Medicine. Antioxidants. https://doi.org/10.3390/antiox12122103
- Kyazimova, N. D., & Kornyakova, V. V. (2024). Influence of polyphenolic compounds on human health and the course of a number of diseases. https://doi.org/10.61634/2782-3024-2024-13-87-91
- Livak, K.J.; Schmittgen, T.D. Analysis of Relative Gene Expression Data Using Real Time Quantitative PCR and the 2-ΔΔCT Method. Methods 2001, 25, 402–408,

doi:10.1006/meth.2001.1262. Duarte-Ayala R.E., Velasco-Rojano E., Sánchez Sosa J.J. Reyes-Lagunes L. I. (2019). Validación psicométrica de la escala de gravedad de fatiga en médicos residentes mexicanos. Revista del Laboratorio de Evaluación Psicológica y Educativa, 17 (2), 113-125. DOI: https://revistas.unc.edu.ar/index.php/revaluar

- López-Roldan P., Mach N. (2012). Efecto del consumo de astaxantina en la salud. Revista Española de Nutrición Comunitaria, 18 (3), 64-177.
- Morera, Luis & Tempesti, Tomas & Pérez, Edgardo & Medrano, Leonardo. (2019). Biomarcadores en la medición del estrés: una revisión sistemática. Ansiedad y Estrés. 25. 10.1016/j.anyes.2019.02.001.
- Shoji, T., Masumoto, S., Miura, T., & Desjardins, Y. (2024). Exploring the potential of apple (poly)phenols: A systematic review of randomized controlled trials on metabolic diseases prevention. https://doi.org/10.1016/j.tifs.2024.104419
- Stringham, N.T.; Holmes, P.V.; Stringham, J.M. Effects of Macular Xanthophyll Supplementation on Brain-Derived Neurotrophic Factor, pro-Inflammatory Cytokines, and Cognitive Performance. Physiol. Behav. 2019, 211, 112650, doi:10.1016/j.physbeh.2019.112650.
- Terao J. (2023). Revisiting carotenoids as dietary antioxidants for human health and disease prevention. Food & function, 14(17), 7799–7824.
- Queen, C.J.J.; Sparks, S.A.; Marchant, D.C.; McNaughton, L.R. The Effects of Astaxanthin on Cognitive Function and Neurodegeneration in Humans: A Critical Review. Nutrients 2024, 16, 826, doi:10.3390/nu16060826.
- Trimarco, V., Battistoni, A., Tocci, G., Coluccia, R., Manzi, M. V., Izzo, R., & Volpe, M. (2017). Single blind, multicentre, randomized, controlled trial testing the effects of a novel nutraceutical compound on plasma lipid and cardiovascular risk factors: Results of the interim analysis. *Nutri- tion, Metabolism, and Cardiovascular Diseases*, *27*(10), 850–857. https://doi.org/10.1016/j.numecd.2017.08.003
- Wendsche J, Ihle A, Wegge J, Penz MS, Kirschbaum C, Kliegel M. (2020). Prospective associations between burnout symptomatology and hair cortisol. Int Arch Occup Environ Health, 93: 779-788. doi:10.1007/s00420-020-01528-3
- Wester V. & Van Rossum, E. (2015). Clinical applications of cortisol measurements in hair. Eur J Endocrinol 2015;173:M1-M10. doi:10.1530/EJE-15-0313
- Yang, H., Song, R., Xie, Y., Qian, Q., Wu, Z., Han, S., & Li, X. (2023). Apple Polyphenol Extract Ameliorates Atherosclerosis and Associated Cognitive Impairment through Alleviating Neuroinflammation by Weakening TLR4 Signaling and NLRP3 Inflammasome in High-Fat/Cholesterol Diet-Fed LDLR-/- Male Mice. Journal of Agricultural https://doi.org/10.1021/acs.jafc.3c01966

- **RESEARCH PROTOCOL: PART 2**

Item	Reagent/Material/justification	Quantity	Cost	Total Cost (IVA)
1	Preparation of dietary suplements and placebo/To supplement study groups and incentive participants (dietary supplements)			$ 1142467.5
2	External services: Blood chemistry determination/Biochemical parameters to metabolic syndrome	360		$67780
3	Human TNF-α ELISA Development kit, clave 900-K25, Peprotech/inflammatory biomarkers	1	13283.5	$13283.5
4	Human IL-4 ELISA Development kit, clave 900-K14, Peprotech/inflammatory biomarkers	1	13283.5	$13283.5
5	Human IL-6 ELISA Development kit, clave 900-M16, Peprotech/inflammatory biomarkers	1	13283.5	$13283.5
6	Human IL-10 ELISA Development kit, clave 900-K21K, Peprotech/inflammatory biomarkers	1	13283.5	$13283.5
7	Human IL-1β ELISA Development kit, clave 900-M95, Peprotech/inflammatory biomarkers	1	13283.5	$13283.5
8	Kit ABTS ELISA Buffer kit, 900-K100, Peprotech/inflammatory biomarkers	3	7650.49	$7650.4
9	Nitric oxide, ADI-917-020, enzo/Oxidative stress biomarker	8	10949.24	$87593.9
10	Kit Elisa cortisol Human (saliva) plate 96 wells ALPCO/Determination of chronic stress biomarker	6	5582.0	$38850.7
11	FeCl3 × 6H2O Ferric chloride hexahydrate RA 500 g/Antioxidative stress biomarker technique	1	$1,495.0	$1734.2
12	±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97%, Sinónimos: Trolox/ For oxidative stress techniques	1	$7789.0	$9035.24
13	Gallic acid (>98%), 100 g,/For oxidative stress techniques	1	$3,235.0	$3752.6
14	ABTS 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt 2 G/For oxidative stress techniques	1	$3,721.0	$4316.3
15	Methanol CMOS 4 L/For oxidative stress techniques	1	$1,040.0	$1206.4
16	ABTS Solution/For oxidative stress techniques	1	$5,905.0	$6,849.8
17	2,4,6-Tripyridyl-s-triazine/For oxidative stress techniques	1	$5026.0	$5830.1
18	Bovine Serum Albumin/For oxidative stress techniques	1	$7128.0	$8268.4
19	Trichloroacetic Acid R. Assay 99% 500 Grams/For oxidative stress techniques	1	$6250	$ 7250
20	α-Tocopherol British Phamacopoeia (BP) Reference Standard α-Tocopherol/For oxidative stress techniques	1	$5953,0	$6905.4
21	2-Thiobarbituric Acid Assay 98% 100 GRAMS/For oxidative stress techniques	1	$8864.0	$10282.2
22	Tube with lilac/purple lid, BD rack with 100/For blood extraction	9	$4,177.7	$4,177.7

