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**HEALTH&WELLNESS COACHING TO CONTROL STRESS LEVEL,  
WITH A POSITIVE IMPACT ON THE QUALITY OF LIFE  
AND EPIGENETIC MARKERS**

**ABSTRACT OF THE DOCTORAL THESIS**

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## INTRODUCTION

Stress, the “health epidemic of the 21st century” (Singh, 2019), is a ubiquitous but poorly understood phenomenon. This research proposes a new approach to understanding and managing stress, having as the main objective the identification of prophylactic solutions for stress control, with the aim of “reducing the incidence of chronic non-communicable diseases, which are responsible for 71% of global deaths.” (WHO, 2024) The traditional medical approach considers stress an associated risk factor, not a generator of chronic diseases. I believe that the unsatisfactory results of most medical programs aiming to control chronic non-communicable diseases may be attributable to their application in a stereotypical way, without being adapted to each individual and especially to the omission of the psychological component.

In this work, I adopted a holistic and integrative perspective, considering stress as a factor generating an altered health status. I carried out interventions on the individual's capacity to manage stress using a modern interventional method: Health&Wellness Coaching (HWC). I also evaluated the effectiveness of non-invasive tools for stress screening and assessment, such as psychometric tests and neuroelectrophysiological and epigenetic investigations.

The research hypotheses were:

- HWC is an effective intervention aimed at controlling stress levels by modeling the psychoemotional component, and thus positively impacting the quality of life and epigenetic markers.
- The non-invasive tools and investigations used (DASS-21R, QOLI, CD, Muse and Mendi devices, miR-21 and miR-26) are effective in stress detection and assessment.

Our results indicated a significant reduction in HWC post-intervention stress levels, correlated with an improvement in the quality of life and positive changes in the stress-associated epigenetic markers. In parallel I ascertained the effectiveness of the tools and investigations used.

# I. THE CURRENT STATE OF KNOWLEDGE

## 1. Health&Wellness Coaching

### 1.1. Definition and types of coaching

Coaching is a form of counselling which has emerged out of a real need to empower individuals, helping them to maximize personal performance by unlocking their human potential. There are three main models of coaching: “

- **The expert model:** the client acquires expertise without responsibility for the outcome.
- **The medical model:** the patient has limited responsibility.
- **The consultative model:** the client has full responsibility, and the coach helps identify client-generated problems.” (Connor, 2007)

Coaching is that consultative model, where the client is responsible for the process of change. The International Coaching Federation categorizes coaching according to the scope of the issues addressed and the category it addresses into "Life Coaching, Business Coaching, Executive Coaching, Career Coaching, Internal Coaching and Team Coaching.” (International Coaching Federation, 2024). The National Association of Health Coaching “makes a clear distinction between Health Coaching and Wellness Coaching in terms of competence and mode of application.” (National Society of Health Coaches, 2024)

### 1.2. Roles and effects of Health&Wellness Coaching

*Health&Wellness Coaching* (HWC) is a technique at the intersection of medicine and wellbeing which facilitates improved health and well-being. “The actual process of coaching involves goal setting by the individual, encourages self-development, and incorporates mechanisms for developing accountability in health behaviours.” (Wolever, 2013) HWC “is rapidly emerging as an adjuvant treatment for lifestyle-related diseases, with great potential in stemming the rising tide of the prevalence of chronic diseases.” (Sforzo, 2017)

The National Consortium for Credentialing Health & Wellness Coaches defines the health and wellness coach (HWC) role as that of “a partner with clients who desire

self-management and lasting change aligned with their values, thereby promoting health and wellness.” (National Board for Health and Wellness Coaching - NCCHWC, 2024)

In this thesis I chose to modulate the psycho-emotional component of the subjects in order to act on stress levels and I proved that HWC is an effective intervention for lowering stress levels in the body.

