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Randomized Controlled Trial

Nutraceutical composition (yeast β-glucan, prebiotics, minerals, and silymarin) predicts improvement of sleep quality and metabolic parameters: A randomized pilot study

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SUMMARY

Background & aims: The search for integrative and natural therapies that favor homeostasis to boost sleep and diet quality took place for young adult populations as a non-pharmacological strategy for long-term good quality of life. Thus, the present pilot study aims to investigate the effects of 90-day consumption of a nutraceutical composition on the neuro-immune-endocrine axis, providing better sleep quality and health improvement.

Methods: For this, from March 2021 to June 2021, twenty-two Brazilian young adult volunteers (women and men) with BMI between 18.5 and 34.9 kg/m^2 were divided into three distinct supplementation groups: NSupple; NSupple plus_S, and NSupple plus. Briefly, the supplement compositions included yeast β-glucan, prebiotics, and minerals in different concentrations associated or not with the herbal medicine silymarin.

Neither nutritional nor physical activity interventions were performed during this pilot study period. The anthropometrics measures, questionnaires answer data, and harvest blood for metabolic, inflammatory, and hormonal tests were collected at baseline time (day zero-T0) and day 90 (T90) post-supplementation.

Results: Our results highlight that the supplementation reduced body mass index (BMI), Waist-to-height ratio (WHtR), waist circumference, AST/ALT ratio, alkaline phosphatase, and HbA1c. Post-supplementation the IL-6 and IL-10 levels and the sleep, humor, and quality of life scores were suggested to improve. Sleep quality improvement seems to predict the reduction of adiposity-related body measures.

Conclusion: In sum, the nutraceutical supplementation might be related to anthropometric, metabolic, and endocrine parameters after 90 days reflecting on perception of humor, sleep, and life quality enhancement. However, it is important to recognize the limitation of the data presented considering that this was a pilot study.

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1. Background

Over the last century, advances in modern medicine have extended the world's populational life expectancy by bringing innovative solutions for diagnosing and treating previously neglected or even unknown diseases. However, it is currently becoming noticeable that increasing life expectancy is not enough, it is also necessary to look for alternatives to promote quality of life and personal independence associated with longevity, seeking to make people live a longer and more fulfilled lifetime [1]. There is a great consensus in the health care area that longevity and maintenance of the quality of life depend on a trivet composed of elements namely good quality sleep, healthy diet, and regular physical activity, besides genetic and environmental factors. Together these factors have proven themselves over time as effective tools for the maintenance of health, autonomy, and a higher quality of life, which leads to healthy aging in the long term [2,3].

However, with the 21st-century lifestyle, keeping good quality sleep, a healthy diet, and regular physical activity becomes increasingly difficult, especially regarding good quality sleep. Sleep quality can be affected by exogenous and endogenous factors. Among the exogenous factors, we can briefly cite alternations of the circadian cycle, exposure to stimulating milieus, and diet patterns. These factors can be managed by exercise practices, healthy diet habits, and sleep hygiene routines involving major lifestyle changes [4,5]. In addition, endogenous factors such as the neuro-immune-endocrine axis play a key role in regulating sleep quality, immune response, and hormonal modulation. The neuro-immune-endocrine axis is a complex and highly regulated system that involves the interaction between the nervous system, the immune system, and the endocrine system in the sleep maintenance process [6].

The central nervous system has neural networks and specific brain regions that control circadian rhythms, responsible for the sleep-wake cycle. The epiphysis mainly influences the circadian rhythm at the central level, which secretes the hormone melatonin, involved in sleep regulation [4,7]. Melatonin production is influenced by the light-dark cycle increasing during the night to promote restful sleep [8]. During sleep, important immune system repair and regeneration processes occur. An imbalance in the immune system, such as chronic inflammation, can lead to sleep disorders like insomnia or sleep apnea [9]. In addition, hormones such as cortisol are related to the sleep-wake cycle and stress control. Proper regulation of cortisol levels is essential for healthy sleep. Hormonal disruptions like adrenal insufficiency, thyroid dysfunction, or chronic stress can affect sleep quality [10,11]. Also, a state of chronic stress can trigger the onset of sleep disorders recognized as a risk factor for the development and worsening of chronic diseases such as obesity, type 2 diabetes, and hypertension, as well as mood disorders such as anxiety and depression [5,12].

Considering this, the search for integrative and natural therapies aiming to favor homeostasis for long-term good quality of life took place for elderly and young adult populations as a non-pharmacological strategy to boost sleep and diet quality. In this sense, some natural compounds stand out in the promotion of the neuro-immune-endocrine axis [2]. Silymarin is a promising phytochemical compound capable of raising dopamine levels and therefore contributes to the modulation of the neuro-immune-endocrine system, and consequently the improvement of sleep quality [8,13]. In addition, minerals such as zinc and magnesium are micronutrients that play an essential role as cofactors in the synthesis of serotonin from tryptophan amino acids. Serotonin is a hormone related to well-being sensation and directly influences sleep quality [14,15]. It is known that the circulating serotonin is produced not only by the epiphysis but also by the large intestine. Modern eating habits are often deficient in dietary fiber and essential minerals for maintaining a balanced intestinal microbiota [16]. Therefore, continuous intake of prebiotic fibers such as β-glucans [17], GOS (galactooligosaccharides) [18], and FOS (fructooligosaccharides) [19] may also represent a natural strategy for the improvement of mineral absorption and maintenance of intestinal health and strengthening of the neuro-immune-endocrine axis [20].

Thus, the present pilot study investigates the effects of 90-day consumption of three different nutraceutical compositions containing sources of prebiotics (β-glucans, GOS, and FOS), minerals (zinc, magnesium, and selenium), and an herbal medicine (Silymarin extract - *Silybum marianum* (L.) Gaertn) [21] for use as a nutritional and nutraceutical supplement aiming to modulate the neuro-immune-endocrine axis, with the main objective of providing better sleep quality and improvement in quality of life.

2. Material and methods

2.1. Ethics Committee approval

This protocol was approved by the HC-FMUSP Research Ethics Committee under the number 5.365.566, which is in accordance with the Helsinki World Medical Declaration [22]. This research was carried out in accordance with the relevant guidelines and regulations and was approved by the Ethics Committee for the Analysis of Research Projects (CAPPesq). This study received approval from the Brazilian National System of Genetic Registration (SisGen), under the number AC29D69, and registered as a Clinical Trial under identification number NCT04810572 (ClinicalTrials.gov) registered on 20th February 2021. All volunteers have read and signed free and informed consent terms before starting the study and can withdraw the consent at any time. The research team has one copy of the document, and a separate copy was provided to the volunteer for safekeeping.

2.2. Volunteers' recruitment and experimental design

The "Novel Nutraceutical Supplement-trial" is a pilot study addressing young adult people comparing baseline and 90-day post-supplementation parameters. Volunteers were recruited by online invitation from March 2021 to June 2021. This study included healthy adult people of any sex, aged between 26 and 42 years old with a BMI between 18.5 and 34.9 kg/m^2, with no recent changes in lifestyle. The exclusion criteria used were: the use of insulin injection, corticoids, and non-steroidal anti-inflammatory drugs for more than 15 days; AIDS or hepatitis diagnosis; pregnancy; patients under chemotherapy treatment and allergy history of any components in the nutraceutical formulation. None of the

volunteers included in this pilot study were diabetic using oral hypoglycemic medications.

To ensure the double-blind feature of the study, the pharmacists' team from the "*Solis Magistral Farmácia Homeopatia Sensitiva*" (São Paulo, SP, Brazil) pharmacy, was responsible for maintaining the blind spot and preparing the capsules delivered to the volunteers. Thus, the team of pharmacists received a precise description of the 3 formulations tested in this study and assigned random codes to the formulations which only the pharmaceutical team had access to, preventing volunteers and researchers from knowing the composition of the formulas.

After baseline data and sample collection pre-supplementation, to ensure a balance of baseline key factors among groups a stratified randomization was performed by the main researcher in this study. Age and sex were set as the key factors for sample stratification before randomization with the assistance of Microsoft Excel 2010 software. A computer-generated randomization list by the software (www.random.org) was used to allocate volunteers, who met the inclusion criteria, among the 3 distinct random codes assigned to the experimental groups by the team of pharmacists abovementioned, keeping the blind spot. Post-randomization, volunteers were assigned numeric IDs ensuring anonymity and privacy.

Volunteers were divided into three groups: NSupple had the Novel Nutraceutical Supplement with *S. marianum*; N Supple plus_S had higher concentrations of the Novel Nutraceutical Supplement without *S. marianum*, and NSupple plus had higher concentrations of the Novel Nutraceutical Supplement with *S. marianum*. Volunteers were advised to take two supplement capsules in the morning and two capsules in the evening. Neither nutritional nor physical activity interventions were performed or stimulated during the study period. The harvested blood, anthropometric measurements, and questionnaire data were obtained at two points: baseline time day 0 (T0) and 90 days (T90) post-supplementation.