23	Green Blood Collection Needle 21G X 38 mm (1 1/2") Vacusystem, 100 Pieces/ For blood extraction/For plasma collection	1	$4999	$4999
24	2 mL centrifuge microtubes, 200 pieces/ For plasma collection	1	3259.6	$3259.6
25	1.5 mL centrifuge microtubes, 500 pieces/ For plasma collection	1	$4,002.0	$4,002.0
26	0.6 mL microtubes, 10,000 pieces/ For plasma collection	1	$7,482.0	$7,482.0
27	SuperScript™ IV One-Step RT-PCR System, invitrogen, 12595100/For molecular biology techniques	1	38,739.6	$38,739.6
28	Trizol LS reagent and Phasemaker Tubes, complete system, no. Catálogo A33253/ For molecular biology techniques	1	15886.1	$15886.1
29	Primer design (Nrf2/ARE, NFκB, AP1, HNF4, P38 MAPK, ACM1,2,3)/ For molecular biology techniques	10	$1500.0	$15000.0
30	SYBR™ Safe DNA Gel Stain, invitrogen, S33102/ For molecular biology techniques	5	$3200.0	$16000.0
31	100 bp DNA ladder-50 µg, Invitrogen/ For molecular biology techniques	1	$2084.7	$2418.3
			Total	**$1,584,488.1 MN**

- **Other support for the Project**

Project	**Register Number**	**Unit of assignment**
1. Efecto de la suplementación con antioxidantes sobre el estrés y fatiga laboral en trabajadores con alteraciones metabólicas. Effect of antioxidant supplementation on stress and work fatigue in workers with metabolic disorders.	20251035	ENMH-IPN
2. Burnout y diabetes mellitus en médicos del primer nivel de atención en consultorios de farmacias en la Ciudad de México. Burnout and diabetes mellitus in primary care physicians in pharmacy clinics in Mexico City.	20253601	ENMH-IPN
3. Evaluación del efecto inmunomodulador, antioxidante y antiinflamatorio de carotenoides y fucoidanos en enfermedades crónicas no transmisibles. Evaluation of the immunomodulatory, antioxidant and anti-inflammatory effect of carotenoids and fucoidans in chronic non-communicable diseases.	Derived from the letter of intent between ENMH-IPN and BioMaussan.	BioMaussan

- **Collaboration with other scientists or research institutions**

Research institutions	**Register Number**
Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Instituto de Biotecnología, Campus Ciudad Universitaria	Hamlet Avilés-Arnaut
Escuela Superior de Medicina, Instituto Politécnico Nacional, Casco de Santo Tomas 11340, Ciudad de México, México.	Virginia Sánchez-Monroy

- **Other research activities of the investigators**

The principal investigator PhD, principal investigator, carries out a multidisciplinary project concerning the “Evaluation of the immunomodulatory, antioxidant and anti-inflammatory effect of carotenoids and fucoidans in chronic non-communicable diseases”, linked with the company BioMaussan.

- **Financing and insurance**

The study is funded by the Secretaría de Investigación y Posgrado, Instituto Politécnico Nacional (IPN), BioMaussan and Valmex through a collaboration agreement with the Instituto Politécnico Nacional (IPN). The funders have no role in the study design, conduct, analysis, or publication. The research team includes the heads of the Research and Development Departments of BioMaussan and Valmex, participating in an individual scientific capacity. Their companies do not intervene in the study.

- **Sponsor and Insurance**

The Instituto Politécnico Nacional (IPN) acts as the sponsor and provides institutional insurance and indemnity for the study.

The study subjects have Social Security with the IMSS and Major Medical Expense Insurance from Inbursa.

