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Research Article

Inositol And High-Dose Water-Soluble Vitamins Supplementation To Improve Psychological Well-Being, Cognitive Function, Perceived Stress, Physical Fatigue, And Sleep Quality.

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Abstract

The general population commonly utilizes nutritional supplementation with one or more water-soluble vitamins to reduce perceived stress and fatigue, improve physical and mental performance, as well as sleep quality. However, to date, no published studies have evaluated the daily use of a nutraceutical formulation combining inositol with high doses of all water-soluble vitamins, aimed at reducing emotional distress, physical and mental fatigue, and perceived stress, while improving psychological well-being, cognitive function, and sleep quality in healthy individuals aged 18 to 65 years. This study provides a preliminary contribution to addressing this gap. In this prospective, comparative, parallel-group study, the effects of daily intake of a nutraceutical tablet containing inositol and high doses of all water-soluble vitamins called Vidrosol•50® (V50) were evaluated on 139 healthy male and female subjects presenting with emotional distress, symptoms of mental and physical tiredness and listlessness, a feeling of general debilitation, and sleep disturbances such as excessive sleepiness and/or difficulty awakening. The enrolled subjects were divided into two groups: one group of 71 subjects was treated with the nutraceutical and compared to a second group of 68 subjects with very similar demographic and medical history characteristics presenting the same conditions who, during the same period, did not take any nutraceutical or drug for the treatment of these conditions. All subjects involved in the study completed the General Health Questionnaire (GHQ-12), the Fatigue Assessment Scale (FAS), the Multidimensional Inventory of Subjective Cognitive Impairment (MISCI), the Perceived Stress Scale (PSS), and a Sleep Quality Scale (SQS) at baseline (T0), and at follow-up assessments conducted after 7 days (T1) and 30 days (T2).

The use of V50 resulted in statistically significant improvements in the total scores of all questionnaires administered already at T1. These improvements were further accentuated at T2. In the untreated subjects, no significant variations in the total questionnaires scores were observed over the same time frame.

The tested nutraceutical was confirmed to be safe and well tolerated: no subject discontinued the intake of V50 due to adverse effects attributable to the nutraceutical.

The study results provide evidence that healthy male and female subjects can benefit from increased intake of inositol and all water-soluble vitamins through the daily administration of a nutraceutical containing inositol and high doses of all water-soluble vitamins. Specifically, this supplementation led to significant improvements, already evident by day 7, in validated assessments of emotional distress and psychological well-being, physical and mental fatigue, cognitive functions, perceived stress, and sleep quality.

Keywords: Inositol, water-soluble vitamins, emotional distress, psychological well-being, physical fatigue, mental fatigue, cognitive function, perceived stress, sleep quality.

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INTRODUCTION

Every vital manifestation is marked and conditioned by biochemical reactions. An appropriate biochemical balance is essential for maintaining homeostasis and the proper functioning of all physiological processes in the human body, and therefore for maintaining or restoring health [1]. In this context, water-soluble vitamins play a crucial role in biochemical functions and cannot be synthesized by the human body, as it lacks the necessary enzymatic pathways. Water-soluble vitamins include thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), vitamin B6, biotin (B7), folate (B9), cobalamin (B12), and ascorbic acid (C).

In cellular biochemistry, enzymes play a fundamental role as they can selectively accelerate chemical reactions by reducing their activation energy. Water-soluble vitamins represent the essential coenzymes for most of enzymatic processes that sustain cellular function [2]. For this reason, the deficiency of even a single water-soluble vitamin has negative consequences on the structure and function of the human body. Except for cobalamin, water-soluble vitamins are not stored in the human body; therefore, it is essential that they are regularly supplied in adequate amounts to meet physiological requirements and support the maintenance of health. Various types of insufficient intake of water-soluble vitamins are described in the scientific literature, and it has been documented that such conditions are common even in apparently well-nourished populations of developed Countries [1]. Although contradicted by epidemiological statistics, it is precisely this situation of apparent well-being that often leads to doubts about the therapeutic utility of the administration of water-soluble vitamins. In developed countries, insufficient intake of water-soluble vitamins is a frequent consequence of an unbalanced diet, often rich in calories but relatively poor in micronutrients [3,4], and of the lability of many water-soluble vitamins, which are destroyed by cooking, storage, contact with acids and alkalis, exposure to sunlight, and humidity [5]. Cross-sectional population studies have shown that vitamin C deficiency affects 10-20% of the Western population and that insufficient vitamin C status is associated with increased all-cause mortality [6-8]. A survey conducted in Italy on the population aged over 65 years reported a prevalence of malnutrition of 25% for thiamine, 20% for riboflavin, 15% for folates and 14% for vitamin C [9]. A study examining the diet of the population of two Italian localities, Bagnara Calabra and Trino Vercellese, reported average daily intake values of folates of 161 µg (from 89 to 259) and 84 µg (from 44 to 104), respectively, well below the recommended values [10]. Another investigation conducted in the United States, Germany, the United Kingdom and the Netherlands found that a significant proportion of the population consumes a dose below the Recommended Dietary Allowance (RDA) for each of the water-soluble vitamins that were considered, namely vitamins B1, B2, B3, B6, B12, C and folate [11]. In the United States, over 30% of a representative sample of individuals over 60 years of age suffers from vitamin B12 deficiency, which may be a consequence of a progressive reduction in intestinal absorption of this vitamin [12]. Among vegetarians, vitamin B12 deficiency has been documented by measurement of methylmalonic acid, holotranscobalamin II, or both in 11%–90% of older adults, 62% of pregnant women, 25%–86% of children, and 21%–41% of adolescents [13]. Even higher deficiency rates have been reported among vegans [13]. In a systematic review of blood cobalamin-based surveys among vegetarians, up to 86.5% of adults and older adults, up to 45% of infants, up to 33.3% of children and adolescents, and 17%–39% of pregnant women were found to be vitamin B12 deficient [14].

Even overnourished subjects are not exempt from water-soluble vitamin deficiencies. Obesogenic diets typically consist of foods rich in fat and simple sugars and low in micronutrients and cause deficiencies of a wide range of vitamins and minerals [3]. This situation is worsened for water-soluble vitamins directly involved in the metabolism of foods high in fat, sugar and protein, such as vitamin B1 and biotin, which play an essential role in glucose metabolism. A large body of research dating back decades, suggests vitamin B1 deficiency is neither rare nor limited to the traditionally defined populations, but that it is simply under-recognized. In these studies, depending upon the population investigated and the assays and cutoff values used to determine thiamine status, frank deficiency has been observed in 10 - 90% of subjects tested [15]. Low biotin levels have been detected in diabetic patients or in individuals with increased fasting plasma glucose levels [16].