We attended the Consolidated Standards of Reporting Trials (CONSORT) [23] and used it to build up a flow diagram to display the trial steps. During the enrollment process, a total of 32 volunteers were assessed. In total, 3 volunteers did not meet the inclusion criteria. Thus, 29 participants were randomized into the three experimental groups: NSupple (n = 9); NSupple plus_S (n = 10), and NSupple plus (n = 10). Between baseline (T0) and post-supplementation period (T90) there were dropouts for no alleged reasons (n = 2) or because of time constraints (n = 5). By the end of the 90-day supplementation period, a total of 22 participants on NSupple (n = 7); NSupple plus_S (n = 8), and NSupple plus (n = 7) completed the supplementation protocol as demonstrated in the CONSORT flowchart (Fig. 1).

2.3. Novel nutraceutical supplements formulations

We conducted three distinct formulations across three experimental groups, namely **NSupple, NSupple plus_S,** and **NSupple plus.** The supplements' composition is presented in Table 1. The percentages used in the formulations were determined following recommendations and parameters published by the European Food Safety Authority (EFSA) [24]. The precise amount of each active component was not disclosed due to the patent register requirements (Patent number: BR 10 2020 016156 3) which can be accessed upon request. The formulations were manipulated by the pharmacy "Solis Magistral Farmácia Homeopática Sensitiva" (São Paulo, Brazil) which kept the double blinding point of this pilot study.

2.4. Anthropometric parameters

To assess the volunteers' physical activity level, we applied the IPAQ - International Physical Activity Questionnaire - Short Version [25]. To perform the anthropometric measurements, the volunteers were positioned in an orthostatic body posture with the head in the Frankfurt horizontal plane. Anthropometrics parameters were performed and analyzed on baseline time (T0) and T90-days post-supplementation. The volunteer's body mass was accessed using the Body Composition Scale 2 (Xiaomi Mi, Beijing, China). The height (m), hips (cm), iliac crest (cm), waist (cm), and neck (cm) circumferences, were obtained using a plastic tape measure. The body mass index – BMI [body mass (kg) ÷ height^2 (m)]; waist-to-height ratio (WHtR) and waist-to-hips ratio (WHR) were calculated based on anthropometric data. To minimize bias all anthropometric measurements were taken by the same trained healthcare professional.

2.5. Questionnaires for sleep and quality of life characterization

The Brazilian Portuguese Version of the Mini-Sleep Questionnaire (MSQ-BR) was used to assess the subjective quality of sleep. It is composed of a ten-item self-report scale that measures frequencies of sleep difficulty. The scores were evaluated as proposed by the literature [26]. Horne & Ostberg's Morningness-Eveningness questionnaire was applied as a self-report instrument to evaluate chronotype. Briefly, the highest score indicates a definite morningness chronotype and the lowest number defines the eveningness chronotype [27]. To assess the participants' perception of quality of life, we applied the World Health Organization Quality of Life instrument-short form (WHOQOL-BREF) [28]. The questionnaires were applied using a self-report model, ensuring that data was obtained directly from participants without the interference of an interviewer, thus guaranteeing privacy and potentially increasing the honesty of responses. Moreover, a healthcare professional researcher was available during the completion of the questionnaires to address any questions from the volunteers.

2.6. Biochemistry and endocrine parameters

Blood samples were collected from volunteers on baseline (T0) and post-supplementation (T90). All samples were collected between 07:00 h and 09:00 h in the morning by a nurse trained in clinical analysis hired solely for this purpose, who remained blind to the groups during sample collection. The samples were processed to perform biochemical analysis and endocrine parameters. Total cholesterol; Triglycerides; HDL (High-Density Lipoprotein)-cholesterol; non-HDL-cholesterol; LDL (Low-Density Lipoprotein)-cholesterol; VLDL (Very-Low-Density Lipoprotein)-cholesterol; AST (Aspartate aminotransferase); ALT (Alanine aminotransferase); Alkaline phosphatase; *Gamma*-GT (*Gamma*-glutamyl transferase); creatinine, insulin, albumin, total proteins, C-reactive protein, IgA (Immunoglobulin -A), IgG (Immunoglobulin -G), IgM (Immunoglobulin -M)were analyzed in plasma glucose levels obtained in sodium fluoride. Biochemical analyses and endocrine parameters were measured by the partner laboratory "*Fleury Medicina e Saúde*" which remained blinded to the analyses by using random codes assigned to volunteers during the initial randomization process. AST/ALT ratio (AAR- Aspartate aminotransferase (AST)/Alanine aminotransferase (ALT) ratio) [29], HOMA index – HOMA-IR = [fasting glucose (mmol/L) x fasting insulin (mU/L)]/405 - [30], and atherogenic index - AI = [log10 (TAG ÷ HDL-c)] - [31] were calculated following the respective references.

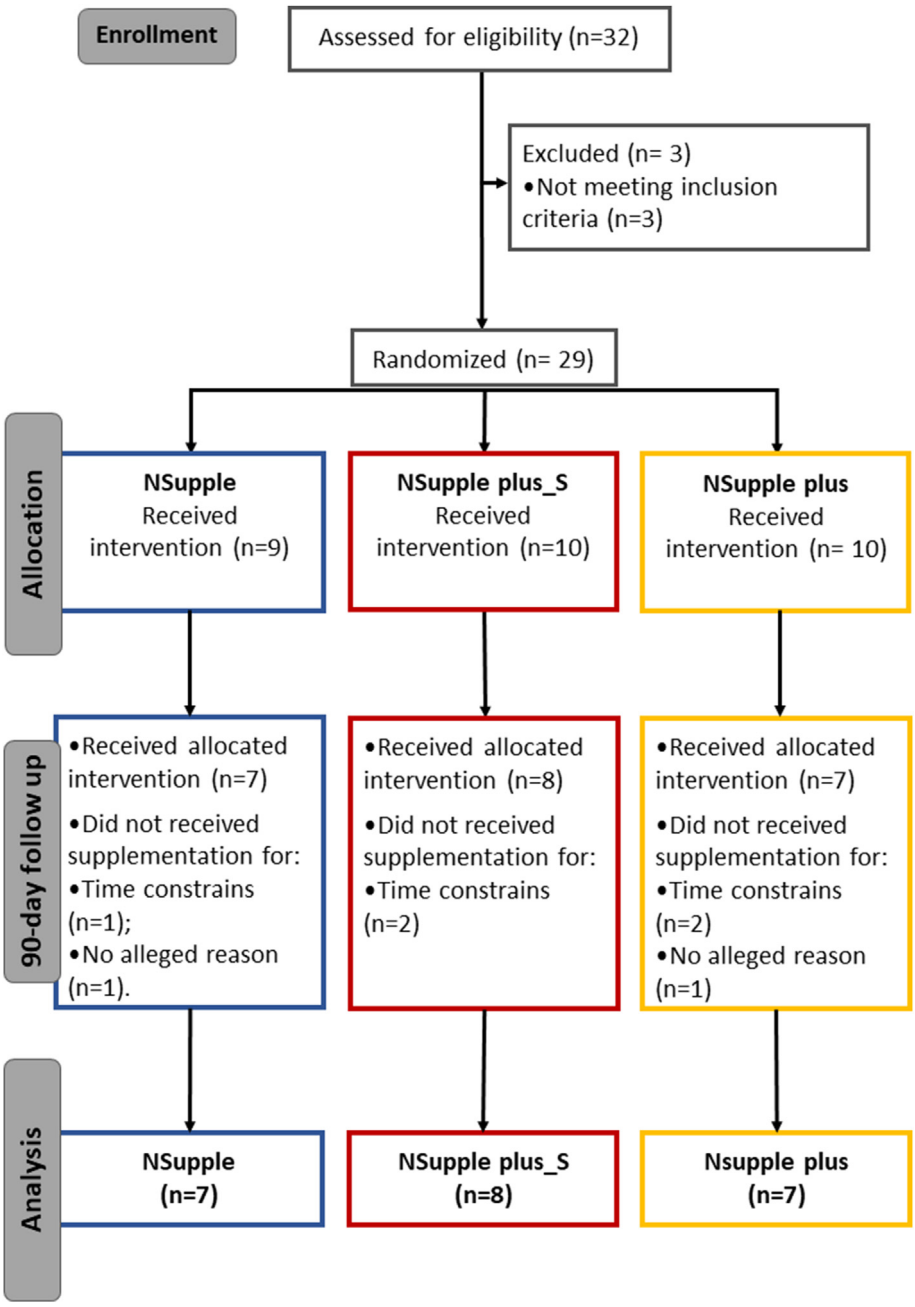

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flowchart describing the recruitment of volunteers and the experimental design carried out on this clinical trial.

Table 1
Supplements formulation components developed and tested in the present study.