- **Curriculum Vitae of investigators**

21.1. Principal investigator

CURRICULUM VITAE. Elvia Pérez Soto, PhD in Health Sciences

Correo electrónico:elvperezs@ipn.mx

ORCID: https://orcid.org/0000-0002-5673-0825

Elvia Pérez Soto is a biologist, holds a Master of Science degree in Molecular Biomedicine, and a Doctorate in Health Sciences. She is affiliated with the National School of Medicine and Homeopathy (ENMH) of the National Polytechnic Institute (IPN). She is a member of the National System of Researchers, Level 1 (SNII-1) in the interdisciplinary area, and Head of the Biomedicine and Occupational Health Laboratory (LABBSO), which belongs to the Master of Science in Occupational Health, Safety, and Hygiene (MCSOSH, in spanish) program at ENMH-IPN. She has coordinated six research projects and participated in seven research projects at the IPN. She also collaborates on research with BioMaussan SA de CV, where she has secured external funding.

Her main lines of research focus on the study of the antioxidant, anti-inflammatory, and immunomodulatory properties of carotenoids, apple polyphenols, plant tocotrienols, and fucoidans in metabolic disorders, steatohepatitis, work-related stress, work-related fatigue, and cancer.

She has supervised 6 undergraduate theses, 4 master's theses, and 1 specialty thesis. His research has won first place in presentations at the Research Forum of the Network of Postgraduate Programs in Occupational Health, the Annual Scientific Conference on Metabolic Syndrome, and the Student Pharmacology Congress. She has also participated in institutional development projects that expand the research capacity of undergraduate and graduate students, promoting dissemination and outreach. She has published 12 international JCR-indexed articles, 5 articles in national journals, and 28 institutional articles, and has participated in 9 international and 42 national conferences.

RECENT PUBLICATIONS

-Lara-Hernández, G., Ramos-Silva, J. A., **Pérez-Soto, E.,** Figueroa, M., Flores-Berrios, E. P., Sánchez-Chapul, L., Andrade-Cabrera, J. L., Luna-Angulo, A., Landa-Solís, C., & Avilés-Arnaut, H. (2024). Anticancer Activity of Plant Tocotrienols, Fucoxanthin, Fucoidan, and Polyphenols in Dietary Supplements. Nutrients, 16(24), 4274. https://doi.org/10.3390/nu16244274

Pascacio-Lugo, A., López-García, M.C. & **Pérez-Soto, E.** (2024). Predisposing, protective factors and strategies to confront burnout syndrome in doctors: an unavoidable reality. Revista de Psicología de la Universidad Autónoma del Estado de México, 13 (37), 103-119. Doi https://doi.org/10.36677/rpsicologia.v13i37.24088.

López-García, M. del C., Sánchez-Sánchez, C. I., Fernández-Martínez, E. & Pérez-Soto, E. (2025). Book Chapter: Work-related stress, burnout, and cortisol in educators during COVID-19: Early detection and recommendations. In (Ed.) Philosophical and social perspectives on mental health (pp. 246-275). Editorial PLAGCIS. 978-628-96829-5-3 ISBN. https://doi.org/10.69821/PLAGCIS.6.c29

- **CURRICULUM VITAE.** María del Carmen López García, PhD in Occupational Health Sciences.

Correo electrónico: mlopezg@ipn.mx

ORCID: http://orcid.org/0000-0002-7309-2186

María del Carmen López García is a Medical Physician, Master of Science in Occupational Health and Doctor of Occupational Health Sciences.

She has been a professor at the National School of Medicine and Homeopathy of the Instituto Politécnico Nacional (IPN) since 2002, at the undergraduate and postgraduate levels, where she is part of the basic core of the Master of Science in Occupational Health, Safety and Hygiene.

Her lines of research are occupational health and psychosocial risk factors at work (job satisfaction; Stress, Biomarkers and Health Effects; Women and Work). She has supervised a total of 29 postgraduate theses in recent years.

As a researcher, she has participated since 2018, as director in both short-term and multidisciplinary research projects at the IPN, and published in national and international journals. The latest publications are:

- Pascacio-Lugo, A., López-García, M.C. & Pérez-Soto, E. (2024). Predisposing, protective factors and strategies to confront burnout syndrome in doctors: an unavoidable reality. Revista de Psicología de la Universidad Autónoma del Estado de México, 13 (37), 103-119. Doi https://doi.org/10.36677/rpsicologia.v13i37.24088.
- López-García, M. del C., Sánchez-Sánchez, C. I., Fernández-Martínez, E. & Pérez-Soto, E. (2025). Book Chapter: Work-related stress, burnout, and cortisol in educators during COVID-19: Early detection and recommendations. In (Ed.) *Philosophical and social perspectives on mental health* (pp. 246-275). Editorial PLAGCIS. 978-628-96829-5-3 ISBN. https://doi.org/10.69821/PLAGCIS.6.c29 .

- **CURRICULUM VITAE.** Monserrat Noemí González Mellado, Physician.

Correo electrónico: momelladoc@gmail.com, mgonzalezm2401@alumno.ipn.mx
ORCID: https://orcid.org/0009-0003-5959-7016

A graduate of Universidad Justo Sierra, her professional career is characterised by a holistic approach that combines high-quality clinical care with the strategic management of occupational health and safety. She is currently pursuing a Master of Science (MSc) in Occupational Health, Safety and Hygiene at the Instituto Politécnico Nacional, consolidating her profile as a leader in healthy work environments.