Even common marginal vitamin deficiencies predispose to an increased risk of disease. Several scientific studies state that neurological and psychological manifestations of vitamin B12 deficiency may be evident at higher plasma levels than those indicating a deficiency condition [17,18]. Using a cutoff of 220 pmol/l, 20% of the US population over the age of 50 is marginally deficient in vitamin B12 [19]. In fact, according to some authors, the lower reference limit for serum vitamin B12 levels may be too low. Evidence suggests that adverse health effects can occur even when vitamin B12 concentrations fall within the apparently normal range [17]. Scientific evidence highlighting the risks associated with marginal deficiency conditions is also available for other water-soluble vitamins, such as vitamin B6 [20] and vitamin B2 [21,22].

The folate and methionine cycles, in which several water-soluble vitamins are essential coenzymes, play a crucial role in methylation processes and in maintaining the biochemical homeostasis of the central nervous system. Water-soluble vitamins, particularly vitamins B1 (thiamine), B6 (pyridoxine),

B9 (folate), and B12 (cobalamin), are essential for the synthesis of neurotransmitters such as norepinephrine, dopamine, serotonin, gamma-aminobutyric acid, and acetylcholine [23,24]. Deficiencies in water-soluble vitamins, particularly vitamins B1, B6, B9, and B12, are associated with mood disorders that can increase the stress perception or cause depression in association with neurotransmitter alterations and increased homocysteinemia [23-25]. Furthermore, a growing body of scientific studies demonstrates that elevated plasma homocysteine levels and low plasma levels of water-soluble vitamins are associated with an increased risk of sleep disturbances [26-29]. Frank deficiencies of most water-soluble vitamins have been associated with lethargy or physical fatigue. These conditions can also be observed in cases of marginal deficiencies but are often overlooked as they are considered nonspecific [30].

Based on the well-known biochemical functions of water-soluble vitamins, the evidence presented so far, and the fact that inadequate daily dietary intake is the main cause of their deficiency, it appears desirable to identify effective strategies to increase the daily intake of water-soluble vitamins. This would aim to optimize the cellular biochemical environment and, consequently, improve overall physiological functions. It is important to emphasize that the simultaneous and daily administration of all water-soluble vitamins, not just some of them, is considered essential. In fact, evidence is available in the literature demonstrating that the administration of a single water-soluble vitamin can cause depletion of other vitamins involved in the same biochemical pathways and may negatively impact health [22,31]. Furthermore, given the biochemical relevance of inositol [32] and its functional interconnection with water-soluble vitamins, even a marginal deficiency may compromise the biochemical activity of these vitamins. Therefore, the co-administration of inositol alongside water-soluble vitamins is considered essential. Although inositol is not classified a vitamin, its deficiency can be caused by inadequate dietary intake, insufficient intracellular absorption, reduced endogenous synthesis or an excessive elimination rate [33].

In conclusion, when the intake of water-soluble vitamins fully meets physiological requirements, their biochemical properties translate into the normal performance of physiological functions. When the intake is lower than physiological needs, actual deficiencies may occur, manifesting with clinical symptoms. There is increasing evidence demonstrating that intermediate situations, often defined as “suboptimal” or “inadequate” nutritional status, “marginal deficiency” or “insufficiency”, may be associated with subclinical functional deficits and an increased risk of pathologies. Targeted dietary supplementation with appropriate nutraceuticals can address both clinical and subclinical deficiencies [34].

The aim of this study is to evaluate the effects of treatment with Vidrosol•50® (V50), a nutraceutical containing inositol and all water-soluble vitamins, in healthy male and female subjects experiencing emotional distress, symptoms of fatigue, mental and physical listlessness, a feeling of general debilitation, and sleep disturbances, such as excessive sleepiness and/or difficulty awakening. By comparing a group of subjects treated with V50 to an untreated control group, we also aim to verify whether the intake of V50 can determine a benefit on the signs and symptoms reported by the subjects, beyond random variation. In addition to demonstrating a statistically improvement in questionnaire scores in the treated subjects compared to the untreated subjects, we believe that a truly effective vitamin supplementation for 30 consecutive days should favourably modify the questionnaires scores of the subjects enrolled in the study by at least 30%.

MATERIALS AND METHODS

Tested Nutraceutical

Vidrosol•50® (V50) is a nutraceutical listed in the Italian Ministry of Health’s National Register of Food Supplements under code 166914. V50 is produced by MèDISIN S.r.l. (Lissone, MB, Italy) and has been marketed in Italy since October 1, 2023. The qualitative and quantitative composition of V50 is reported in **Table 1**.

Table 1. Qualitative and quantitative composition of V50.

Ingredient	Dose per tablet
Inositol	200 mg
Vitamin C (L-Ascorbic Acid)	160 mg
Vitamin B3 (Niacin)	54 mg
Vitamin B1 (Thiamine)	25 mg
Vitamin B2 (Riboflavin)	25 mg
Vitamin B5 (Pantothenic Acid)	18 mg
Vitamin B6 (Pyridoxine)	10 mg
Biotin	450 µg
Vitamin B9 (L-methylfolate)	400 µg
Vitamin B12 (Methylcobalamin)	25 µg
Vitamin B12 (5’-Deoxyadenosylcobalamin)	25 µg

According to current Italian regulations, the authorized health claims for the individual ingredients of the nutraceutical are listed on the V50 packaging. Niacin, pantothenic acid, and vitamins B2, B6, B12, and C contribute to normal energy-yielding metabolism and the reduction of tiredness and fatigue. Biotin contributes to normal macronutrient metabolism. Folate contributes to normal amino acid synthesis. Vitamin B6 contributes to normal protein and glycogen metabolism. Folate, Vitamin B6, and B12 contribute to normal homocysteine metabolism. Niacin, Thiamine, Biotin, and Vitamins B6, B12, and C contribute to the normal functioning of the nervous

system and normal psychological function. Vitamin B6 contributes to the regulation of hormonal activity. Pantothenic acid contributes to the normal synthesis and metabolism of steroid hormones, Vitamin D, and some neurotransmitters. Folate contributes to normal hematopoiesis. Vitamins B6 and B12 contribute to normal red blood cell formation. Vitamin B2 contributes to the maintenance of normal red blood cells and normal iron metabolism. Vitamin C enhances iron absorption. Thiamine contributes to normal cardiac function. Folate and vitamins B6, B12, and C contribute to the normal function of the immune system. Niacin, vitamin B2, and biotin contribute to the maintenance of normal skin. Niacin, vitamin B2, and biotin contribute to the maintenance of normal mucous membranes. Vitamin C contributes to normal collagen formation for the normal function of blood vessels, bones, teeth, cartilage, gums, and skin. Vitamins B2 and C contribute to the protection of cells from oxidative stress.