## **2. Stress, the quality of life and epigenetics**

### **2.1. Stress - definition, effects and 'anti-stress' therapies**

#### **2.1.1. Definition**

Stress has been defined as “a nonspecific response of the organism to any demand.” (Selye, 1976) Stress can also be seen “ as a particular relationship between the person and the environment, appraised by the individual as exceeding his or her resources, jeopardizing his or her well-being.” (Lazarus, 1984) However, “attempts to define stress have remained elusive, and stress has become a negatively charged term, being used to describe both a stimulus and a response, inevitably leading to confusion.” (Mustafa, 2016)

#### **2.1.2. “Anti-stress” effects and therapies**

Stress is all-pervasive in modern life, and studies conducted in the US between 1983 and 2009 “have shown a significant increase in stress levels (10-30%), it is associated with an increased frequency of myocardial infarction, hypertension, obesity, addictions, anxiety, depression and other disorders.” (Fink, 2016)

Resilience to stress is often established in childhood, and “anti-stress” strategies such as mindfulness and relaxation training have been shown to have beneficial effects on stress reactivity and overall health. Recent studies also “suggest that implementing a comprehensive stress management program can significantly improve depression and anxiety scores.” (Xenaki, 2018)

## **2.2. Quality of life - definition, dimensions and indicators**

### **2.2.1. Definition**

Quality of Life (QoL) is “a concept that aims to capture the well-being of a population or an individual, with both positive and negative elements, and encompasses several areas of life such as: personal health (physical, mental and spiritual), relationships, educational status, work environment, social status, wealth, a sense of security and safety, freedom, autonomy in decision making, social belonging and physical surroundings.” (Teoli, 2023) And according to WHO, QoL is “an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns.” (World Health Organization, 2024)

### **2.2.2. Dimensions and indicators**

In Romania, “the quality of life is the object of research for the Research Institute for Quality of Life (ICCV), with the aim of creating a comprehensive picture of quality of life using objective and subjective indicators.” (Institutul de Cercetarea a Calității Vieții, 2024)

Accordingly, they have been structured on several measurable dimensions: “emotional, material, physical well-being, interpersonal relations, personal affirmation, independence, social integration and the provision of fundamental rights, which are illustrated by certain indicators: happiness, self-esteem, job security, income, social support and autonomy, etc.” (Lupu, 2006)

## **2.3. The interrelationship between stress and epigenetics**

### **2.3.1. Epigenetics – definition**

Epigenetics is defined “as heritable changes in gene function that cannot be explained by changes in DNA sequence but alter the transcriptional phenotype of a cell.” (Tronick, 2016). Epigenetics is a “bridge between genotype and phenotype, and cell differentiation can be considered an epigenetic phenomenon rather than alterations in genetic inheritance.” (Goldberg, 2007). It is also important to know that “not everything that is inherited is genetic and that there are other self-sustaining systems that contain information and function as inheritance systems. Most obvious are the systems by which culture is maintained and inherited.” (Jablonka, 1995)

## **2.3.2. MicroRNAs**

### **2.3.2.1. Biogenesis, classification and functions**

miRs are “short single-stranded RNAs that regulate the expression of messenger RNAs by destabilizing the transcript, inhibiting translation, or both, and a single miR can regulate the expression of numerous genes associated with the same physiological process.” (Miguel, 2018) It is considered that “miRNA functions can be divided into two types: homeostatic regulation of gene expression and robustness of cellular responses. The first type involves the regulation of gene expression through the precise adjustment of the cell's the requirements, and the second type of regulation is present on processes such as cell fate, its differentiation state and stress responses.” (Bautista-Sánchez, 2020) It has been found that "miRs regulate almost all cellular functions, including cell proliferation, growth, differentiation and apoptosis." (Ranganathan, 2014)

### **2.3.2.2. The interrelationship between stress and miR-21 & miR-26**

“The concepts of stress and stressors have brought our understanding of psychological dysfunction to the postmodern world, i.e., in the modern conception, a mentally ill person was an individual with a disease, and in the postmodern conception, a psychologically dysfunctional person is an individual overwhelmed by stress.” (Elkind, 1995) Illness is often “the result of an aberrant or inappropriate response to physiological and pathophysiological stress, and studies in recent years have uncovered a recurrent paradigm in which miRs regulate cellular behavior under these conditions, suggesting a significant role for them in pathological conditions.” (Mendell, 2012)

“Acute stress can induce protective epigenetic responses, whereas chronic stress can disrupt epigenetic mechanisms and even favour the pathogenesis of stress-related depression by disrupting epigenetic mechanisms of the stress response.” (Rusconi, 2018)