She possesses extensive experience in patient screening and outpatient follow-up , notably through her active participation in the National COVID-19 Vaccination Strategy and the management of chronic-degenerative conditions, for which she received recognition from the Mexico City Ministry of Health for her valuable contribution. Her work has extended to the voluntary sector, where she has led early disease detection brigades for vulnerable populations, demonstrating a strong social commitment and high organizational capacity.

In the corporate sector, she specialises in health campaign management, data analysis, and implementing psychosocial wellbeing and ergonomics programmes. Her continuous professional development is extensive, including diplomas in “The Integral Approach to Chronic Diseases”, “Probiotics in Non-Alcoholic Fatty Liver Disease and Other Functional Digestive Disorders”, “Healthy Ageing”, “Geriatric Medicine”, and “Medical Treatment of Obesity”, accredited by institutions such as Carlos Slim Foundation, the National Regulatory Committee for General Medicine (CONAMEGE), and the National Institute of Geriatrics in México.

Furthermore, she holds multiple certifications and training from the Mexican Social Security Institute (IMSS), such as “Occupational Health”, “Psychosocial Wellbeing in the Workplace and the New Normal”, and “Promoter of Healthy Work Environments”. Additionally, she has completed the “Introduction to Food and Health” course at Stanford University.

Beyond her clinical practice, she is an active member of the Mexican College for the Study of Metabolism and Cellular Nutrition. She possesses advanced leadership , scientific writing, and health and safety regulatory management skills, enabling her to contribute effectively to organisational productivity and health.

- **CURRICULUM VITAE.** Eduardo Nateras Molina

Medical Doctor

Correo electrónico: enaterasm@ipn.mx, drnateras@hotmail.com

ORCID: https://orcid.org/0009-0005-8045-2318

Medical Doctor

PROFESSIONAL PROFILE

Physician with extensive experience in clinical care within both institutional and private settings. Specialized in metabolic syndrome and addictions. Dedicated to providing comprehensive, high-quality medical attention to staff members at the Instituto Politécnico Nacional (IPN) and to patients in private practice.

EDUCATION

Bachelor of Medicine (General Physician and Midwife)

Instituto Politécnico Nacional (IPN) – Escuela Superior de Medicina, 1986

Specializations:

- Metabolic Syndrome
- Addictions

PROFESSIONAL EXPERIENCE

Academic Secretariat – Instituto Politécnico Nacional (IPN)

Staff Physician

- Provided medical care to academic, administrative, and operational personnel.
- Conducted clinical evaluations, follow-up, and referrals when needed.
- Delivered comprehensive medical attention within the institution.

Sanatorio Yukon

Private Practice Physician

- Delivered private medical care to patients with diverse clinical needs.
- Performed diagnosis, treatment, and follow-up of chronic and acute conditions.
- Specialized management of metabolic syndrome and addiction-related cases.

LANGUAGES

- French: A1

- **CURRICULUM VITAE.** Gabriel Lara Hernández, MS

Correo electrónico: gabobmol@gmail.com

Training

Medical doctor with a Master's degree in Biomedical Sciences, specializing in Molecular Biology, from the Military School of Graduate Health of the Secretariat of National Defense (SEDENA) with an honorable mention.

Various courses including: Aerospace Medicine; Aerospace Medicine and Psychology; Neonatal Resuscitation; Prehospital Life Support (PHTLS) Advanced Trauma Life Support (ATLS) Microarrangements in Obesity and Diabetes, all taught at SEDENA.

Experience

- Personnel medical of the Emergency Department of the High Specialty Naval General Hospital, Mexico City.
- Personnel medical at the Naval Hospital of Tuxpan, Tuxpan Veracruz.
- Full Professor of Medical Microbiology at the Military School of Dentistry, Mexico City.
- Full Professor of Medical Biochemistry I and II at the Naval Medical School, Mexico City.
- Full Professor of Research Methodology I and II at the Naval Medical School, Mexico City.
- Full Professor of Research Methodology I at the Naval School of Nursing, Mexico City.
- Full Professor of Research Methodology I and II at the School of Postgraduate Studies in Naval Health, Mexico City.
- Head of Research Development at the Naval Health Postgraduate School, Mexico City.
- General Coordinator of Incorporation into the National Program of Quality Postgraduate Studies of CONACyT of the School of Postgraduate Studies in Naval Health, Mexico City.
- Medical and Scientific Director of Research and Development at BioMaussan, Mexico City.

Publications

- Anticancer Activity of Plant Tocotrienols, Fucoxanthin, Fucoidan, and Polyphenols in Dietary Supplements, December 11, 2024 / Nutrients 2024, 16, 4274. https://doi.org/10.3390/nu16244274
- The cytotoxic effect of quercetin-induced apoptosis on lung metastatic cells from giant cell tumor of bone, May 31, 2025 / Cell. Mol. Biol. 2025, 71(5): 6-12, http://dx.doi.org/10.14715/cmb/2025.71.5.2
- A case report: Dietary supplement 2.0 (astaxanthin, fucoxanthin, and apple polyphenols) reduces stress, work fatigue, and metabolic disorders. July 8, 2025 / South Florida Journal of Development, Miami, v.6, n.7. p. 01-12, 2025. ISSN 2675-5459, 10.46932/sfjdv6n7-046