Study design

This is a prospective, comparative, parallel-group study comparing parameters measured in a group of subjects called the V50 Group, at the time of starting V50 intake (baseline), after 7 days of taking the nutraceutical, and after 30 days of taking the nutraceutical. For a total of 30 consecutive days, subjects in the V50 Group took one tablet of the V50 daily with a glass of water, between meals. The parameters measured in this Group were compared with those measured at the same time points in a parallel, untreated Group called the Control Group.

The study was conducted in compliance with the guidelines for Good Clinical Practice [35] and the Declaration of Helsinki [36]. In the case of the present study, ethical approval is not applicable. The 2015 ministerial guidelines, based on the indications of the European Food Safety Authority (EFSA) state that the ethics committee approval is required only for studies aimed at evaluating the efficacy and safety of specific components (i.e. food ingredients) of the dietary supplement, and therefore to attribute specific health claims for those components, or for studies aimed at refuting the health claims previously attributed to them. In the current study, we investigated how a food supplement (and thus a dietary recommendation given by the experimenter) can affect the well-being of the subjects who take the supplement rather than focusing on the activity of a specific component and therefore on the legitimacy of attributing or not a health claim to such component [37].

In accordance with the usual practice of the physicians authors of this study and in compliance with current regulations, the enrolled subjects were informed about the characteristics and benefits of the nutraceutical they were taking and signed an informed consent form, including the processing of their personal data.

At baseline, a medical history form was completed for each subject, detailing all the collected data. A form was attached to be completed at the next check-up.

Subjects Evaluated

The V50 and Control Groups included adult male and female subjects. At enrollment, subjects were required to be 18 years of age or older and under 65 years of age. Subjects had to present symptoms of mental and physical fatigue and listlessness, a feeling of general debilitation, and sleep disturbances, such as excessive sleepiness and/or difficulty waking up. They also had to have a GHQ-12 score of 9 or greater.

Subjects were enrolled if they had not yet started any therapies for the aforementioned conditions or had discontinued any therapies for at least 15 days.

Subjects who, in addition to taking V50, were also taking medications, dietary supplements, or medical devices that could interfere with the effect of V50 during the observation period were excluded from the V50 Group. All subjects who had taken any medication, food supplement, or medical device for the treatment of physical or mental fatigue or sleep disturbances or that could affect physical or mental fatigue and sleep quality during the observation period were excluded from the Control Group.

Subjects who answered “never” in all items of the FAS questionnaire except items 4 (“I had enough energy for my daily activities”) and 10 (“When I did something, I was able to concentrate quite well”) were excluded. Also subjects suffering from prolonged (1-6 months) or chronic (more than 6 months) fatigue, anemia, myalgic encephalomyelitis, systemic exercise intolerance disease, chronic fatigue syndrome, or post-viral fatigue syndrome were excluded. All subjects with asthenic symptoms associated with the following causes were excluded: cancer (including metastases, chemotherapy, and radiotherapy), depression or other psychiatric conditions, hypercalcemia, hypernatremia, hyperkalemia, dehydration, anorexia, cachexia, endocrine disorders, infections, heart and/or lung disease. Subjects undergoing immunotherapy, bone marrow transplantation, chronic use of opioids, steroids, antiemetics, beta-blockers, sedatives, and psychotropic drugs in general were excluded. Subjects with food intolerances or allergies, a BMI > 35 kg/m2 (grade II obesity), already undergoing therapies other than V50 for the treatment of asthenia, pregnant or breastfeeding, mentally incapacitated, with language barriers that could compromise data collection, or known allergies or hypersensitivity to any component of the nutraceutical were excluded.

Medical History and Physical Examination

The subjects underwent a physical examination and a thorough medical history, including an assessment of

symptoms, allergies, diet, fruit and vegetable intake, alcohol consumption, smoking, occupational activity, comorbidities, and current medications. Alcohol consumption was reported for each subject based on the number of alcoholic units typically consumed over a week. One alcoholic unit corresponds to 12 grams of pure alcohol and is equivalent to: a glass of wine (125 ml with an alcohol content of 12% ABV); a can of beer (330 ml with an alcohol content of 4.5% ABV); an aperitif (80 ml with an alcohol content of 38% ABV); and a shot of spirits (40 ml with an alcohol content of 40% ABV). Alcohol, fruit, and vegetable consumption levels were recorded at T0 and confirmed at T2 by self-reporting to ensure that they reflected the subjects' habits in the previous period and during the 30-day observation period, after clarifying the definitions of an alcoholic unit and a portion of fruit and vegetables.

General Health Questionnaire (GHQ-12)

The GHQ-12 is of the most widely used standardized instruments for measuring the level of emotional distress and psychological well-being in epidemiological studies [38]. It is a self-administered questionnaire composed of 12 items, each of which assesses the severity of a psychological problem in the past few days using four predefined responses such as: not at all; no more than usual; more than usual; much more than usual; or: more than usual; as usual; less than usual; much less than usual. The scores typically used are a binary scale (0-0-1-1) and a 4-point Likert scale (0-1-2-3). The scores of all items are summed to obtain a total score ranging from 0 to 12 (binary scale) or from 0 to 36 (Likert scale), with higher scores indicating a more severe degree of impairment. A score above a specific cutoff (3/4 for the bimodal scale and 13/14 for the Likert scale) indicates psychological distress and suggests further investigation for potential mental disorders. In this study, the 4-point Likert scale was used for scoring.

Fatigue Assessment Scale (FAS)

The FAS is a 10-item scale that assesses the symptoms of chronic fatigue [39]. Unlike other similar measures, the FAS treats fatigue as a unidimensional construct and does not separate its measurement into different factors. However, to ensure that the scale assesses all aspects of fatigue, the developers chose items that represent both physical and mental symptoms. The scale has been validated on a population of men and women with a mean age of 45 ± 8.4 years and 43 ± 9.5 years, respectively. The FAS is a self-administered scale that takes just a few minutes to complete. Each FAS item is rated using a five-point scale, from 1 ("never") to 5 ("always"). Items 4 and 10 are reverse scored. The total score can range from 10, indicating the lowest level of fatigue, to 50, indicating the highest level.