Components	NSupple	NSupple plus_S	NSupple plus
zinc (Zn)[a]	1%	1%	1%
magnesium (Mg)[a]	1%	9%	9%
fructooligosaccharide (FOS)[b]	45%	39%	39%
selenomethionine (0.2% Se)[c]	0.01%	8%	8%
galactooligosaccharide (GOS)[c]	10%	16%	16%
1.3/1.6-(β-glycosidic bonds) yeast β-glucans (*Saccharomyces cerevisiae*)[c]	6%	14%	14%
Silybum marianum (L.) Gaertn (silymarin extract 3.11%)[d]	9%	–	11%

[a] Purifarma Distribuidora Química e Farmacêutica, São Paulo, Brazil.
[b] NutraFlora®, Westchester, USA.
[c] Biorigin, São Paulo, Brazil was the supplier of selenomethionine, Galactooligosaccharide, and yeast β-glucans.
[d] SM Empreendimento Farmacêutica LTDA., São Paulo, Brazil supplied Silybum marianum (silymarin extract 3.11%).

Table 2
Descriptive sample characterization, chronotype classification according to the *Horne and Ostberg morningness-eveningness questionnaire*, measure of daytime sleepiness Epworth Sleepiness Scale (EES), and physical activity level according to the *International Physical Activity Questionnaire* (IPAQ).

	NSupple	NSupple plus_S	NSupple plus
Sample size (M/F)	7 (3/4)	8 (3/5)	7 (5/2)
Age (years)	35 ± 4.75	32 ± 4.82	36 ± 3.86
Height (m)	1.79 ± 0.07	1.66 ± 0.08	1.78 ± 0.08
Horne & Ostberg's Morningness-Eveningness Chronotype			
	NSupple *% (n)*	**NSupple plus_S** *% (n)*	**NSupple plus** *% (n)*
Definitely morning	28.57 (2)	25 (2)	42.85 (3)
Moderately morning	14.29 (n = 1)	12.5 (1)	–
Intermediate	28.57 (2)	62.5 (5)	42.85 (3)
Moderately evening	28.57 (2)	–	14.29 (1)
Definitely evening	–	–	–

Epworth Sleepiness Scale (ESS)

	NSupple		NSupple plus_S		NSupple plus	
	T0	T90	T0	T90	T0	T90
Mean ± SD	7.71 ± 1.44	8.57 ± 2.38	8.71 ± 2.05	6.43 ± 1.43	9.50 ± 1.48	9.87 ± 1.87
CI 95%	11.24–4.18	14.42–2.73	13.74–3.68	9.92–2.93	12.99–6.01	14.31–5.44

International Physical Activity Questionnaire (IPAQ)

	NSupple		NSupple plus_S		NSupple plus	
	T0	T90	T0	T90	T0	T90
	% (n)	*% (n)*	*% (n)*	*% (n)*	*% (n)*	*% (n)*
Very active	–	–	–	–	–	–
Active	33 (2)	50 (3)	50 (4)	88 (7)	71 (5)	71 (5)
Irregularly Active	67 (4)	50 (3)	50 (4)	13 (1)	29 (2)	29 (2)
Sedentary	–	–	–	–	–	–

2.7. Cytokines and chemokines levels

The CBA test was used to quantitatively determine the cytokine concentration in plasma samples. The plasma was kept at −80 °C till estimation. The preparation of beads, standards, reagents, plasma samples, and the protocols for flow cytometer setup and data acquisition were performed according to the manufacturer's instructions. Cytokine levels in the serum samples were detected by BD FACS Canto II flow cytometer (Becton Dickinson Holdings Pte Ltd, USA) using commercially available kits from BD™ Cytometric Bead Array (CBA) for "552990 - Human Chemokine (RRID AB_2868970)" and "551811 - Human Inflammatory Cytokines CBA kit (RRID AB_2868941)" kits (BD Biosciences, USA). In brief, cytokine standards were prepared using the method of serial dilutions following the manufacturer's instructions. Capture bead was added into each tube that is samples, standards, and negative control and was incubated in the absence of light. The flow cytometer was calibrated using cytometer setup beads and the assay was performed. The results were calculated using an CBA Analysis software (SoftFlow, Pecs, Hungary) and were expressed in pg/mL.

2.8. Statistical analysis

The sample size enrolled was based on a previous calculation using the G*Power software [32] assuming an F test (ANOVA: repeated measures, within factors) with a type I error of 0.05, power of 0.8, and a success rate (effect size) of 0.3, the required total sample size was 24 people. Considering a potential dropout of 20%, the sample size was inflated to at least 29 participants. Initially, the Grubbs test was applied to detect outliers. Data were classified as parametric or nonparametric based on the Shapiro–Wilks and Smirnov-Kolmogorov test. Analyses were performed using GraphPad Prism 9.5.0 (GraphPad Software, La Jolla, CA, USA) software. To compare the differences in the paired groups, parametric (Student T-Test and ANOVA repeated measures, within factors) or nonparametric (Wilcoxon or Friedman) tests were conducted when indicated. Categorical data were analyzed using the Chi-Square or Fisher Exact test. Pearson or Spearman correlation was used to verify associations between variables. Multiple linear regression was applied to determine the predictors presented as β coefficient, $R^{2,}$ and 95%CI values. For all analyses, significance was determined as $p \leq 0.05$. Data are presented as mean ± standard deviation or mean levels ±95% confidence interval of the mean.

3. Results

3.1. Anthropometric data improvement by the nutraceutical composition

At the beginning of the supplementation period, the volunteers were characterized by sex, age, and height. Volunteers were also classified according to their chronotype. The sample group of this study included male and female people with ages between 26 and 42 years old. Also, all the experimental groups included both sexes. The sample was predominantly composed of intermediate or definitely morning chronotypes according to the classification of *Horne and Ostberg*. The sample did not include volunteers having an evening chronotype which can be considered a limitation of this study The intermediate chronotype presents adaptability characteristics, being more flexible regarding sleep schedule. This distribution adequately reflects the population average since the chronotype can be influenced by genetic, environmental, and behavioral factors. The sample characteristics are displayed in Table 2.

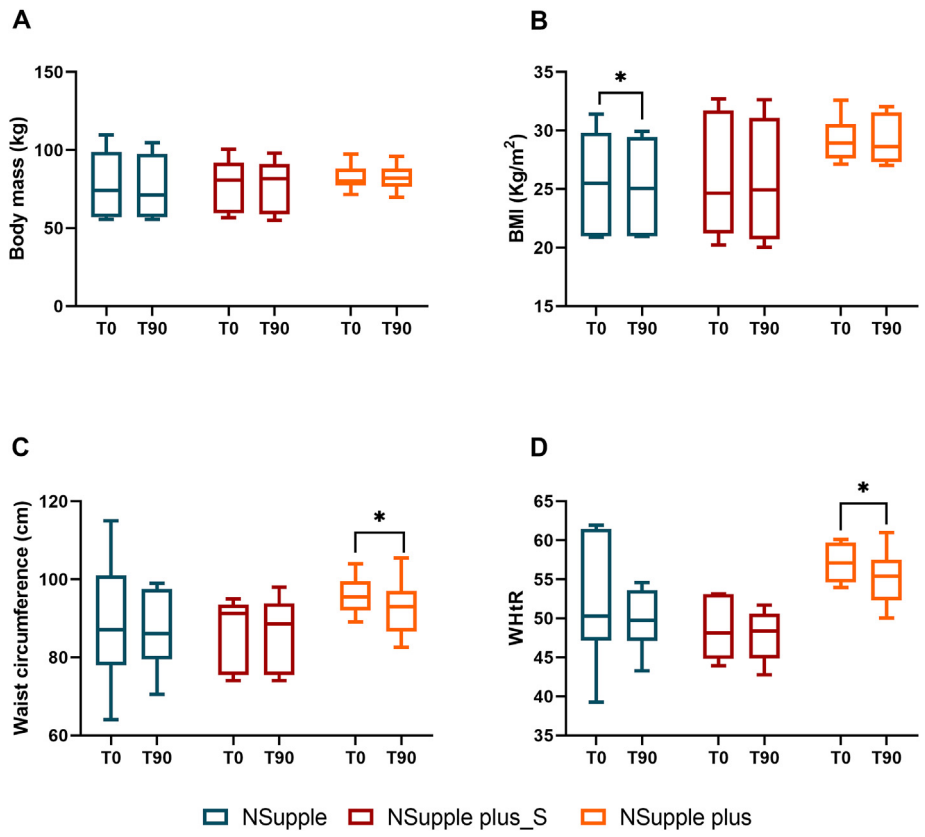

Fig. 2. Anthropometric parameters evaluated before and after the 90 days between the different supplementation groups: **[A]** body mass, **[B]** body mass index (BMI) **[C]** waist circumference at the iliac crest **[D]** Waist-to-height ratio (WHtR).

For anthropometric data follow-up of the sample group, body mass (Fig. 2A), waist circumference (Fig. 2C), and waist-to-height ratio (WHtR) (Fig. 2D) were evaluated, the waist circumference and WHtR were reduced solely by the NSupple plus group post-supplementation. Weight and height data were used to calculate the body mass index (BMI) shown in Fig. 2B. The BMI allows us to suggest that this research sample group was composed of individuals classified between normal weight, overweight, and obesity. Notably, the NSupple group, encompassing individuals of normal weight, overweight, and obesity showed a statistically significant reduction in BMI after 90 days of supplement consumption. The anthropometric parameters of neck and hip circumference, abdominal circumference at the iliac crest (WC-IC), and waist/hip ratio (WHR) were also evaluated, which did not differ over time (Table 3).