- **CURRICULUM VITAE.**

Dra. Ericka Patricia Flores Berrios

Correo electrónico: eflores@biovalmex.com

Doctor of Science. Specialization in Molecular and Cellular Plant Biology. Polytechnic Institute of Toulouse, France. 1999. Award for best dissertation presented at the national level in France, 2000.
Bachelor's Degree. Biology. Faculty of Higher Studies Zaragoza, UNAM. 1995. Gustavo Baz Prada Award for Excellence in Social Service.
Diploma in Effective Linkage and Technology Transfer. ADIAT (2012)
Diploma in Social Development (USEM) 2023

WORK EXPERIENCE

−Partner and founder of BIODESARROLLOS VALMEX S.A. de C.V., a company dedicated to the design and manufacture of nutritional supplements - Current -

−Partner of SIMBIOHEALTH SAPI de CV, a company that markets and exports nutritional supplements - Current -

−Senior Researcher at the Center for Research and Assistance in Technology and Design of the State of Jalisco A.C., March 2000 to August 2010. Technology Transfer and Innovation.

−Undergraduate and Graduate Professor: University of Guadalajara, Autonomous University of Guadalajara, UNAM; Technological Institute of Tlajomulco, ITESO.

CERTIFICATIONS AND RECOGNITIONS

-Graduate in High-Tech Entrepreneurship from UNAM, 2017
-Distinguished Professor, UAG, 2012-2015
-National System of Researchers Level 1, 2001-2009
-Project Evaluator in the area of GMOs for CIBIOGEM
-Technological Consultant Specialist for the National Council of Science and Technology (Code RCCT-E00662) in the area of Biotechnology.

ACADEMY

-15 published indexed scientific articles
-15 supervised undergraduate and graduate theses
-Co-author of 3 books in different areas of biology
-Contributions to various popular science journals
-Co-author of the book WOMEN WHO INSPIRE (2024)

VALMEX BIODEASURING DISTINCTIONS

− 2022 Food Award from the Jalisco Chamber of Food, Supplements Section
− 2022 Jalisco Entrepreneurship Award, Outstanding Entrepreneur Category
− 2023 JALISCO WITHOUT GAPS Distinction (Gender Equality)
− 2024 JALISCO RESPONSIBLE Distinction (Good Labor Practices)
− SOCIALLY RESPONSIBLE COMPANY DISTINCTION (ESR - CEMEFI)

- **CURRICULUM VITAE.** Virginia Sánchez Monroy, PhD in Molecular Biomedicine

Correo electrónico: vickysm17@hotmail.com, vsanchezm@ipn.mx
ORCID: 0000-0003-1969-1342

RECENT PUBLICATIONS

1. Aranda-Lara L et al. Small Interfering RNA Carriers for Oncotherapy: A Preclinical Overview. Pharmaceutics. 2025, doi:10.3390/ pharmaceutics17111408
2. Rodríguez- Romero BI et al Link between multiple human papillomavirus 16 and 18 infection and prostate cancer, and relevance of tumor characteristics. Mol Clin Oncol. 2025, doi: 10.3892/mco.2025.2880
3. Rodríguuez-Romero BI et. al. Infección de Virus de Papiloma Humano y cáncer de próstata en México. Rev Mex Urol, 2025
4. Corzo-Cruz et al. Association of Endogenous PCR Final Product in HPV Human Samples with Ebis Measurements. Computación y Sistemas 2025, doi: 10.13053/CyS-29-2-5674
5. Vazquez-García et al. EBiS Characteristic Frequency Using AD5933EBZ for Characterization of DNA Concentration and Purity. Computación y Sistemas 2025, doi: 10.13053/CyS-29-2-5701
6. Gonzalez-Díaz CA et al. The Human 8-oxoG DNA Glycosylase 1 (OGG1) Ser326Cys Polymorphism in Infertile Men. Biomedicines 2024, https://doi.org/10.3390
7. Pérez-Mora S et al. Entamoeba histolytica: In Silico and In Vitro Oligomerization of EhHSTF5 Enhances Its Binding to the HSE of the EhPgp5 Gene Promoter Int J Mol Sci 2024, doi: 10.3390/ijms25084218.
8. Torres-Ramírez D. et al Polimorfism Ser326Cis de la 8-oxoguanina ADN glucosilasa 1 (ogg1) en hombres con teratozoospermia Perinatol Reprod Hum. 2024, doi:10.24875/PER.24000003
9. Villagómez-Sánchez PA et al. Generalidades del antígeno leucocitario humano g y su relación con la pérdida gestacional recurrente Perinatol Reprod Hum. 2024, doi: 10.24875/PER.24000007
10. Nadia Mabel Pérez-Vielma NM et al Genotoxicidad por tratamiento con ortodoncia fija Rev.Odont.Mex, 2023, doi:10.22201/fo.1870199xp.2023.27.4.85376
11. Angeles-Estrada L, et al. Oxidative stress and genotoxicity in oral epithelial cells from subjects undergoing orthodontic treatment with fixed appliances. Clinical oral investigations, 2023, doi:10.1007/s00784-023-05039-6
12. Maritza Velásquez-Torres et al. Riluzole, a Derivative of Benzothiazole as a Potential Anti-Amoebic Agent against Entamoeba histolytica. Pharmaceuticals. 2023, doi:10.3390/ph16060896.
13. Sivakumaran Karthikeyan,et al.Dynamic response antibodies SARS-CoV-2 human saliva studied using two-dimensional correlation (2DCOS) infrared spectral analysis coupledwith receiver operation characteristics analysis. Molecular Basic of Disease 2023. doi:10.1016/j.bbadis.2023.166799
14. Didilia Rodríguez Cruz, et al. Evaluation of the Pro12Ala polymorphism of PPARg the gene in women with gestational diabetes mellitus. Ginecol Obstet Mex. 2023, doi:10.24245/gom.v91i8.8768
15. Saavedra Morales A, et al. Prolonged computer use by office workers induces ocular symptoms associated with tear film alterations and overexpression of mucin 5 AC and J Occup Environ Med. 2023, doi: 10.1097/JOM.0000000000002653.