Multidimensional Inventory of Subjective Cognitive Impairment (MISCI)

The MISCI is a self-administered questionnaire designed to measure subjective cognitive dysfunction in patients with fibromyalgia [40]. Although the subjects included in this study do not suffer from fibromyalgia, this questionnaire was chosen because the enrolled subjects temporarily experience a condition characterized by persistent fatigue, accompanied by sleep disturbances and cognitive difficulties comparable to those chronically experienced by patients with fibromyalgia. Furthermore, the questionnaire is simple and self-administered, which significantly facilitates data collection. The first six items are positively worded and reflect perceived cognitive abilities, while the last four items are negatively worded and reflect perceived cognitive difficulties. There are two items for each cognitive domain, listed in the following order: mental clarity, memory, attention/concentration, executive functions, and language. The same score is used for both response scales; however, scores for items 7-10 must be reverse coded before summing the response scores to obtain the total score, due to the negative wording of these items. The possible total score range is 10 to 50, with higher scores indicating better perceptions of cognitive functioning.

Perceived Stress Scale (PSS)

The PSS is the most widely used psychological instrument for measuring perceived stress. It is a self-administered questionnaire to assess subjective perceptions of specific everyday situations and reactions to events perceived as destabilizing and risky [41]. It consists of 10 items that address feelings and thoughts over the past few days. Each item must be rated by choosing from five options on a 5-point scale ranging from 0 to 4 depending on severity. The scores are reversed for the responses to the four positively worded items (items 4, 5, 7, and 8). The total score is calculated by adding the scores of the ten responses and ranges from 0 to 40. A higher score indicates a higher level of perceived stress. A score between 0 and 13 indicates a low level of stress; between 14 and 26 indicates moderate stress; between 27 and 40 indicates a high level of stress.

Sleep Quality Scale (SQS)

To assess sleep quality, a seven-point scale, previously used in a clinical study [42], was used. Subjects rated their overall sleep quality over the past two weeks using the following scale: 0 (very poor), 1 (very poor), 2 (poor), 3 (fair), 4 (good), 5 (very good), and 6 (excellent).

Timeline of Follow-Up

Subjects in the V50 Group and those in the Control Group completed the GHQ-12, FAS, MISCI, PSS, and SQS questionnaires on three occasions: at baseline, which in the

case of subjects in the V50 Group also represented the start date of V50 intake (T0), after 7 days (T1), and after 30 days (T2). At T1 and T2, information on any adverse effects induced by the nutraceutical was collected.

Statistical Analysis

Descriptive statistics were used to summarize cohort characteristics in terms of median, mean, and standard deviation (SD), or frequencies when appropriate.

The treatment effect was estimated as the change in outcome in treated patients between questionnaire scores at T0 and questionnaire scores at T1 and T2. The significance of differences was determined by applying the nonparametric Wilcoxon test for paired data from subjects belonging to the same group and the nonparametric Mann-Whitney test for unpaired data when comparing changes between subjects belonging to different groups. For all analyses conducted, results were considered statistically significant at $P < 0.05$.

GraphPad Prism version 8.0.2 for Windows software, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com, was used for statistical analysis.

RESULTS

Subjects enrolled between November 2023 and March 2025 who were compliant with the study protocol during the observation period were included in the study. A total of 150 subjects were enrolled in the study, all presenting symptoms of mental and physical fatigue and listlessness, a feeling of general debilitation, sleep disturbances, and had a GHQ-12 score greater than or equal to 9. The subjects were equally divided into two groups of 75 subjects each. When the results were processed, four subjects from the V50 Group and seven from the Control Group were excluded due to incomplete data. The analyses were therefore conducted using data from 71 subjects from the V50 Group and 68 subjects from the Control Group. The demographic and medical history data of the subjects included in the study did not differ statistically between Groups and are reported in **Table 2**.

The medications regularly taken by the subjects included in the study are listed in **Table 3**.

In the V50 Group, the T1 check-up was performed on average 7.25 ± 1.44 days after T0; the T2 check-up was performed on average 22.9 ± 1.26 days after T1. In the Control Group, the T1 check-up was performed on average 6.81 ± 0.50 days after T0; the T2 check-up was performed on average 23.7 ± 2.09 days after T1.During the treatment period, no adverse effects attributable to the nutraceutical taken were reported in the V50 Group.

Table 2. Demographic and medical history data of the subjects included in the study. The two Groups were homogeneous for all demographic and medical history parameters. Data are expressed as mean ± SD, unless otherwise indicated. P values not statistically significant are italicized.

Variable	V50 Group (n=71)	Control Group (n=68)	P-value
Age (years)	42.4 ± 13.2	42.8 ± 15.1	*0.7438*
Sex, % Female (n)	57.7% (41)	54.4% (37)	*0.7343*
Height (cm)	170.0 ± 8.57	170.0 ± 9.58	*0.9891*
Weight (kg)	67.9 ± 11.5	67.1 ± 14.7	*0.7375*
BMI (kg/m2)	23.5 ± 3.09	23.0 ± 3.51	*0.3404*
Fruit and vegetable, servings per day (n)	2.02 ± 1.01	2.10 ± 0.93	*0.6573*
Smoking, cigarettes per day (n)	2.00 ± 4.64	2.48 ± 5.32	*0.4167*
Alcohol intake, units per week (n)	1.72 ± 2.85	1.97 ± 2.42	*0.1351*
Allergies % (n)	15.5% (11)	19.1% (13)	*0.6559*
Comorbidities % (n)	26.8% (19)	33.8% (23)	*0.4602*
Regular intake of medication % (n)	26.8% (19)	29.4% (20)	*0.8505*

Table 3. Medications regularly taken by study subjects, divided by Group.

Drug category	V50 Group (n=71)	Control Group (n=68)
ACE inhibitors	5	7
Proton pump inhibitors	6	5
Sartans	4	3
Statins	4	3
Oral Contraceptives	2	4
Low-dose acetylsalicylic	2	2
Mineral supplements (Ca)		1
Vitamin D		1
Diuretics		1
PDE5 inhibitors	1	
Aminosalicylates	1	

General Health Questionnaire (GHQ-12)

At T0, the comparison of the total GHQ-12 scores between the two Groups revealed no statistically significant differences (**Table 4, Figure 1**).

In the V50 Group, the total GHQ-12 score decreased significantly at T1 compared to T0 and further statistically significantly at T2 compared to T1, demonstrating a reduction in emotional distress and an improvement in psychological well-being. In the Control Group, the score variations were not statistically significant between T0 and T1, T1 and T2, or T0 and T2.