Anthropometric parameters can be directly affected by the regular practice of physical activity. Therefore, the volunteers were evaluated regarding the level of physical activity at the baseline and post-supplementation period. Table 2 shows that the sample does not include sedentary individuals or individuals with intense physical activity (Very active) neither before nor after supplementation. All experimental groups were composed of active or irregularly active volunteers and this characteristic was maintained throughout the experimental period. This result demonstrates that the positive effects of the supplement were not influenced by changes in lifestyle related to the practice of physical activity. In addition, the supplement was effective in promoting changes in anthropometric parameters even in the case of volunteers with an active lifestyle. This data might be limited by the small sample size; however, it may bring to light the supplementation effect.

3.2. Nutraceutical composition effect on metabolic and endocrine bloodstream parameters

In addition, biochemical markers accessible to clinical practice were assessed for the metabolic evaluation of volunteers through the consumption of tested supplements. As this is a group of young, physically active, and mostly eutrophic/overweight adults, it was not possible to detect alterations in the serum parameters of triglycerides, total cholesterol, HDL-cholesterol, non-HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, atherogenic index among groups or over time (Table 3). This result demonstrates that there were no metabolic alterations at the beginning of the study and that the supplement did not promote deleterious alterations in these parameters.

We also evaluated markers related to the presence of insulin resistance before and after supplementation in different groups. There was no statistical difference in fasting blood glucose and insulin levels (Table 3). Serum HbA1c levels were reduced post-supplementation in the NSupple plus group (Fig. 3E). From the calculation of HOMA-IR, it is possible to assess the presence of insulin resistance. In this sense, the NSupple group showed a statistical increase in this index after supplementation (Fig. 3F). However, it is noted that despite this increase, the HOMA-IR mean values obtained remained within the reference values of (HOMA-IR <3.6) proposed in the literature. Thus, we can say that the volunteers did not show insulin resistance after consuming the supplement.

Evaluating liver markers, we observed a decrease in AST levels in the NSupple plus group after supplementation (Fig. 3B). On the other hand, serum ALT values did not differ between groups throughout treatment (Fig. 3C). By calculating the AST/ALT ratio, a

Table 3
Anthropometrics and serum parameters were analyzed before and after the supplementation.

Group	NSupple			NSupple plus_S			NSupple plus		
	T0	T90	*p*	T0	T90	*p*	T0	T90	*p*
Anthropometrics									
Neck (cm)	36.07 ± 1.71	34.61 ± 1.22	-	35.80 ± 0.90	35.82 ± 1.01	-	37.25 ± 1.92	36.83 ± 1.52	–
Hip (cm)	103.4 ± 3.70	103.8 ± 2.21	-	104.1 ± 4.92	107.0 ± 4.77	-	107.8 ± 3.04	109.8 ± 3.59	-
WC-IC (cm)	96.07 ± 4.79	93.49 ± 7.41	-	97.0 ± 5.35	98.5 ± 5.85	-	101.3 ± 1.20	103.4 ± 1.34	-
WHR	0.86 ± 0.04	0.84 ± 0.03	-	0.83 ± 0.02	0.80 ± 0.02	-	0.89 ± 0.03	0.87 ± 0.04	-
Glucose tolerance									
Glycemia (mg/dL)	86.5 ± 3.80	89.29 ± 2.45	-	88.0 ± 5.23	89.17 ± 2.04	–	86.0 ± 2.82	87.57 ± 2.42	–
Insulin (mU/L)	10.00 ± 1.85	12.29 ± 1.94	-	14.67 ± 2.60	14.0 ± 1.96	–	10.14 ± 1.74	12.0 ± 1.98	–
Serum lipids profile									
Total Cholesterol (mg/dL)	190.2 ± 5.21	181.7 ± 14.60	–	194.7 ± 11.4	190.3 ± 10.91	–	178.0 ± 5.38	176.9 ± 7.59	–
HDL-C (mg/dL)	47.7 ± 5.05	46.3 ± 4.16	–	51.40 ± 2.84	50.0 ± 3.27	–	47.29 ± 4.06	47.86 ± 4.80	–
LDL-C (mg/dL)	114.3 ± 8.97	116.1 ± 11.72	–	122.7 ± 9.24	118.3 ± 8.92	–	110.6 ± 5.77	108.7 ± 6.26	–
VLDL-C (mg/dL)	18.57 ± 1.13	19.14 ± 1.77	–	20.6 ± 1.47	22.0 ± 2.14	–	20.14 ± 1.93	18.33 ± 1.12	–
Non-HDL-C (mg/dL)	132.9 ± 9.43	135.3 ± 12.38	–	143.2 ± 10.52	140.3 ± 10.32	–	130.7 ± 7.47	129.0 ± 7.30	–
Triglycerides (mg/dL)	88.71 ± 7.99	83.67 ± 6.94	–	99.83 ± 9.05	112.8 ± 16.17	–	98.57 ± 13.24	86.33 ± 8.41	–
Atherogenic index	0.27 ± 0.04	0.30 ± 0.06	–	0.33 ± 0.02	0.33 ± 0.07	–	0.30 ± 0.08	0.31 ± 0.10	–
Immunoglobulins									
IgA (mg/dL)	218.7 ± 31.83	227.7 ± 24.53	-	236.2 ± 21.59	231.7 ± 20.01	–	231.3 ± 31.39	265.6 ± 39.10	–
IgG (mg/dL)	1152 ± 107.7	1133 ± 246.6	–	1322 ± 93.57	1398 ± 42.34	–	1141 ± 67.21	1204 ± 96.50	–
Serum protein profile									
Albumin (g/dL)	4.59 ± 0.08	4.62 ± 0.06	–	4.66 ± 0.08	4.57 ± 0.08	-	4.49 ± 0.09	4.14 ± 0.07	–
C-Reactive protein (mg/dL)	0.20 ± 0.05	0.21 ± 0.07	-	0.14 ± 0.05	0.06 ± 0.02	–	0.16 ± 0.07	0.23 ± 0.10	–
Liver and Kidney function markers									
Creatinine (mg/dL)	0.79 ± 0.03	0.83 ± 0.04	–	0.88 ± 0.09	0.84 ± 0.10	–	0.71 ±.0.4	0.73 ± 0.04	–
Gamma-GT (U/L)	13.7 ± 2.70	26.0 ± 6.92	–	14.33 ± 1.33	13.67 ± 1.56	–	21.50 ± 2.63	24.86 ± 5.02	–

reduction in this ratio was observed only in the NSupple plus group post-supplementation. This relationship represents a reduction in cardiovascular risk and preservation of liver health in volunteers (Fig. 3A). Furthermore, there was a reduction in serum alkaline phosphatase levels after supplementation in the NSupple_S group compared to their baseline levels (Fig. 3D). Creatinine and gamma-GT values did not differ between groups over time (Table 3).

3.3. Proteins and inflammatory cytokines levels in the bloodstream

We also evaluated the presence of protein and inflammatory markers in the bloodstream before and after the supplementation. A reduction in total protein levels was observed in the NSupple plus group post-supplementation period (Fig. 3G). The NSupple group also demonstrated activation of the immune system through an increase in IgM post-supplementation (Fig. 3H). Serum albumin, C-reactive protein (CRP), IgA and IgG values did not differ between groups (Table 3).

Addressing the cytokines and chemokines levels in the bloodstream, we find an increase in IL-6 in the NSupple plus group post-supplementation (Fig. 4B). There was also an increase in IL-10 levels in the NSupple and NSupple plus post-supplementation (Fig. 4C). We also evaluated the levels of IL-1β, TNF-α and IL-12p70, CXCL/IL8, CCL5/RANTES, CXCL9/MIG, and CXCL10/IP10 which did not change throughout the supplementation period (Fig. 4A, D-I).

3.4. Sleep and quality of life enhancement by the nutraceutical composition

Evaluating the sleep pattern of the volunteers included in this research, the Epworth Sleepiness Scale (ESS) questionnaire was applied to provide a measure of the general level of daytime sleepiness (Table 2). Total ESS scores adequately distinguish individuals who have adequate sleep patterns from individuals who have sleep disorders such as obstructive sleep apnea syndrome (OSAS), narcolepsy, and idiopathic hypersomnia. The absence of statistical differences in this parameter demonstrates that the sample includes individuals who did not have sleep disorders from the beginning to the end of the period of supplementation consumption.

The sleep quality of these individuals was also evaluated using the Mini-Sleep Questionnaire (MSQ-BR). In this instrument, high scores represent worse sleep quality. The results presented in Fig. 5F demonstrate that the NSupple and NSupple plus groups promoted a reduction in the MSQ-BR score, demonstrating an improvement in the quality of sleep in these participants post-supplementation. It is important to consider that the absence of a control/placebo group, and the small sample size might be a bias of the results.