- **CURRICULUM VITAE.** Hamlet Avilés Arnaut in Biotechnology, CINVESTAV, México.

Correo electrónico: hamlet.avilesarn@uanl.edu.mx
ORCID: https://orcid.org/0000-0001-6813-6869
Instituto de Biotecnología, Facultad de Ciencias Biológicas Universidad Autónoma de Nuevo León (UANL)
Personal Website: https://www.uanl.mx/investigadores/hamlet-aviles-arnaut/ LinkedIn: www.linkedin.com/in/hamlet-avilés-arnaut Laboratory Website: https://lab5uanl.wixsite.com/laboratorio-05---ins

Professional Summary

Biotechnology researcher with expertise in cancer cell biology, natural bioactive compounds, and microbial biotechnology. Extensive experience in vitro cytotoxicity assays, 3D spheroid models, and molecular biology techniques. Active in scientific dissemination and interdisciplinary research integrating plant, algal, and microbial systems.

Professional Experience

Research Professor Institute of Biotechnology, UANL - Lead research on bioactive compounds and cellular mechanisms in cancer and plant systems - Supervise undergraduate and graduate students - Develop academic courses and scientific outreach initiatives

Research Interests

- Cancer cell biology and cytotoxicity mechanisms - Natural products and dietary supplements with bioactive properties - Microbial consortia and plant growth promotion - 3D cell culture models and spheroid-based assays - Algal biotechnology and pigment production

Selected Publications

1. López-Reyes, P. K., De la Torre-Zavala, S., Cortés-González, M. M., Galán-Wong, L. J., Avilés-Arnaut, H. (2024). Biological Control of Streptomyces sp. PR69 Against Phytophthora capsici and Its Growth-Promoting Effects on Plants. Horticulturae, 10(12), 1365. https://doi.org/10.3390/horticulturae10121365
2. Lara-Hernández, G., Ramos-Silva, J. A., Pérez-Soto, E., Figueroa, M., Flores-Berrios, E. P., Sánchez-Chapul, L., Andrade-Cabrera, J. L., Luna-Angulo, A., Landa-Solís, C., Avilés-Arnaut, H. (2024). Anticancer Activity of Plant Tocotrienols, Fucoxanthin, Fucoidan, and Polyphenols in Dietary Supplements. Nutrients, 16(24), 4274. https://doi.org/10.3390/nu16244274
3. Kynurenines and Inflammation: A Remarkable Axis for Multiple Sclerosis Treatment (2024). Carrillo-Mora P, Landa-Solís C, Valle-Garcia D, Luna-Angulo A, Avilés-Arnaut H, Robles-Bañuelos B, Sánchez-Chapul L, Rangel-López E. Pharmaceuticals. 2024; 17(8):983. https://doi.org/10.3390/ph17080983
4. Pharmacological Treatments and Therapeutic Targets in Muscle Dystrophies Generated by Alterations in Dystrophin-Associated Proteins (2024). Luna-Angulo, A.; Landa-Solís, C.; Escobar-Cedillo, R.E.; Estrada-Mena, F.J.; Sánchez-Chapul, L.; Gómez-Díaz, B.; Carrillo-Mora, P.; Avilés-Arnaut, H.; Jiménez-Hernández, L.; Jiménez-Hernández, D.A.; Miranda-Duarte, A. Medicina 2024, 60, 1060. https://doi.org/10.3390/medicina60071060
5. Alkaline-Tolerant Bacillus cereus 12GS: A Promising Polyhydroxybutyrate (PHB) Producer Isolated from the North of Mexico (2024). San Miguel-González GdJ, Alemán-Huerta ME, Martínez-Herrera RE, Quintero-Zapata I, de la Torre-Zavala S, Avilés-Arnaut H, Gandarilla-Pacheco FL, de Luna-Santillana EdJ. Microorganisms. 2024; 12(5):863. https://doi.org/10.3390/microorganisms12050863

- **Transparency and International Compliance**

This additional section ensures that the study complies with **ISRCTN Registry requirements** and **SPIRIT 2013 guidelines**, strengthening the ethical and methodological rigor of the trial.