Thus “miR-21 is considered a versatile regulator in the progression of CNS disorders and could be a promising predictive and diagnostic biomarker as well as a therapeutic target for these disorders. " (Bai, 2022)

An interesting role of miR-26 is related to neurogenesis, “thus, mature miR-26b generation is activated during neurogenesis. Finally, miR-26a and miR-26b have also been

implicated in the differentiation of other cell types, stemness and cancers, not just neurons.” (Dill, 2012)

Stress is considered “an important co-factor in the genesis and maintenance of many diseases, with an effect on gene expression through epigenetic regulation. miR-21 might be involved in the cellular stress response - oxidative stress - which, in turn, also appears to be a result of perceived psychological stress. In addition, a significant correlation between miR-26b and miR-21 was revealed, both of which appear to be associated with sympathetic activation.” (Wiegand, 2018)

## **II. PERSONAL CONTRIBUTIONS**

### **3. Pilot study - The effects of the HWC intervention on the quality of life**

#### **3.1. Introduction - the working hypothesis and specific objectives**

The pilot study was conducted in order to evaluate the effectiveness of the HWC intervention on the QOL in individuals who present with an altered QOL due to high levels of perceived stress. The specific objectives of the study included: increasing self-esteem and self-confidence; improving levels of communication and socialization; achieving an overall sense of well-being by increasing levels of physical energy and muscle toning; and optimizing psycho-emotional state, all with the ultimate goal of increasing personal QOL.

#### **3.2. Material and method**

The study was conducted according to the “ethical procedures” recommended by the World Medical Association (Helsinki), after the subjects gave their written informed consent. (World Medical Association, 2023)

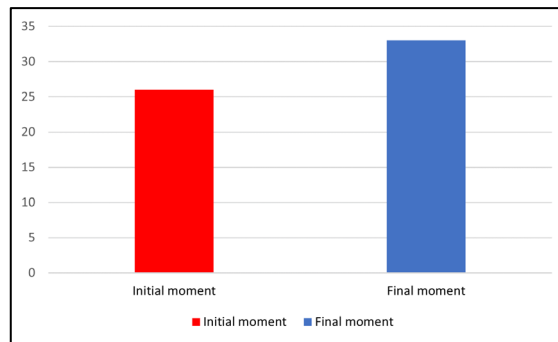
The HWC intervention included 12 sessions, with one every 3 weeks, of which the first was a group training, followed by 11 individual sessions: 7 combined coaching and training, and 4 coaching. The study was conducted between May 2018 and February 2019, at a private practice in Bucharest as well as online.



The psychometric instruments used were the scale for self-esteem (Rosenberg, 1965) and the scale for Personal Communication Styles (Marcus, 1997), applied at the beginning (T1) and at the end (T2) of the intervention. The sessions lasted approximately 60 minutes and were conducted in an informal setting, with general and specific information being provided in the training sessions according to the subject's needs, while in the coaching sessions the subject was empowered and guided towards setting and achieving the outlined goals. Statistical processing was carried out using SPSS (Statistical Package for the Social Sciences) statistical software. As I was dealing with a single subject, I used non-parametric statistical methods, namely the following tests: Friedman Two Way Analysis of Variance by Ranks and Cochran's Q Test.

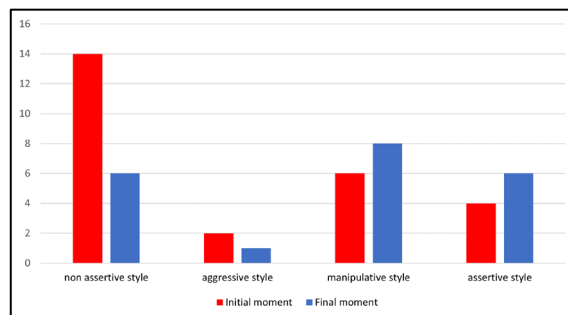
### 3.3. Results

The results showed an important improvement in self-esteem, with an increased score from 26 to 33 (Fig. 3.1.).



**Fig. 3.1.** Self-esteem

The communication styles also showed a positive change from non-assertive and aggressive styles to assertive and manipulative styles (Fig. 3.2.). These results are statistically significant ( $p < 0.05$ ), demonstrating the effectiveness of the HWC intervention.