We also evaluated the study participants' reported quality of life through the WHOQOL-brief questionnaire developed by the WHO to determine the quality of life of populations based on 5 domains involving physical, psychological, social, and environmental aspects and their impact on quality of general life. The results show that there were no changes in the perception of the participants' general quality of life (Fig. 5A). However, evaluating the aspects separately, there was an improvement in the perception of the physical aspect that involves self-satisfaction with the personal perception of physical capacity in the NSupple plus_S group after the supplementation period (Fig. 5B). Evaluating the psychological aspect, there was a decrease in the perception of quality of life in the psychological aspect in the NSupple group (Fig. 5C). In terms of social relationships, there was an improvement in this aspect in the NSupple plus_S group post-supplementation (Fig. 5D). Finally, the environmental aspect did not differ post-supplementation (Fig. 5E).

Evaluating the Brunel Mood Scale (BRUMS), the domain related to the sensation of vigor was improved in the NSupple group (Fig. 6D). The NSupple plus group also demonstrated a decrease in the feeling of fatigue post-supplementation (Fig. 6E). The domains of tension, depression, anger, and confusion did not differ in the groups (Fig. 6A–C and F).

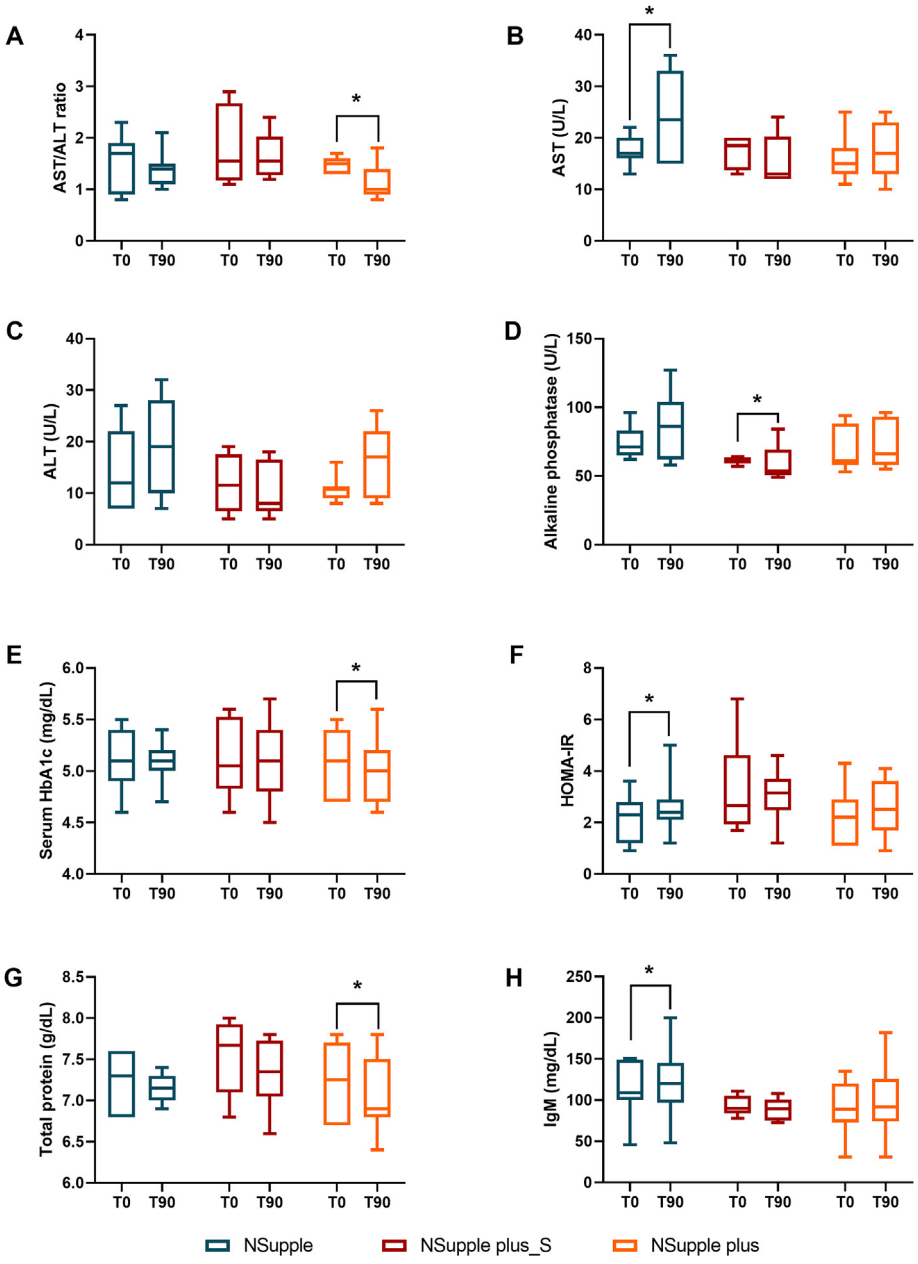

Fig. 3. Serum parameters before and after the 90 days between the different supplementation groups: **[A]** AST/ALT ratio, **[B]** AST, **[C]** ALT, **[D]** alkaline phosphatase, **[E]** serum HbA1c, **[F]** HOMA-IR, **[G]** total protein, **[H]** IgM.

3.5. Nutraceutical composition leads to anthropometric measures as predictive indicators of improved sleep quality

After the Pearson correlation test, multiple linear regression analysis was performed to check if the anthropometric measures such as BMI, WHtR, waist circumference, or body mass would be predictors of MSQ-BR in the different supplemented groups. In this sense, the NSupple group has shown a positive association of BMI with the MSQ-BR score. Also, the NSupple plus demonstrated an association of WHtR, waist circumference, and body mass variable with the MSQ-BR score, as shown in Table 4. Nevertheless, it is important to consider during this pilot study assessment that results might be influenced by the small sample size and lack of a control group.

4. Discussion

The pursuit of integrative medicine strategies aimed at sustaining long-term health is steadily expanding. The recognition that maintaining a balanced state of health can potentially reduce the occurrence of age-related pathologies has led to the exploration of compounds and therapies adopting a holistic approach to well-being [1]. Research in the field of integrative medicine has notably increased, particularly in the utilization of supplements and nutraceutical combinations that promote health by maintaining homeostasis, minimizing contraindications, and presenting few adverse effects [33,34]. Consequently, there is a growing need to thoroughly investigate the impacts of new nutraceutical compositions on health. In this context, this pilot study delves into