The mean percentage change in the total GHQ-12 score between T0 and T1 in the V50 Group was -41.7±26.8%, while in the Control Group it was -0.26±27.5%. The mean percentage variation in the total GHQ-12 score between T0 and T2 in the V50 Group was -53.6±40.0%, whereas in the Control Group it was -3.12±23.2%.

At T0, 56.3% of the subjects in the V50 Group scored 14 or higher on the GHQ-12, confirming that the study population perceived a condition of emotional distress and temporary psychological discomfort (patients with psychiatric disorders were excluded from the study). At T1, the percentage of subjects scoring 14 or higher dropped to 8.4% and remained unchanged at T2. After 30 days of V50 intake, 91.6% of the subjects scored 13 or lower on the GHQ-12, indicating they were not experiencing emotional distress or psychological discomfort.

Fatigue Assessment Scale (FAS)

At baseline (T0), total FAS scores did not differ significantly between the two Groups (**Table 4, Figure 1**).

In the V50 Group, the total FAS score decreased significantly at T1 compared to T0 and it further decreases in a statistically significant way at T2 compared to T1, demonstrating a reduction in physical and mental fatigue. In the Control Group, the variations in score were not statistically significant between T0 and T1, T1 and T2, or between T0 and T2.

The mean percentage variation in the total FAS score between T0 and T1 in the V50 Group was -23.7 ± 22.7%, whereas in the Control Group it was +5.69 ± 25.2%. The mean percentage change in the total FAS score between T0 and T2 in the V50 Group was -30.9 ± 27.2%, whereas in the Control Group it was +0.82 ± 16.0%.

Multidimensional Inventory of Subjective Cognitive Impairment (MISCI)

There was no statistically significant difference between the total MISCI scores of the two Groups at T0 (**Table 4, Figure 1**).

In the V50 Group, the total MISCI score increased significantly at T1 compared to T0 and it further increases in a statistically significant way at T2 compared to T1, demonstrating an improvement in perceived cognitive abilities. In the Control Group, the variations in score were not statistically significant between T0 and T1, T1 and T2, or T0 and T2.

The mean percentage variation in the total MISCI score between T0 and T1 in the V50 Group was +26.1 ± 30.4%, while in the Control Group it was +1.91 ± 10.0%. The mean percentage change in the total MISCI score between T0 and T2 in the V50 Group was +37.8 ± 39.8%, while in the Control Group it was +2.35 ± 10.8%.

Perceived Stress Scale (PSS)

There was no statistically significant difference between the total PSS scores of the two Groups at T0 (**Table 4, Figure 1**).

In the V50 Group, the total PSS score decreased significantly at T1 compared to T0 and decreased significantly again at T2 compared to T1, demonstrating a reduction in the subjects' perceived stress level. In the Control Group, the variations in score were not statistically significant between T0 and T1, T1 and T2, or T0 and T2.

The mean percentage variation in the total PSS score between T0 and T1 in the V50 Group was -33.2 ± 41.5%, while in the Control Group it was +2.57 ± 27.3%. The mean percentage variation in the total PSS score between T0 and T2 in the V50 Group was -42.4 ± 80.6%, while in the Control Group it was +10.9 ± 66.2%.

At T0, the total PSS score of 84.5% of the subjects in the V50 Group was equal to or greater than 14, confirming that the study population perceived a moderate to high level of stress. At T1, the percentage of subjects perceiving a moderate to high level of stress decreased to 45% and further decreased to 22.5% at T2. After 30 days of taking V50, 77.5% of subjects scored 13 or less on the PSS questionnaire, indicating a low or absent level of perceived stress.

Sleep Quality Scale (SQS)

There was no statistically significant difference between the total SQS scores of the two Groups at T0 (**Table 4, Figure 1**).

In the V50 Group, the total SQS score increased significantly at T1 compared to T0 and further increased significantly at T2 compared to T1, demonstrating an improvement in sleep quality. In the Control Group, the variations in score were not statistically significant between T0 and T1, T1 and T2, or T0 and T2.

The mean percentage variation in the total SQS score between T0 and T1 in the V50 Group was +47.7 ± 75.0%, whereas in the Control Group it was +9.56 ± 35.2%. The mean percentage variation in the total SQS score between T0 and T2 in the V50 Group was +74.5 ± 92.5%, while in the Control Group it was +7.72 ± 50.6%.

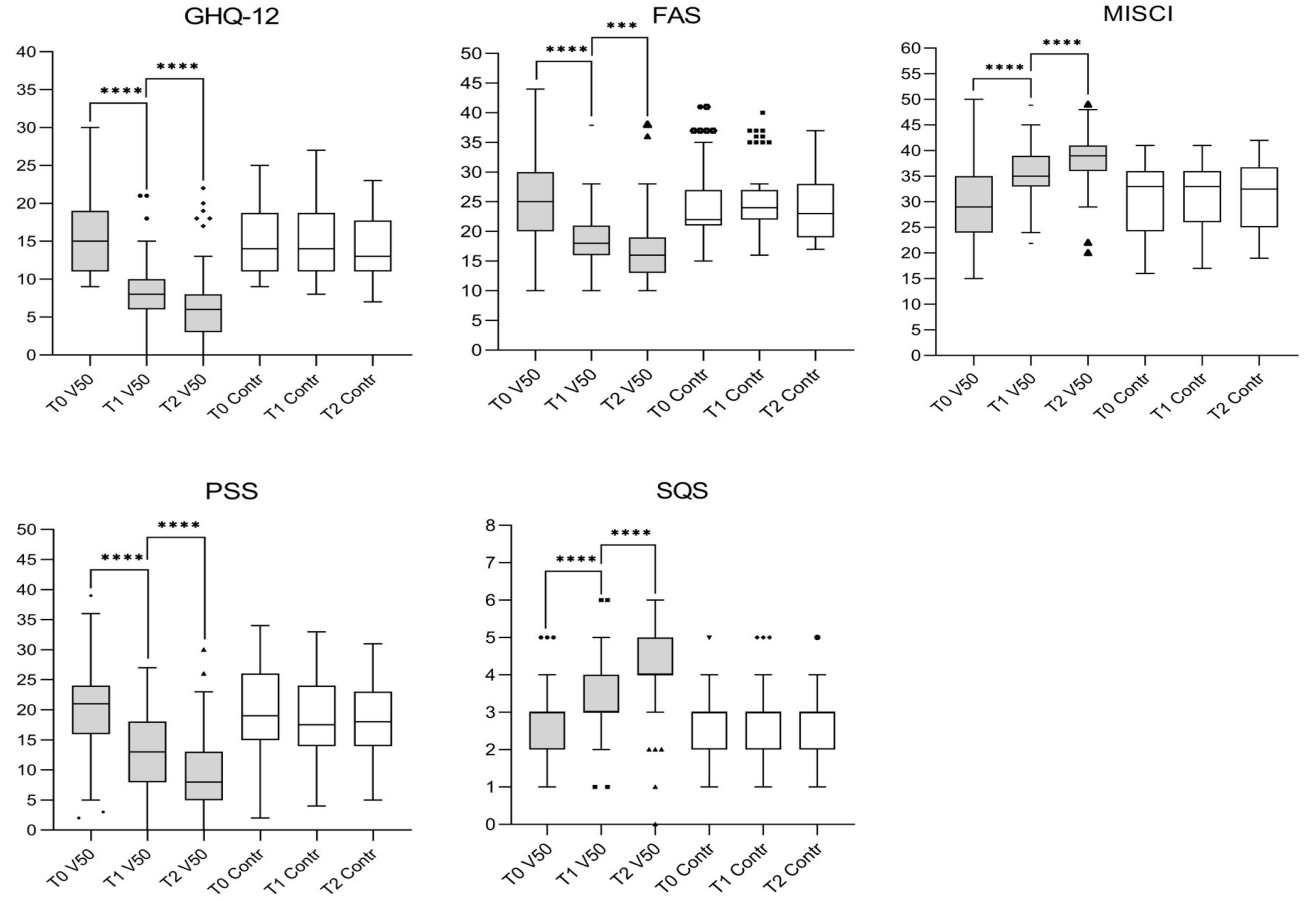
At T0, only 15.5% of subjects in the V50 Group rated their sleep quality as 4 or 5 (good or very good), and none rated it as 6 (excellent). This confirms that the enrolled subjects exhibited poor sleep quality on average at T0. At T1, after 7 days of taking V50, 47.9% of subjects rated their sleep quality a score between 4 and 6, thus defining their sleep quality as good or excellent. At T2, after 30 days of taking V50, this percentage increased to 77.9%.