**Fig. 3.2.** The communication style

### **3.4. Discussion**

The intervention had a significant and positive impact on the subject, a young woman who had suffered a lot, yet with a great potential for personal development and evolution, a potential which had been blocked due to her present condition. The results showed a considerable improvement in self-esteem and a favourable and statistically significant change in communication styles, with a decrease in non-assertive and aggressive styles and an increase in assertive and manipulative styles.

### **3.5. Conclusions of the pilot study**

1. Following the HWC intervention, the subject achieved a very high degree of personal satisfaction by gaining self-confidence and self-esteem
2. The evolution of the communication style was further proof that HWC had a positive impact on all communication styles, resulting in a healthier communication style, which brought multiple benefits to the subject
3. **HWC** is a modern and effective intervention method in the process of lifestyle reorganization, resulting in improved quality of personal life with positive physical, psychological, emotional and social impact
4. Stress-related mental disorders are the main cause of the overall health burden and finding effective solutions is crucial for optimizing the quality of life
5. The early identification of stress-related symptoms with a negative impact on the personal quality of life is particularly important in order to be able to adopt timely and effective “anti-stress” measures.

## **4. The main study - HWC to control stress levels, with a positive impact on the quality of life and epigenetic markers**

### **4.1 Introduction - the working hypothesis and general objectives**

The present work builds on a number of theoretical and practical observations which have shown that there is a close link between stress and the onset or exacerbation of various chronic medical conditions. Furthermore, we are going through a period of multiple challenges, which can lead to psycho-emotional imbalances, with the potential to turn into illness if not addressed in time. In recent years, numerous studies have emerged demonstrating that “interventions for the prevention and management of stress, could produce, including, altering the expression of genes related to the modulation of the psychobiological stress response.” (Stoffel, 2022)

Thus, I have set the following objectives: *The main objective*: to demonstrate the effectiveness of the HWC intervention method in optimizing psycho-emotional affective states in order to control stress levels, with a positive impact on the quality of life and epigenetic markers and *the secondary objective*: to test the effectiveness of the measurement tools and the non-invasive investigations used: psychometric tests (DASS-21R, QOLI and CD), epigenetic investigations (miR-21 and miR-26), and neuroelectrophysiological investigations (Muse and Mendi devices), with a view to extend their use for the early identification and assessment of high stress levels in the body.

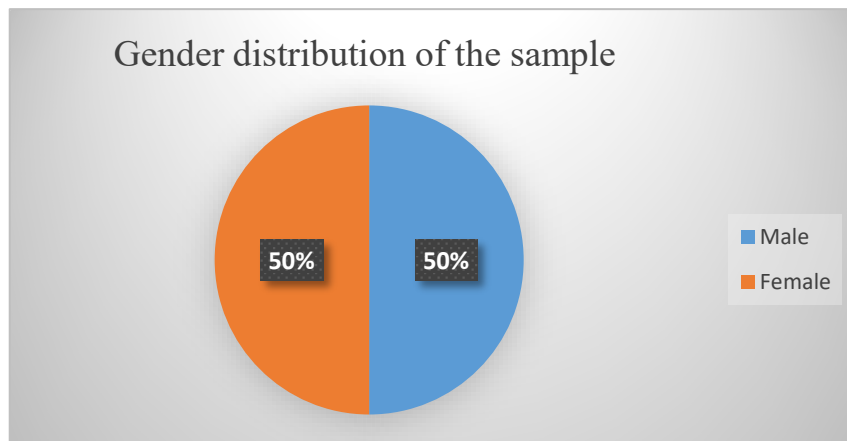
### **4.2 The general methodology of the study**

After conducting the pilot study, I recalibrated the terms of the intervention to make it easier for subjects. Therefore, as the research was focused on the area of prevention, the HWC intervention took place online and did not require the use of drugs and/or invasive medical procedures, being a research at the border between medicine and wellness, it did not require the opinion of the ethics committee of the "Carol Davila" University of Medicine and Pharmacy.