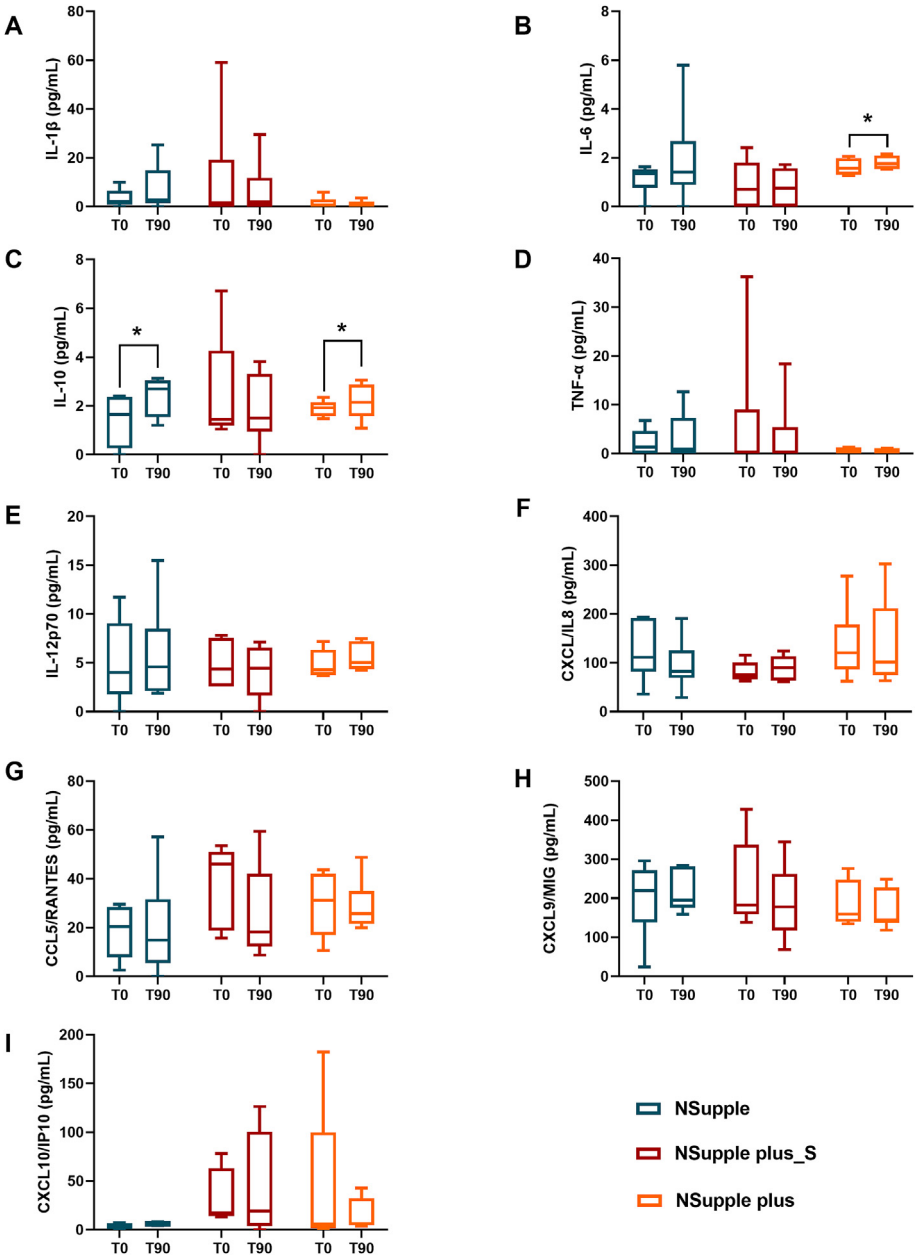

Fig. 4. Serum inflammatory parameters evaluated before and after supplementation **[A]** IL-1β, **[B]** IL-6, **[C]** IL-10, **[D]** TNF-α, **[E]** IL-12p70 **[F]** CXCL/IL8, **[G]** CCL5/RANTES, **[H]** CXCL9/MIG, **[I]** CXCL10/IP10.

exploring the potential and enhanced effects of a specific nutraceutical blend comprising prebiotics (β-glucans, GOS, and FOS), essential minerals (zinc, magnesium, and selenium), and an herbal extract (Silymarin from *S. marianum*).

This pilot study focuses on assessing its influence on parameters associated with the neuro-immune-endocrine axis and its implications for mood, sleep patterns, and perceived quality of life. To lay a robust foundation for the scientific evidence, this pilot study was undertaken to assess the feasibility and efficacy of employing these nutraceutical supplements in human applications [35]. As a preliminary investigation, it's crucial to acknowledge inherent limitations before delving into its effects. These constraints include a small sample size, a short-term supplementation, and uneven distribution in chronotype and BMI across groups due to the double-blind randomization process applied for allocation. Despite these limitations, we find value in sharing and discussing the obtained results, considering their relevance to this burgeoning field of research prior to the development of a bold clinical trial.

The individual components within the formulas examined here are acknowledged for their distinct health-promoting bioactive properties when utilized in isolation. Fructooligosaccharides (FOS) [19], galactooligosaccharides (GOS) [18], yeast β-glucans [17], minerals like magnesium [15], zinc [14], and selenium [36], as well as plant-derived compounds like *S. marianum* (Silymarin) [21], has been related to improved metabolic markers recovering the health status. Noteworthy, the nutraceutical compositions tested here display a complementary effect that potentiates the compound's benefits The preliminary findings, recently published in a preclinical model of obesity [33,34,37] examined the effects of the nutraceutical composition across a spectrum of metabolic disruption

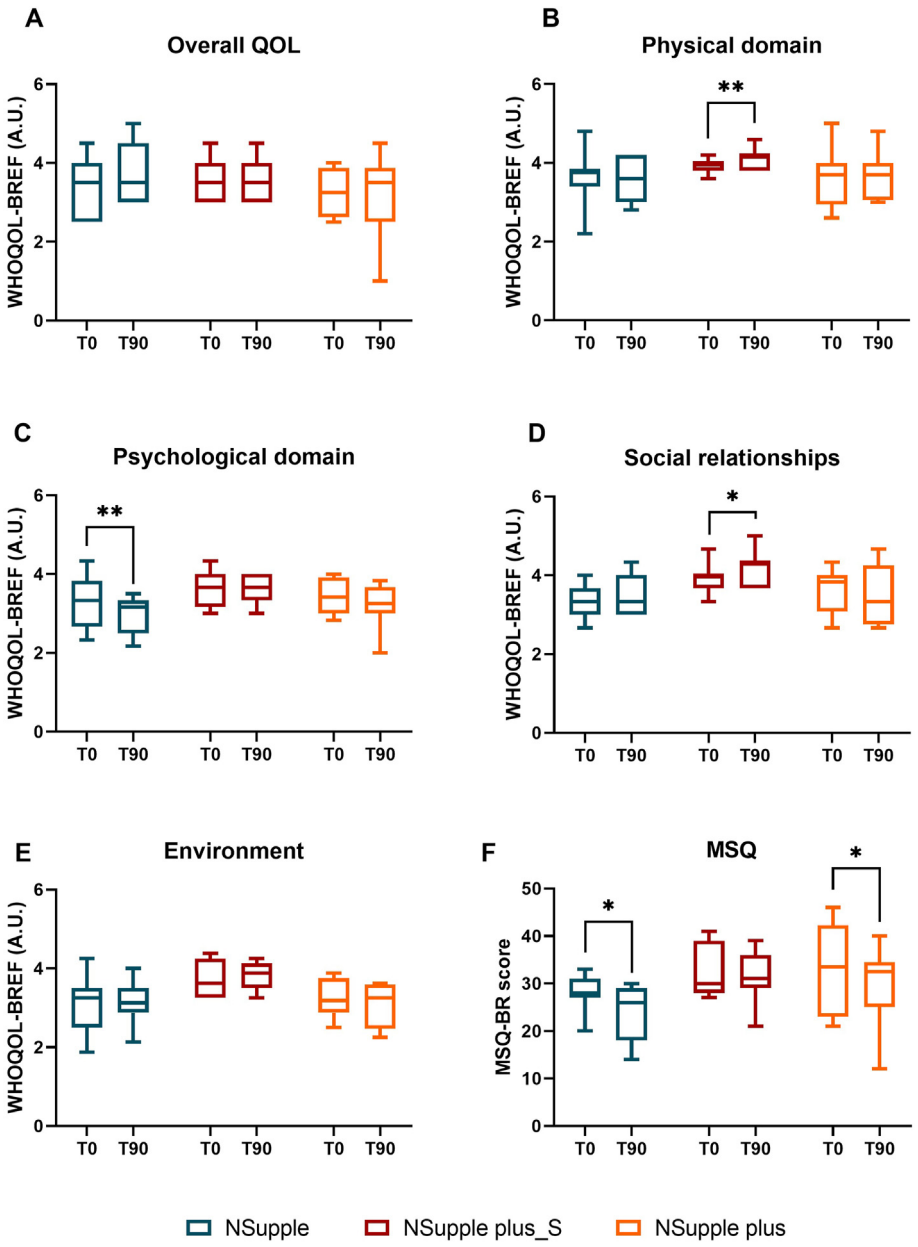

Fig. 5. Assessment of quality of life through the WHOQOF-brief questionnaire in aspects of **[A]** overall quality of life **[B]** physical domain, **[C]** psychological aspects, **[D]** social relationships, and **[E]** environmental aspects. Assessment of sleep quality through the **[F]** *Mini-Sleep Questionnaire* (MSQ).

scenarios. These initial findings have demonstrated favorable outcomes in countering weight gain associated with obesity, as well as aiding in the recovery of metabolic [34], inflammatory, and endocrine markers [33]. Additionally, they have shown potential for reshaping of gut microbiota [37,38]. Thus, the nutraceutical compositions demonstrate promising effectiveness in promoting health improvement and impacting markers that contribute to remission or reduction in the prevalence of chronic metabolic diseases highly incident in modern society, such as metabolic syndrome, type 2 diabetes (T2D), cardiovascular diseases, and metabolic dysfunction-associated fatty liver disease (MAFLD). Thus, this research aims to translate the pre-clinical findings into a clinical pilot study to establish a solid base for a double-blind and randomized clinical trial in the subsequent phase.