Table 4. Medians, 25th and 75th percentiles, and means ± SD of the total GHQ-12, FAS, MISCI, PSS, and SQS scores measured in the V50 Group and the Control Group at baseline (T0), after 7 days (T1), and after 30 days (T2), and the statistical significance of the comparisons. Non-significant P-values are italicized.

Instrument	Parameter	T0		T1		T2	
		V50 Group	Contr Group	V50 Group	Contr Group	V50 Group	Contr Group
GHQ-12	Median [25th – 75th]	15.0 [11.0-19.0]	14.0 [11.0-18.8]	8.0 [6.0-10.0]	14.0 [11.0-18.8]	6.0 [3.0-8.0]	13.0 [11.0-17.8]
	Mean ± SD	15.6±5.39	15.0±4.62	8.51±3.92	14.6±4.62	6.51±4.80	14.1±4.50
	P-value vs T0			<0.0001	*0.0775*	<0.0001	*0.0902*
	P-value vs T1					<0.0001	*0.3630*
	P-value T0 V50 vs T0 Contr	*0.6453*					
	P-value T1 V50 vs T1 Contr			<0.0001			
	P-value T2 V50 vs T2 Contr					<0.0001	
FAS	Median [25th – 75th]	25.0 [20.0-30.0]	22.0 [21.0-27.0]	18.0 [16.0-21.0]	24.0 [22.0-27.0]	16.0 [13.0-19.0]	23.0 [19.0-28.0]
	Mean ± SD	25.8±7.56	24.4±6.48	18.6±4.49	25.1±5.63	16.7±5.19	24.1±5.10
	P-value vs T0			<0.0001	*0.6814*	<0.0001	*0.5887*
	P-value vs T1					0.0001	*0.2039*
	P-value T0 V50 vs T0 Contr	*0.2100*					
	P-value T1 V50 vs T1 Contr			<0.0001			
	P-value T2 V50 vs T2 Contr					<0.0001	
MISCI	Median [25th – 75th]	29.0 [24.0-35.0]	33.0 [24.3-36.0]	35.0 [33.0-39.0]	33.0 [26.0-36.0]	39.0 [36.0-41.0]	32.5 [25.0-36.8]
	Mean ± SD	29.5±7.44	30.6±6.62	35.5±5.08	31.0±6.46	38.3±5.48	31.1±6.54
	P-value vs T0			<0.0001	*0.0779*	<0.0001	*0.0801*
	P-value vs T1					<0.0001	*0.5822*
	P-value T0 V50 vs T0 Contr	*0.1736*					
	P-value T1 V50 vs T1 Contr			0.0001			
	P-value T2 V50 vs T2 Contr					<0.0001	

PSS	Median [25th – 75th]	21.0 [16.0-24.0]	19.0 [15.0-26.0]	13.0 [8.0-18.0]	17.5 [14.0-24.0]	8.0 [5.0-13.0]	18.0 [14.0-23.0]
	Mean ± SD	20.5±7.62	19.3±8.39	12.7±6.21	18.6±7.86	9.63±6.32	18.2±6.59
	P-value vs T0			<0.0001	*0.2069*	<0.0001	*0.0705*
	P-value vs T1					<0.0001	*0.4094*
	P-value T0 V50 vs T0 Contr	*0.3993*					
	P-value T1 V50 vs T1 Contr			<0.0001			
	P-value T2 V50 vs T2 Contr					<0.0001	
SQS	Median [25th – 75th]	3.0 [2.0-3.0]	3.0 [2.0-3.0]	3.0 [3.0-4.0]	3.0 [2.0-3.0]	4.0 [4.0-5.0]	3.0 [2.0-3.0]
	Mean ± SD	2.66±0.97	2.74±0,94	3.55±1.07	2.82±0.94	4.14±1.16	2.68±0.94
	P-value vs T0			<0.0001	*0.3449*	<0.0001	*0.6501*
	P-value vs T1					<0.0001	*0.0968*
	P-value T0 V50 vs T0 Contr	*0.4932*					
	P-value T1 V50 vs T1 Contr			<0.0001			
	P-value T2 V50 vs T2 Contr					<0.0001	

Figure 1. In the V50 Group, treatment with V50 for 7 days, from T0 to T1, and for 30 days, from T0 to T2, induced a statistically significant variation in the total scores of the GHQ-12, FAS, MISCI, PSS, and SQS. In the Control Group, no statistically significant change in the total scores of the questionnaires was observed between T0 and T1, T1 and T2, or T0 and T2 (****P<0.0001, ***P= 0.0001).