Appendix 1. The TIDieR (Template for Intervention Description and Replication) Checklist

Item	Name	Description (applied to supplements dietary)
1	Dietary supplement 2.0 Dietary supplement 2.1 Dietary supplement 3.0	*2.0 Marine Algae"* – Dietary supplement 2.1 Premium Seaweed-Dietary supplement 3.0 Astaxanthin, special formula
2	Why: Theoretical Justification	The study will evaluate the beneficial effects of three antioxidant supplements containing carotenoids, anthocyanins, and apple polyphenols (COFEPRIS authorization: Notice of Establishment of Products and Services No. 2509055019X00049). These compounds act independently on psychological stress, fatigue, and metabolic syndrome.
3	Materials	2.0 Dietary supplement, 600 mL, formulated with astaxanthin, fucoxanthin, and apple polyphenols. 2.1 Dietary supplement, 120 mL, formulated with anthocyanins, xanthophylls and apple polyphenols. 3.0 Dietary supplement, 120 mL, formulated with 3.0 astaxanthin, special formula.
4	Procedures	Oral intake of 5 ml, twice daily, for 6 months.
5	Who	Self-administered by participants under supervision of the study physician.
6	How	Supplements delivered in person at the corporate site; daily adherence monitored via videoconference.
7	Where	Private Information Technology corporation, Mexico City.
8	When and How Much	2.0 dietary supplement: 5 ml twice daily (containing 84 mg of apple polyphenols, 40 mg of astaxanthin, 13.2 mg of fucoxanthin) for 6 months. 2.1 supplement dietary: 5 ml twice daily (containing 166 mg of anthocyanins, 194.8 mg of total xanthophylls and 125 mg of apple polyphenols) for 6 months. 3.0 dietary supplement: 5 ml twice daily (containing 500 mg/day of astaxanthin from *Haematococcus pluvialis*)
9	Dosage	Same dosage for all participants.
10	Modifications	No changes in supplier or dosage during the study.
11	Adaptation	If participants experience intolerance to taste, the dose may be diluted in plain water.
12	Fidelity	No leftover material at the end of the protocol.

Appendix 2. INFORMED CONSENT

ANTIOXIDANT SUPPLEMENTATION ON STRESS AND FATIGUE AT WORK AND METABOLIC SYNDROME IN WORKERS OF A PRIVATE CORPORATION

Mexico City, _____ _____, 20__.

Principal Investigator: Montserrat Noemí González Mellado (Professional License No. 12527231, email: mgonzalezm2401@alumno.ipn.mx). If you have any questions or issues arise, you can contact 5535373300. Consulting researchers: María Del Carmen López García and Elvia Pérez Soto.

Explanation of the protocol: The beneficial effects of three antioxidant supplements based on carotenoids, anthocyanins, and apple polyphenols (COFEPRIS permit - Notice of Operation of Establishment for Products and Services - 2509055019X00049) will be evaluated. These supplements target stress, work-related fatigue, and metabolic syndrome. Four groups of employees from a private corporation in Mexico City will be included. The study will last six months, with evaluations (comprehensive medical assessment, biochemical analysis, and quantification of various inflammatory and oxidative stress biomarkers) at baseline, and at 3 and 6 months after supplementation. Hair and blood samples will be collected, and instruments measuring work-related stress and the Work-Related Fatigue Severity Scale will be administered.

OBJECTIVE: To analyze the effect of 3 dietary supplements on stress, work fatigue, and metabolic syndrome in workers of a private corporation in Mexico City.

JUSTIFICATION OF THE STUDY: The high prevalence of obesity, work stress, and metabolic syndrome in Mexico represents a challenge for public health and negatively affects work productivity and lifestyle. Likewise, the relationship between psychological stress and increased oxidative damage has been established as an important factor in the development of metabolic alterations. On the other hand, a few supplements help prevent oxidative damage, metabolic alterations, stress, and occupational fatigue, providing an opportunity to explore the possible synergistic effects of these antioxidants.

EXPECTED BENEFITS: Physical, physiological, and psychological symptoms related to stress and work fatigue will be reduced, improving productivity. In addition to regulating metabolism, reducing the risk of metabolic syndrome, diabetes mellitus, and obesity. In addition, there are other health benefits of using antioxidants, such as improved skin health, reduced visual fatigue, benefits for the cardiovascular and nervous systems, protection against cellular ageing, and strengthened immunity.

POSSIBLE SIDE EFFECTS: Allergic reactions in people sensitive to algae or iodine.

Possible drug interaction with blood thinners or thyroid medications.

In some people, supplements can cause mild and temporary gastrointestinal discomfort such as nausea or diarrhea, especially at the beginning of consumption.

DURATION OF SUPPLEMENTATION: 10 mL of the food supplement should be provided daily for 6 months.

GUARANTEES: Your rights and well-being will be respected, and you will receive answers to any doubts or questions about research and supplementation. The participant is free to withdraw their consent at any time and to allow participation in the study, without prejudice to their care and treatment. The worker will not absorb payment for the research, but rather from the research budget.

CONFIDENTIALITY: Personal information will be safeguarded in accordance with privacy regulations.

IN CASE OF EMERGENCY OR MEDICAL EMERGENCY: Samples will be taken by medical physicians and laboratory technicians who will have first aid equipment to attend to any emergency. In the event of adverse effects to the supplements, their administration will be suspended immediately. Medical personnel will provide first aid or call the emergency services (911). If necessary, the person designated as your emergency contact will be notified, and you will be transferred to the nearest medical centre. Any adverse event will be recorded and reported to the regulatory authorities and the ethics committee, in accordance with established procedures. By signing this informed consent, you declare that you have read and understood the information provided, and that you freely and voluntarily accept your participation in the study, including procedures and the consumption of dietary supplements.