Collaboration agreements were only signed with the “Stefan S. Nicolau” Institute of Virology and the University of Bucharest, Faculty of Psychology and Educational Sciences, in order to carry out the necessary investigations for doctoral research. The research was

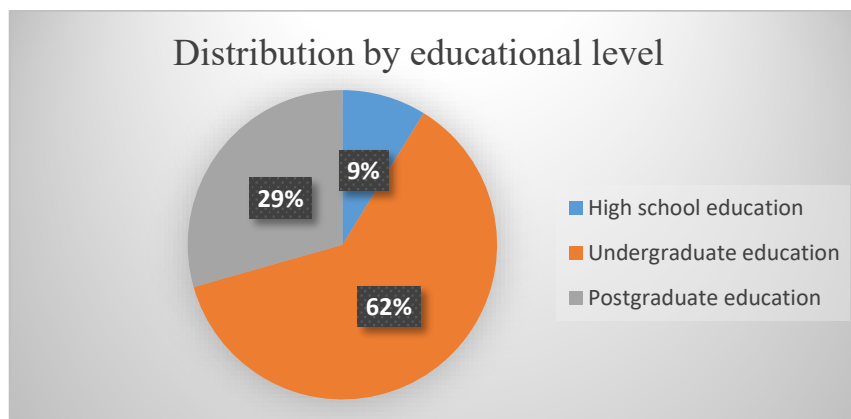
conducted according to the ethical rules recommended by the World Medical Association (Helsinki) (World Medical Association, 2023).

The actual implementation and investigation period of the HWC intervention was approximately 3-4 months (June 2023 - September 2023). Subjects were enrolled in the study following an online webinar - Is stress good or bad? - which was attended by several dozen people, following which 34 adult subjects, aged between twenty and seventy-two years, with a mean age of 49.06 years, female and male, with education ranging from high school to undergraduate, including postgraduate, were voluntarily enrolled for this research, and the intervention was offered free of charge to everybody. The gender distribution was roughly equal, meaning that half of the sample were male and half female (Fig. 4.1).



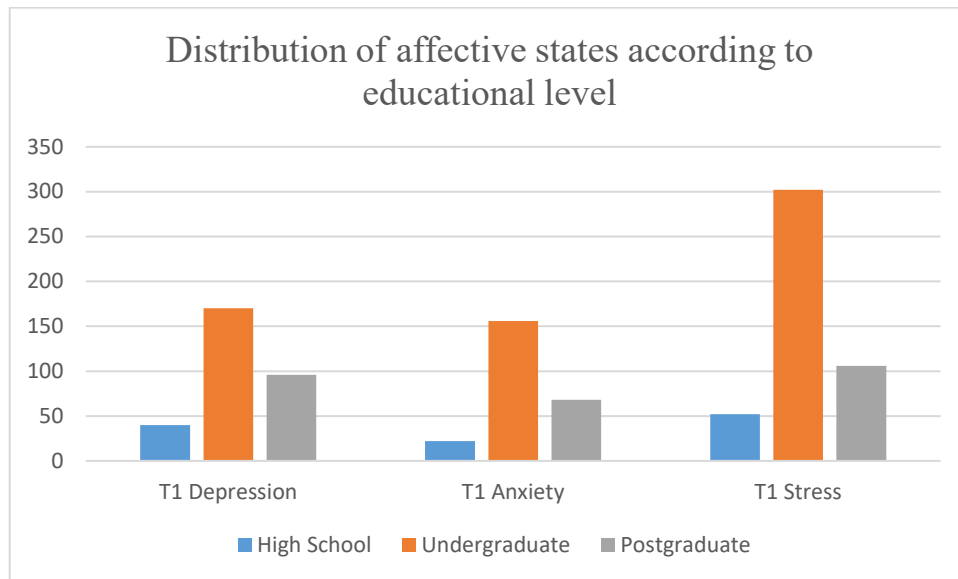
**Fig. 4.1.** Gender distribution of the sample

The participants also have a high level of education, (Fig. 4.2.), with those with a university degree forming the majority.



**Fig. 4.2.** Distribution of the sample by educational level

Affirmatively, at T1, the subjects suffered from a high level of stress (Fig. 4.3) and had no chronic diseases.