DISCUSSION

This study investigated the effect of V50: after 7 days of treatment, the total GHQ-12, FAS, MISCI, PSS, and SQS scores improved significantly compared to T0, varying on average by -41.7 ± 26.8%, -23.7 ± 22.7%, +26.1 ± 30.4%, -33.2 ± 41.5%, and +47.7 ± 75.0%, respectively. The variations in the FAS and MISCI questionnaire scores did not reach the 30% threshold we considered essential for V50 to demonstrate effectiveness. After 30 days of treatment with V50, the total scores of GHQ-12, FAS, MISCI, PSS and SQS further improved compared to T0, varying on average by -53.6 ± 40.0%, -30.9 ± 27.2%, +37.8 ± 39.8%, -42.4 ± 80.6%, +74.5 ± 92.5% respectively. After 30 days of taking V50, the variations in the total scores of all questionnaires exceeded the 30% threshold set in this study as the criterion for considering V50 effective.

Within the V50 Group, all questionnaire scores showed a statistically significant improvement between T0 and T1, as well as between T1 and T2. This suggests that, in healthy subjects experiencing physical and mental fatigue, high levels of stress, and poor sleep quality, the intake of inositol and high doses of the entire group of water-soluble vitamins exerts a broad effect, resulting in a reduction in emotional distress, an increase in psychological well-being, a reduction in physical and mental fatigue and perceived stress, and an improvement in cognitive function and sleep quality.

A comparison between the V50 Group and the Control Group revealed significant differences: the untreated subjects showed no improvements in questionnaire scores either 7 days or 30 days from the start of the observation period.

This confirms the effectiveness of inositol and high doses of all water-soluble vitamins in inducing a significant improvement in quality of life related to the perception of physical and mental fatigue.

The improvements in the V50 Group determined by the intake of inositol and high doses of all water-soluble vitamins, and the total absence of improvements in the Control Group, suggest that the cohort of healthy subjects enrolled in both Groups must have had suboptimal water-soluble vitamin status at enrollment. It is important to note that demographic and medical history characteristics, fruit and vegetable consumption, and questionnaire scores at enrollment were equivalent between the two Groups, suggesting firstly that the nutritional status in the two Groups at enrollment was similar and secondly that the study results are not due to pre-enrollment differences in nutritional status between the Groups.

Overall, the results of this study suggest that improving nutritional status through supplementation with inositol and all water-soluble vitamins may be beneficial to the general population. There is a lack of information to determine whether these benefits result from compensation of damage caused by overt or marginal deficiencies or from improvement in suboptimal levels of essential nutrients, which, according to current guidelines, would not even be classified as marginal deficiencies.

As stated in the introduction, the authors of this study did not identify any studies in the literature verifying the efficacy of a nutraceutical containing exclusively inositol and high doses of all water-soluble vitamins in the treatment of healthy non-elderly subjects experiencing physical and mental fatigue, high levels of stress, and poor sleep quality. Therefore, it is not possible to make a direct comparison with existing literature. Alternatively, we provide a review of several studies testing multivitamin dietary supplements with compositions at least partially similar to that of V50 and which may contribute to clarify the benefits associated with the intake of a nutraceutical such as the one tested in this study.

Two articles describe the effects on mood and cognitive function induced by 12 months of supplementation with a high-dose multivitamin (10 times the recommended daily dose). The study demonstrates that, compared to a placebo, supplementation improves subjective mood as measured by the Profile of Mood States (POMS), psychological health as measured by the GHQ questionnaire, and cognitive performance in attention tasks [43,44]. In a double-blind, placebo-controlled study in 300 healthy adults, it was reported that the 4-week administration of a complex of water-soluble vitamins and minerals improved subjective ratings of stress, anxiety, and psychological well-being [45]. A further study involving 80 healthy men showed that the 4 week administration of a complex of water-soluble vitamins and minerals reduced subjective stress as measured by the PSS questionnaire and anxiety as measured by the Hospital Anxiety and Depression Scale (HADS) [46]. In a subsequent randomized, double-blind, placebo-controlled study involving 210 healthy, full-time employed men, the same complex of water-soluble vitamins and minerals was used and the effects of 33 days of supplementation on cognitive performance, mood, and well-being were evaluated. Supplementation significantly improved general psychological health assessments (GHQ-12), reduced subjective perception of stress (PSS), and increased “vigor” (POMS), with a strong trend toward overall mood improvement. Cognitive functions assessed by administering a cognitive demand battery designed to induce mental fatigue also improved. This was accompanied by a reduction in “mental tiredness” ratings during performance of the cognitive demand battery tasks and a trend toward reduced mental fatigue [47]. A randomized, double-blind, crossover controlled clinical trial comparing two groups of healthy subjects of both sexes demonstrated that the administration of some water-soluble vitamins (vitamin B1, B2, B6, and B12) for 28 consecutive days increased endurance performance during physical exercise

and reduced plasma concentrations of metabolites (lactate and ammonia) during exercise and at rest after exercise [48]. In a randomized, double-blind, placebo-controlled clinical trial, 108 Japanese older adults (mean age 72.2 years) were given daily vitamin B3 or a placebo for 12 weeks. Sleep quality was assessed with the Pittsburgh Sleep Quality Index (PSQI), and, in participants treated with vitamin B3 supplementation, the study authors found a significant main effect of time on sleep duration, sleep disturbance score, daytime dysfunction score, sleep quality score, and total score [49]. In a randomized, double-blind, placebo-controlled trial, the impact of 12-week vitamin B3 supplementation on physical function and sleep using the PSQI and physical functioning questionnaires was evaluated. Participants were 60 Japanese older adults, men and women, aged 65 to 75 years. Participants receiving vitamin B3 showed improvements in physical function and a significant improvement in the daytime dysfunction score and PSQI global score [50].

In a randomized, double-blind, placebo-controlled study involving 60 pregnant women with a gestational age of at least 14 weeks, participants were divided into two groups and took either a supplement containing myo-inositol and folic acid or a placebo for 10 weeks. Sleep quality was measured using the PSQI. Significant differences were found between the two groups in total sleep quality score, subjective sleep quality, sleep duration, and habitual sleep efficiency [51].

The most original aspect of this study is that it tested the effect of administering all water-soluble vitamins together with inositol. Many published studies have focused on a single water-soluble vitamin or a small group of them, overlooking the fact that water-soluble vitamins and inositol perform closely intertwined activities within the cell. This study aims to emphasize that the combination of inositol and all water-soluble vitamins represent an inseparable biochemical unit and, therefore, aims to verify whether the simultaneous administration of inositol and high doses of all water-soluble vitamins can produce a significant benefit in healthy subjects.