Currently, it has been known that holistic therapies may exhibit efficacy in treating and preventing symptoms and diseases, potentially without the adverse effects commonly associated with allopathic medicine, necessitating close monitoring. The scientific evidence supporting holistic treatments and therapies has fostered the potential for an alignment between conventional and integrative medicine strategies [39]. Thus, our present results suggest the safety of chronic consumption of nutraceutical supplementation, which did not trigger side effects or negative outcomes for young adults. Indeed, health professionals must have a comprehensive understanding of the benefits and possible side effects associated with nutraceutical supplement compositions and natural compounds before considering their applications in clinical practice. Our results regarding biochemical analysis cover a wide spectrum of routine exams for clinical practice used for the diagnosis of several endocrine, metabolic, and immunological pathologies. These analyses were selected due to their accessibility to healthcare professionals who may not be directly involved in scientific

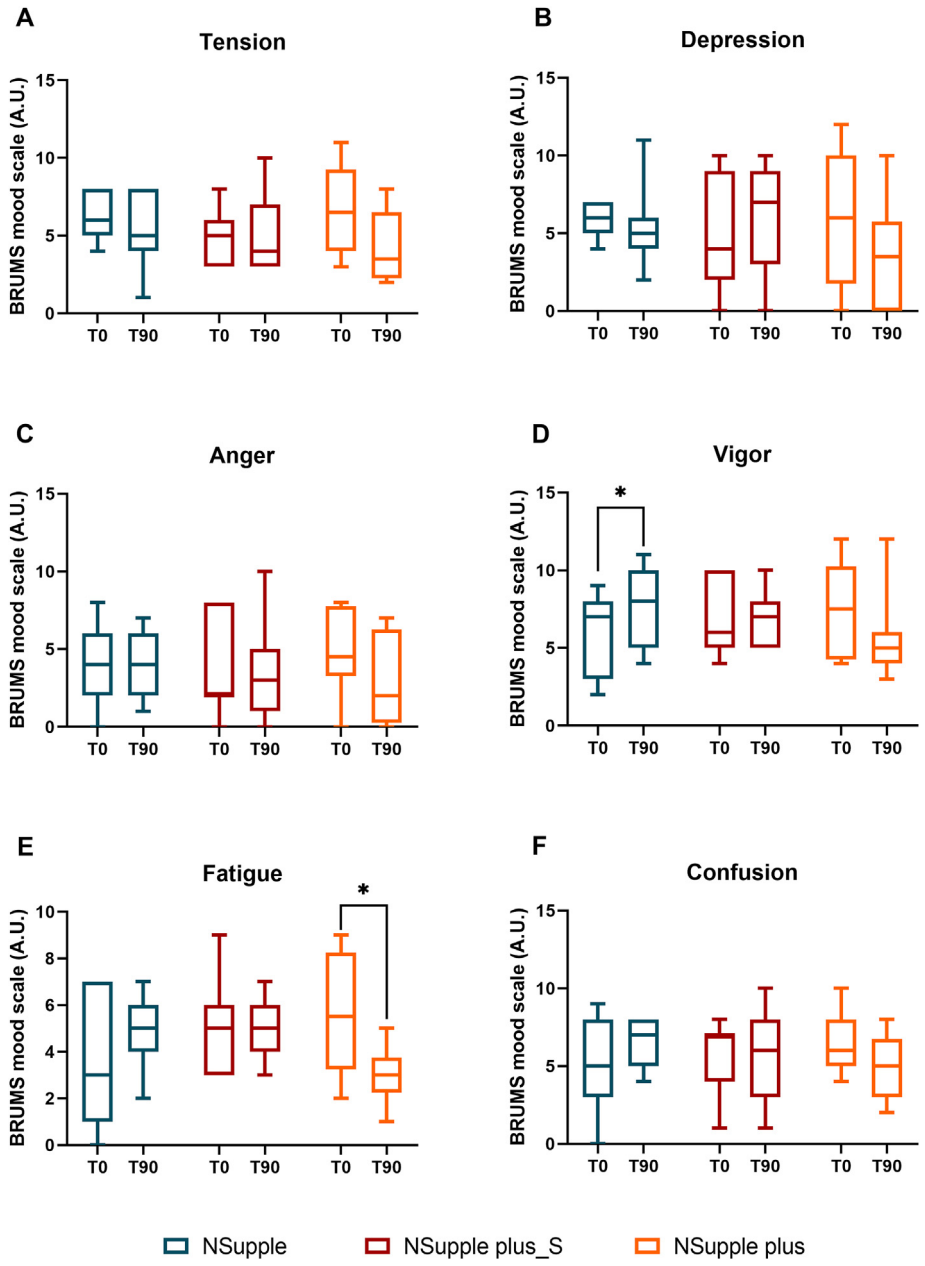

Fig. 6. Evaluation of the Brunel Mood Scale (BRUMS) in the aspects of **[A]** tension; **[B]** depression, **[C]** anger, **[D]** vigor, **[E]** fatigue, **[F]** confusion.

Table 4
Multiple linear regression analysis of Mini-Sleep Questionnaire (MSQ-BR) score and anthropometric parameters in supplemented overweight people.

	Mini-Sleep Questionnaire (MSQ-BR) score			
		β	95%CI	*p*
NSupple	**BMI**	40.76	12.05 to 69.46	0.0132
NSupple plus	**WHtR**	−43.46	−74.13 to −12.78	0.0008
	Waist circumference	23.89	6.415 to 41.37	0.0007
	Body mass	−13.97	−23.94 to −3.991	0.0140

research. Hence, we deem it pertinent to present and discuss these pilot study results here, bridging the gap between scientific findings and clinical practice. In this context, we did not detect changes in markers employed for diagnosing dyslipidemia, glycemic regulation, renal function, or immune response. This underscores the possible safety of continuous consumption of these supplements for 90 days, potentially aligning with a chronic intake pattern. Also, evaluating biochemical markers of liver health and function, we observed a reduction in the AST/ALT ratio in the NSupple plus group. In this sense, silymarin seems to potentiate the efficacy of lipoproteins and liver function enzymes recovery, since it is well established by the literature that silymarin has the potential for liver metabolism enhancement in robust research models [40]. This result suggests the recovery of liver health, preventing the onset of MALFD and NAFLD and contributing to the reduction of the risk of cardiovascular diseases such as ischemia [29]. This result is promising since the supplements components seem to act together, promoting a metabolic improvement in this population, despite the lack of lifestyle changes related to diet and exercise, which represents a potential tool to assist in intervention and treatment to reduce metabolic and cardiovascular disease risk in wider sample

sizes clinical trials. These results align with the anthropometric parameters evaluated, allowing us to discuss the importance of the interaction among nutrients. Our findings allow us to suggest the efficacy of supplementation in decreasing easily measurable anthropometric parameters directly associated with visceral adiposity, thereby potentially reducing cardiovascular risk. Both waist circumference and WHt ratio are recognized indicators of cardiovascular risk and metabolic syndrome prevalence [41]. Also, the BMI data distribution reveals that the NSupple and NSupple plus_S groups comprised volunteers within the eutrophic BMI range, whereas the NSupple plus group exhibited a minimum BMI placing individuals within the overweight and obesity classification which might be also considered a bias for this pilot study along with the reduced sample size. However, it is important to consider that the NSupple group had statistically reduced BMI and it includes volunteers ranging from eutrophic to obesity BMI. The anthropometric markers reduction comes along with the AST/ALT ratio results, suggesting a real reduction in the risk of cardiovascular disease in the NSupple plus group in just 90 days without diet or exercise intervention. These results are of great relevance for health research since, according to the WHO, cardiovascular diseases represent the greatest global cause of death nowadays [42]. Nevertheless, these findings must be further confirmed in a stronger sample size, including people with cardiovascular risk or metabolic syndrome diagnosis.

It is known that metabolic diseases related to overweight such as metabolic syndrome, type 2 diabetes, and MALFD have a strong interaction with inflammatory processes, mediated by the immune system [43]. It is understood that the most significant alterations take place within the innate immune system (neutrophils, dendritic cells, macrophages, and epithelial cells). Nonetheless, recent studies have discussed the involvement of the adaptive immune system (B and T cell lymphocytes) in initiating the process of metainflammation [44]. This pilot study indicates the immune system and inflammatory parameters effect through the modulation of immunoglobulin M (IgM) and cytokines such as IL-6 and IL-10. IL-10 plays an important anti-inflammatory role, reducing metainflammation associated with metabolic diseases [45]. The increase in IL-10 evidences the anti-inflammatory potential of the nutraceutical supplement, in addition to corroborating the preclinical results which demonstrated similar findings [33], enforcing the translational potential of this research. Furthermore, increased IL-6 was also observed in preclinical studies and demonstrates the pleiotropic action of this cytokine [33]. Circulating IL-6 plays a key role in central inflammatory processes and can directly affect hunger and satiety systems, as well as the circadian cycle, influencing sleep quality through the modulation of serotonin production at the central level [46]. These results may be related to the improvement in sleep quality perception reported by the NSupple plus group associated with increased cytokines in this same group.

Moreover, IgM stands as the primary antibody generated by B cells response. The elevation in IgM levels might suggest the potential enhancement in targeted immune responses, manifesting through escalated antibody production against infectious antigens. Recent research indicates that immunoglobulins like IgM can directly influence the restructuring of the microbiota by modulating the intestinal immune response, potentially suppressing or facilitating the proliferation of specific colonies crucial for maintaining eubiosis [47]. In this sense, IgM antibodies seem to play a crucial role in the immunometabolism of obesity and type 2 diabetes via modulating the microbiota [48]. Thus, the increase in IgM observed may be related to the microbiota reshape promoted by the nutraceutical supplementation previously published in a preclinical model of obesity [37]. These results once again seem to be relevant for clinical practice, since obtaining data on cytokines such as IL-6 and IL-10 may be unusual, but measuring markers such as IgM are accessible for the daily routine of health practitioners and may show evidence of deeper changes at a molecular level.