**Fig. 4.3.** Distribution of affective states according to educational level

The data collection was performed using the following tests, investigations and methods: psychometric: DASS-21R, Decisional Capacity (CD) and QOLI; neuroelectrophysiologic: the Muse device (EEG) and the Mendi device (fNIRS) and epigenetic: miR-21 and miR-26.

The HWC intervention method: the HWC intervention was carried out online, the aim was to convey information and empower subjects to reduce stress levels in order to improve the personal quality of life for prophylactic purposes. The intervention took place over 8 sessions, one session every approximately 2 weeks, 7 individual coaching&training or simple coaching only sessions and one group session.

The statistical analysis was performed using the GraphPad Prism 9.3.0 software (GraphPad Software Inc., San Diego, U.S.A.) Paired t-test and Chi-square test were used and p values < 0.05 were considered statistically significant. Microsoft Office Professional Pro Plus 2019 (Microsoft Excel) and SPSS (Statistical Package for the Social Sciences version 24) were also used. We performed descriptive statistics, statistical tests (Pearson Correlation, Anova etc), simple linear regressions as well as multiple regressions.

## **4.2.1. The effectiveness of HWC as evidenced by psychometric assessments**

### **4.2.1.1. Introduction – the working hypothesis and specific objectives**

It is essential to understand stress taking into consideration “the human brain - the central component of our identity” (Pittella, 2024) as well as the most sophisticated central nervous system of all species, therefore, the psycho-emotional arousal is one of the most frequent initiators of somatic stress. Thus, I initially evaluated the effectiveness of HWC on the psycho-emotional component through psychometric tests. Subsequently, depending on the instruments and investigations used, the subjects were grouped into three groups. The initial group, consisting of all 34 subjects (LOT1) participated in the psychometric tests: DASS-21R, QOLI and CD at both T1 and T2. Then they were also further evaluated by epigenetic investigations (miR-21 and miR-26), and only 27 subjects (LOT2) remained, as for the other 7 subjects the collected data were not sufficient, and of these only 24 (LOT3) accepted the neuroelectrophysiologic evaluation. I point out that the HWC intervention method was applied to all subjects participating in this study.

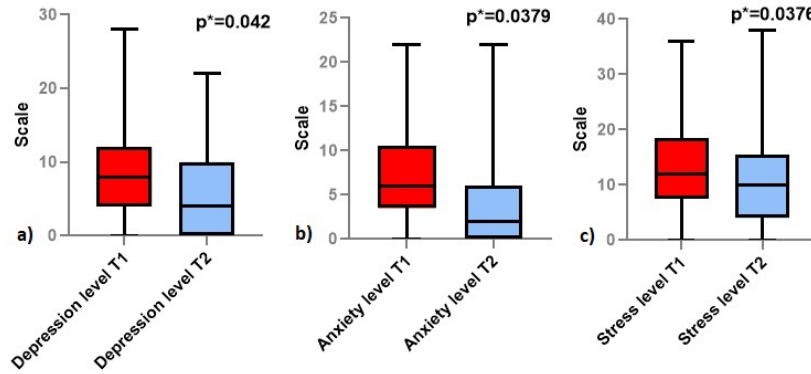
Thus, the **specific objectives** pursued for LOT1 through the application of the three psychometric instruments were to highlight the impact of HWC on: perceived stress, anxiety and depression; quality of life; decision-making capacity; as well as to evaluate the effectiveness of the three questionnaires: DASS-21R, QOLI and CD in relation to stress.

### **4.2.1.2. Assessment methods (LOT1)**

Each subject received an envelope at home before the start of the intervention, which contained the three questionnaires they had completed at T1, during the first session, in the first 15-20 minutes. All three psychometric instruments were purchased under license for this research and were adapted, standardized and validated on the Romanian population.