Another original aspect of this study is the goal of achieving a favourable variation in the total score of the questionnaires of at least 30%. A comparison between patients' pre- and post-treatment status is often considered sufficient. If the therapeutic interventions adopted in the study have already proven effective in treating the disorder under consideration, a statistically significant improvement in the parameters chosen for clinical evaluation is easily achievable. However, such a statistically significant improvement should not be considered sufficient: a patient who has experienced a statistically significant variation in the total score of a symptom assessment questionnaire is not necessarily satisfied with the result obtained. Therefore, the goal of achieving a favourable change in the total score of the questionnaires used in this study of 30% or more is intended to at least partially obviate this potential bias in assessing the efficacy of inositol and high doses of all water-soluble vitamins and to provide a useful contribution to determining how long these substances should be taken to achieve the best results.

The subjects enrolled in this study did not undergo blood tests to measure plasma concentrations of inositol and the water-soluble vitamins prior to their administration. Therefore, within the two Groups, it was not possible to identify subjects with overt, marginal, or functional deficiencies of one or more water-soluble vitamins. This could be considered a significant limitation of this study. In fact, the approach adopted is aligned with the practices commonly followed by physicians in their routine clinical work. Blood tests that investigate the concentrations of all water-soluble vitamins are not routinely performed in clinical practice and are difficult to find outside the context of academic research. In standard medical practice, under certain conditions, attention is directed exclusively to specific water-soluble vitamins, focusing diagnostic (measuring plasma concentrations) and therapeutic (administration of drugs or supplements) efforts solely on these vitamins.

The current study aims to challenge the common practice of focusing attention exclusively on only a few of the water-soluble vitamins. Further aspects are highlighted: for some water-soluble vitamins, the plasma concentration ranges currently used in blood tests are considered inadequate, and just the blood test alone does not allow to determine whether the amount of vitamin present in the plasma is sufficient to meet the increased requirements that may arise in various conditions, including the elevated levels of perceived stress observed in the patients enrolled in the current study [52].

As noted in a review [53], even government agencies responsible for setting dietary recommendations recognize that the benefits of micronutrient consumption can accrue along a continuum well above the RDA. Clearly, common sense dictates that the optimal intake of any nutrient is not simply the amount that prevents disease related to a frank deficiency, or even a marginal deficiency, of that nutrient. Consistent with this, a large body of epidemiological evidence suggests associations between increased consumption and biochemical levels of several vitamins and benefits for cardiovascular function, cognitive function, as well as reduced incidence of dementia, clearly showing that individuals derive relevant physiological benefits from consuming micronutrients well above the RDA and at biochemical levels well above those indicating a deficiency [54,55]. It should be noted that this study did not enroll elderly subjects or subjects suffering from conditions that suggest a deficiency of one or more micronutrients. The enrolled population consisted of healthy subjects with a normal diet; therefore, overt deficiencies of water-soluble vitamins were not expected. Indeed, the aim of this study is to determine whether,

regardless of initial vitamin status, increasing the daily doses of inositol and water-soluble vitamins can result in health benefits that are reflected in significant improvements in the parameters evaluated.

In conclusion, we believe that the results of a blood test cannot be the guiding criterion for deciding whether to administer a multivitamin containing all water-soluble vitamins. The well-documented widespread prevalence of insufficient intake of water-soluble vitamins among the population and the already demonstrated benefit of supplementation with these vitamins, regardless of initial vitamin status, in improving physical and mental performance, reducing perceived stress, and improving sleep quality are considered sufficient conditions to guide the decision to administer these vitamins to a fatigued and stressed individual. It is considered appropriate to apply the "*ex adiuvantibus*" criterion, whereby the favourable results obtained confirm the appropriateness of the choice made. The results of this study provide a further contribution intended to strengthen this approach.

The lack of a placebo-treated control arm can be considered a limitation of the present study. We would like to point out that we do not consider a comparison with a placebo-treated group necessary, first of all because water-soluble vitamins have already been tested, as single vitamins or in small groups, in clinical trials versus placebo in the reduction of perceived stress, in the improvement of psychological well-being and in the improvement of cognitive functions, physical performance and sleep quality [45-51]. In these studies, water-soluble vitamins have proven to be more effective than placebo and this allows us to presume that V50, which combines in its formula all water-soluble vitamins in high doses, can easily determine effects in treated subjects that are superior to those of placebo. Furthermore, the goal of the authors of the present study is not to prescribe nutraceuticals that exceed the efficacy of placebo, a result that is easy to achieve, but rather to try to bring patients back to an optimal state of health. Finally, this study was conducted as part of routine clinical practice and administering a placebo to individuals who turn to their physician in the hope of alleviating bothersome symptoms was not considered ethically acceptable.

CONCLUSIONS

Increased mental distress, temporary reduction in psychological well-being, worsening physical and mental performance, heightened perception of stress, and worsening sleep quality outline a very common phenotype in the general population, attributable to the life conditions to which a significant proportion of the population is exposed daily. Alongside this initial evidence, it emerges that the apparently healthy population is exposed to a suboptimal daily dietary intake of water-soluble vitamins, substances essential for the proper functioning of the entire body.

The effectiveness of lifestyle changes in improving quality of life and dietary changes in improving bodily functions are well known. However, given the objective difficulty of implementing lifestyle and dietary changes, there is an increasing need to adopt simple, repeatable, effective, and safe measures in the short, medium, and long term. In the introduction to this study, the hypothesis was formulated that such measures could include the intake of a nutraceutical containing inositol and high doses of all water-soluble vitamins, nutrients that contribute to optimizing the cellular biochemical functions of all organs in the body. In conclusion, the hypothesis that was formulated appears to be confirmed by the results of the study: the intake of V50, a nutraceutical containing inositol and high doses of all water-soluble vitamins, for 30 consecutive days is able to increase psychological well-being, reduce emotional distress, physical and mental fatigue, and perceived stress, and improve cognitive function and sleep quality in healthy subjects.

The authors are aware that confirmation of the results obtained in this study requires further clinical studies with adequate statistical power.

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Conflict of interest: Giuseppina Garbelli, Federico Maffezzoni, and Serena Miglio, who collected the data presented in the study, declare that they have no commercial or other relationships that could pose a conflict of interest in relation to the article. Patrizia Giordano, who organized the collected data and performed the statistical analysis, was rewarded by MèDISIN S.r.l. for the work done. Stefano Agostini, who wrote the article, is the Scientific Director of MèDISIN S.r.l.

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