Full name and signature **PRINCIPAL INVESTIGATOR**	*Full name and signature* **PROTOCOL PARTICIPANT**
Full name and signature **WITNESS**	*Full name and signature* **WITNESS**

Appendix 3. CONSENTIMIENTO INFORMADO (SPANISH)

SUPLEMENTACIÓN CON ANTIOXIDANTES SOBRE EL ESTRÉS Y FATIGA LABORAL Y SÍNDROME METABÓLICO EN TRABAJADORES DE UN CORPORATIVO PRIVADO

Ciudad de México a, _____ de _____ del 2025.

Investigador Principal: Montserrat Noemi González Mellado, mgonzalezm2401@alumno.ipn.mx con cédula profesional: 12527231. Ante cualquier duda o situación que se presente podrá comunicarse al teléfono 5534660900. Investigadoras asesoras: D. en C María Del Carmen López García y D. En C. Elvia Pérez Soto

Explicación del protocolo: Se evaluará el efecto benéfico de tres suplementos antioxidantes a base de carotenoides, antocianinas y polifenoles de manzana (permiso COFEPRIS-Aviso de funcionamiento de establecimiento de productos y servicios- 2509055019X00049), que actúan sobre estrés, fatiga laboral, y síndrome metabólico. Se integrarán 4 grupos con trabajadores del corporativo privado en CDMX. El estudio tendrá una duración de seis meses, con evaluaciones (valoración médica integral, análisis bioquímico y cuantificación de diversos biomarcadores inflamatorios y de estrés oxidativo) al inicio, a los 3 y 6 meses posteriores a la suplementación. Se tomarán muestras de cabello y sangre, y se aplicarán instrumentos que miden estrés laboral, la escala de severidad de la fatiga laboral.

OBJETIVO: Analizar el efecto de 3 suplementos alimenticios en el estrés, fatiga laboral, y síndrome metabólico en trabajadores de un corporativo privado de la Ciudad de México.

JUSTIFICACIÓN DEL ESTUDIO: La elevada prevalencia de obesidad, estrés laboral y síndrome metabólico en México representa un desafío para la salud pública, además afecta negativamente la productividad laboral y estilo de vida. Así mismo, se ha establecido la relación entre estrés psicológico y el aumento de daño oxidativo, siendo un factor importante en el desarrollo de alteraciones metabólicas. Por otro lado, existen pocos suplementos que prevengan el daño oxidativo, la inflamación, así como el desarrollo de alteraciones metabólicas y por tanto, el estrés, la fatiga laboral, por lo que los suplementos aquí mencionados, son una opción para explorar los posibles efectos sinérgicos de los antioxidantes y su impacto a largo plazo.

BENEFICIOS ESPERADOS: Se disminuirán los síntomas físicos y fisiológicos relacionados al estrés y fatiga laboral, mejorando la calidad de sueño, la de vida laboral y productividad. Sumado a regular el metabolismo, disminuyendo la predisposición al síndrome metabólico, la diabetes mellitus y la obesidad. Además, se tendrán otros beneficios en la salud por el uso de antioxidantes, como en la piel, vista al evitar la fatiga visual, en sistemas cardiovascular y nervioso, e incluso prevenir el envejecimiento celular, y fortalecer el sistema inmunológico.

POSIBLES EFECTOS ADVERSOS: Reacciones alérgicas en personas sensibles a las algas o al yodo.
Posible interacción medicamentosa con anticoagulantes o medicamentos para la tiroides.
En algunas personas, los suplementos pueden causar molestias gastrointestinales leves y temporales como náuseas o diarrea, especialmente al inicio del consumo.

DURACIÓN DE ADMINISTRACIÓN DE SUPLEMENTOS: se proporcionarán 10 mL del suplemento alimenticio diariamente durante 6 meses.

GARANTÍAS: Se respetarán sus derechos y bienestar y recibirá respuestas a cualquier duda o pregunta sobre la investigación y suplementación. El participante tiene la libertad de retirar su consentimiento en cualquier momento que así lo decida y de dejar participar en el estudio, sin que se creen prejuicios para su cuidado y tratamiento.
Los gastos adicionales de la investigación no serán absorbidos por el trabajador; sino por el presupuesto destinado a la investigación.

CONFIDENCIALIDAD: La información personal será resguardada conforme a la normativa de privacidad.

EN CASO DE URGENCIA O EMERGENCIA MÉDICA: Las muestras serán tomada por médico y técnicos de laboratorio que contarán con equipo de primeros auxilios para atender cualquier emergencia. En caso de presentar efecto adverso a los suplementos, se procederá a suspender de inmediato la administración de los mismos. El personal médico dará primeros auxilios o se llamará al servicio de emergencias (911) y se notificará de ser necesario a la persona designada como su contacto de emergencia y si así lo amerita, será trasladado al centro médico más cercano. Se registrará y reportará todo evento adverso a las autoridades regulatorias y al comité de ética, conforme a los procedimientos establecidos.

Al firmar este consentimiento informado, usted manifiesta haber leído y comprendido la información contenida, así como aceptar de manera libre y voluntaria su participación en el estudio, colaborando en procedimientos y el consumo de los suplementos alimenticios otorgados.

Nombre completo y firma **INVESTIGADOR PRINCIPAL**	*Nombre completo y firma* **PARTICIPANTE DE PROTOCOLO**
Nombre completo y firma **TESTIGO**	*Nombre completo y firma* **TESTIGO**