### **4.2.1.3. Results**

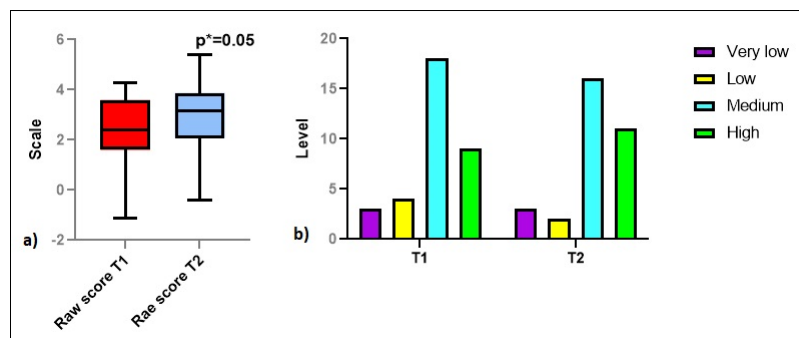
All three negative affective states/constructs or syndromes, assessed by the DASS-21R, had a statistically significant ( $p < 0.05$ ) favourable trend, the most significant being the level of perceived stress (c), followed by anxiety (b) and depression (a), as shown in Fig. 4.4.



**Fig. 4.4.** The three levels of specific effects at T1/T2 moments:

a) Depression b) Anxiety and c) Stress

Overall, a significant increase in the quality-of-life score (Fig. 4.5.) is observed (a), and in the overall quality of life parameter (b), a decrease in those with low and average levels in favour of an increase in those with higher quality of life, post HWC.



**Fig. 4.5.** Level of quality of life at T1/T2 time points

a) Raw score and b) Overall quality of life

In terms of  $\hat{CD}$ , the results obtained show a favourable evolution, without statistical significance, but it is interesting to note that the very poor level has completely disappeared, and the good and very good levels of decisional capacity have increased.

#### 4.2.1.4. Discussion

The results suggest that HWC is an effective intervention in reducing stress, anxiety and depression levels as well as improving quality of life. It can also be stated that the DASS-21R and QOLI are useful tools both in the assessment of the initial state and in the

evaluation of the HWC intervention, with the only amendment on the renaming of the psychological construct of „stress“.

#### **4.2.2. The efficiency of HWC as evidenced by epigenetic assessments (miR-21 and miR-26)**

##### **4.2.2.1. Introduction - the working hypothesis and specific objectives**

I utilized epigenetic markers to highlight the effects of stress at the molecular level, as well as to highlight the possibility that these markers could be included in screening models to detect stress levels in the body.

At this stage I have chosen the following as **specific objectives**: to evaluate the impact of HWC on miR-21 and miR-26 levels, as well as to evaluate the efficacy of miR-21 and miR-26 investigations for their use as pathognomonic salivary biomarkers for stress in screening tests.

##### **4.2.2.2. Assessment methods (LOT2)**

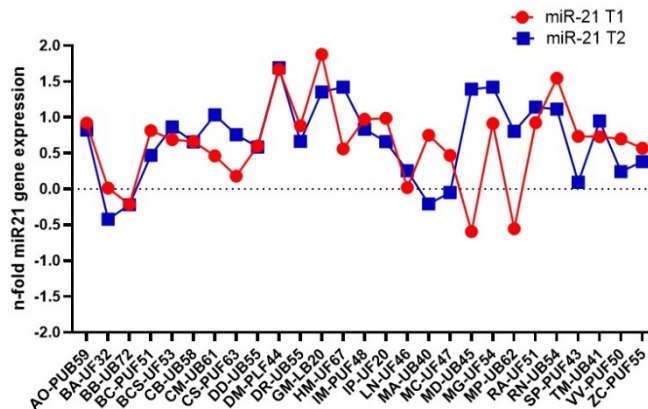
The 27 subjects (LOT 2) used the saliva harvester and the collection modality at both T1 and T2. I also chose these miRs because the salivary level is correlated with the blood level, thus facilitating the collection procedure. In order to obtain the necessary information from the collected salivary samples, the following methods were used for miR-21 and miR-26 expression: RNA isolation and cDNA synthesis (DNA copy) followed by quantitative real-time PCR (qRT-PCR/Polymerase Chain Reaction)

##### **4.2.2.3. Results**

In this study I observed an interesting evolution of the two miRs (21 and 26), with individual variations in most subjects, mostly correlated with the state of psychoemotional impairment. Thus, as shown in Fig. 4.6, in the subjects who presented a relatively higher level (extremes) of miR-21 expression at T1, at T2, the miR-21 level decreased, while in those who presented a relatively lower, even negative, level at T1, at T2, the miR-21 level increased. In contrast, those with values between 0 and 1 at T1, had smaller changes in