Regarding the results of mood, sleep, and quality of life presented here, it is noteworthy some volunteers' sample characteristics. Firstly, the sample chronotype evaluated by the Horne and Ostberg morningness-eveningness questionnaire shows that the volunteers were predominantly intermediate chronotypes. Chronotype refers to individual preference concerning the time of day when they perceive better performance in physical, mental, and social activities. This classification describes natural inter-individual differences in their ability to adapt to different circadian rhythms (24-h cycles that rule the body's biological and behavioral processes) [27]. The intermediate chronotype benefits the social functionality and work routine of these people who naturally favor performing activities during the daylight period and prefer to sleep between midnight and 8 a.m [49]. This chronotype normally faces fewer difficulties in adapting to the schedules imposed by commitments to activities in society. This sample characteristic may also be considered a bias of this pilot study results on sleep quality and should be kept in mind by the reader since the randomization did not consider the volunteers' chronotype. We also evaluated the presence of sleep disturbances such as insomnia or hypersomnia through the Epworth Sleepiness Scale (ESS-BR) questionnaire [50]. In this sense, we observed that our sample in general does not demonstrate important changes in sleep patterns before or after the supplementation period, emphasizing that our samples do not include important sleep disorders issues that must be closely monitored and could represent a bias for this research. Nevertheless, our results suggest a potential improvement in sleep quality by the reduction in the Mini-Sleep Questionnaire (MSQ-BR) score [26], especially in the NSupple and NSupple plus groups. Improved sleep quality can bring many benefits to health and well-being, both physical and mental. Some of the main benefits encompass enhancing the immune system, alleviating stress, enhancing memory and learning capabilities, and mitigating the risk of chronic illnesses [51].

Sleep disorders can be triggered by a state of chronic stress, which impacts immune functions and health in general. Additionally, inadequate sleep quality has emerged as a risk factor contributing to the onset or exacerbation of chronic degenerative conditions such as obesity, diabetes, and hypertension, as well as mental health disorders like anxiety and depression. These conditions significantly diminish the long-term quality of life for affected individuals. Neuroendocrine factors notably contribute to compromised sleep quality [10,52]. Enhancements in sleep quality can directly correlate with the synthesis of melatonin. This hormone facilitates the conversion of adenosine, generated throughout the day, within brain terminals, inducing a state of drowsiness conducive to sleep [51]. Stress can be considered a major trigger for the development of sleep disorders. During periods of stress, there is the bloodstream release of corticosteroids and pro-inflammatory cytokines such as interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin 1 beta (IL-1β), which lead to increased peripheral and central production of kynurenine. Kynurenine is a metabolite of L-tryptophan which is a precursor to serotonin, consequently reducing serotonin production in the brain. Serotonin produced in the brain is a neurotransmitter, which has the function of maintaining the balance between states of anxiety and depression [12,53]. In addition, serotonin is a precursor to melatonin, a key hormone to promote good sleep quality. It also plays a very important role in achieving deep, restorative NREM sleep. Apart from neuroendocrine stress, other forms of stress characterized by inflammation or oxidative processes also detrimentally impact the brain's serotonin production

[8]. In this sense, some phytochemical compounds in the nutraceutical formulation, such as silymarin, present in the NSupple and NSupple plus formulas, may have an antidepressant effect. Silymarin has been described to contribute to raising dopamine levels, as well as inhibiting the oxidative deamination of monoamines, such as serotonin and dopamine [54]. Serotonin, in particular, has a central action related to feelings of satisfaction and well-being. Circulating serotonin is stored and transported by platelets, and derived from the amino acid tryptophan used as cofactors, minerals such as zinc and magnesium present in the nutraceutical compositions tested, which might partially explain this pilot study's findings. Also, magnesium is especially important in the process of serotonin production by enterochromaffin cells, in addition to being a key mood regulator, directly participating in the production and secretion of neurotransmitters [55]. Some bacteria found in the intestinal microbiota, known as psychobiotics, also produce neurotransmitters, such as norepinephrine, dopamine, serotonin, and GABA (gamma-aminobutyric acid), which might have positive effects on mental health state [20]. Hence, we may infer that the alterations observed in the intestinal microbiota, facilitated by the nutraceutical supplement in the preclinical model [37], could potentially relate to the reported enhancements in sleep quality and the observed changes in mood as per the BRUMS scale in our pilot study. This association is likely due to the involvement of bacteria within the brain-gut communication pathway. Thus, stable and balanced microbiota can also contribute to the modulation of the neuro-endocrine system to improve sleep. Other components of the nutraceutical formulation, such as FOS and GOS, have a prebiotic role, favoring the growth of beneficial colonies that may contribute to the results observed in this study [20]. Nevertheless, the microbiota modulation hypothesis raised here must be confirmed in future in-depth research regarding the prebiotic potential of the nutraceutical formulas in clinical trials.

According to the regression analysis performed it is possible to suggest that the NSupple and NSupple plus groups presented an association with body measures like BMI, waist circumference, WHtR reduction, and the improvement of sleep quality after post-supplementation. The relationship between overweight and impaired sleep quality is not new since sleep disorders such as obstructive sleep apnea among others are closely linked to obesity [56]. Thus, the reduction of visceral adiposity body measures indicators might be related at some level to the improvement of sleep quality perceived by the volunteers in our results although it is not imperative and more analysis is necessary to make such statements. Moreover, the body measures reductions may also affect the immune system related to inflammatory pathways activation [43]. Overweight/obesity is directly linked to chronic subclinical inflammation development and maintenance; thus, we suggest that the inflammatory biomarkers reduced might be linked to the improvement of anthropometrics. It is interesting to note that the effect found in this pilot study might be mostly attributed to the supplementation intake since the volunteer did not change their lifestyle regarding diet or exercise practice which represent crucial factors for long-term high quality of life [55]. Here, it is noteworthy that the supplementation intake was able to promote an improvement of anthropometric markers similar to those attributed to lifestyle changes enforcing the improvement of sleep quality and some facets of mood and quality of life promoted by the supplements. Despite these encouraging results, it is necessary to once again warn about the limitations of this pilot study and emphasize that these results must be observed with caution to avoid inappropriate extrapolations of these initial clinical findings. Therefore, the neuro-immune-endocrine axis plays a crucial role in sleep quality, with complex and regulatory interactions among the nervous immune, and endocrine systems. The proper balance and interaction of these systems are essential for good sleep hygiene and maintaining a healthy sleep pattern.

5. Conclusions

Summing up, this pilot study allows us to suggest that after 90 days of the nutraceutical composition intake, there may be an improvement in the quality of sleep as well as some domains of mood and quality of life assessment, indicating an improvement in the participant's perception of well-being. Nevertheless, it's crucial to bear in mind that the assessment of sleep quality, quality of life, and mood - although conducted using validated questionnaires from existing literature - captures subjective aspects reported by volunteers. These perceptions can be susceptible to external factors beyond the scope of the experimental design and the control of researchers and must be carefully evaluated considering the limited sample size of this pilot study. In addition, our results suggest that the nutraceutical composition could potentially act in reducing visceral adiposity markers, protecting liver health, and reducing risks of cardiovascular diseases by reducing the AST/ALT ratio and WHtR in our limited population of young healthy adults. These results might be more valuable if we consider that there were no lifestyle changes, allowing us to wonder whether the effects found can be directly attributed to the supplement intake in this pilot study. We believe that the data presented here may be appreciated by healthcare practitioners, contributing to an up-to-date conscious inclusion of integrative medicines in clinical services. Moreover, it is important to consider that this pilot study acknowledges inherent limitations in its experimental model, that must be stated such as the limited sample size, absence of a control or placebo group, a short-term supplementation period, and uneven chronotype and BMI distribution among groups. Nevertheless, the findings presented here underwent meticulous scientific scrutiny to ensure their reliability. These flaws must be better elucidated in future research bringing to light in-depth analyses of the physiological mechanisms discussed in this article, in addition to a larger sample size as well as long-term effects of the nutraceutical composition in an appropriate double-blind randomized and controlled trial enrolling a stronger sample size.

Ethics statement

The research with human participants, underwent review and approval by the HC-FMUSP Research Ethics Committee, with the CAAE number 39984320.5.0000.0068. It was also registered as a Clinical Trial with identification number NCT04810572 on ClinicalTrials.gov. All patients/participants provided written informed consent to take part in this study.

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Author contributions

ABS and AFMP performed data analysis, intellectual support, writing, and discussion of findings; JAF, GMM, AFG, and EHRO performed experiments, data analysis, and technical assistance; DAM, ES and JPO contributed with intellectual support; ESO and

DPD performed statistical data analysis and contributed with technical assistance; BFRBS and SLS performed data curation; AFMP and VNF gave conception and design of the study and was adviser and supervisor in the whole project.

Data availability statement

The data supporting this article will be made available by the authors, without undue reservation.

Declaration of competing interest

This study received funding from EFEOM Nutrition S.A. The funder had the following involvement with the study: financial support for experimental protocol development and financial resources for analysis performed. All authors declare no other competing interests.

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