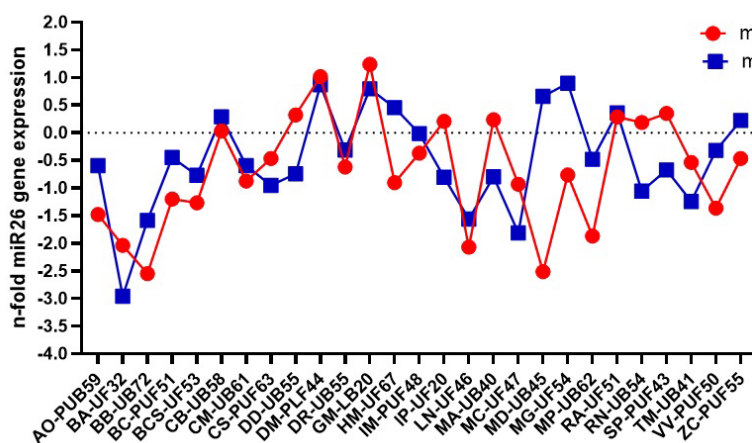
miR-21, slightly increasing or decreasing, post HWC intervention (T2). There were also a few stationary cases at T2 compared to T1.



**Fig. 4.6.** – Evolution of miR-21 for each subject at T1/T2

To achieve statistical significance, given the small number of subjects, I used regression analysis and obtained an F-value = 4.228 and p-value = 0.050, suggesting that the regression model using miR-21 at T1 to determine the change in miR-21 at T2 is statistically significant. This means that there is a significant change in miR-21 at T2 compared to T1. The HWC intervention, therefore, had a significant positive impact on miR-21, which is consistent with the favourable change in the three negative affective states (stress, depression and anxiety).

miR-26 also showed individual variations in most subjects, with a similar evolution to miR-21, as shown in Fig. 4.7., with the specification that the range of values is predominantly negative.



**Fig. 4.7.** - Evolution of miR-26 for each subject at T1/T2 times

As in the case of miR-21, for miR-26 I used regression analysis. Thus, I obtained an F value =4.904, and **p value = 0.036**, which highlights statistically significant changes, and the relationship is not random. In conclusion, this significant variation of miR-26 at T2 compared to T1, also certifies the impact of HWC intervention at molecular, epigenetic level in this case.

#### **4.2.2.4. Discussion**

The p-value = 0.050 for miR-21, indicates that the HWC intervention had a significant impact, including at the molecular, epigenetic level, even if this variation is at the limit of significance. Therefore, maintaining an optimal level of miR-21, I would estimate the value somewhere between of 0 and 1, could maintain a balance between proliferation and cell death processes. This balance is imperative for the optimal functioning of the immune system. Values too high or too low are usually associated with disease.

miR-26 appears to play an important role in cell survival in a hypoxic environment. The predominantly negative values could be explained by neuronal damage. I appreciate that all these impairments are *subclinical*, the subjects are compensated, i.e. they do not present chronic diseases, but could be considered as potentially clinical states. The HWC intervention had a significant impact ( $p = 0.036$ ), indicating an improvement or recovery, which goes hand in hand with the reduction of negative affective states and could be explained by a process of neuronal regeneration, through neurogenesis. Therefore, by maintaining an optimal level of miR-26, I would estimate somewhere between -0.5 and 0.5, one could support an optimal balance between cell proliferation and cell death processes, a balance necessary for the ability to cope with stressful psycho/emotional/mental conditions or situations. Both miR-21 and miR-26 showed significant changes at T2, indicating that the HWC intervention was effective in reducing stress levels, including at the molecular, epigenetic level. In order to be considered as predictive and diagnostic biomarkers for a high level of stress in the body, I believe that it would be necessary to correlate them with other stress-related parameters.

### **4.2.3. The efficiency of HWC as evidenced by neuroelectrophysiological assessments**

#### **4.2.3.1. Introduction – the working hypothesis and specific objectives**

(LOT3) accepted to be additionally assessed by mental/brain measurements with the Muse-EEG and Mendi-fNIRS devices, both at T1 and T2, and as **specific objectives** at this stage, I aimed at a favourable evolution of some parameters that could be correlated with the stress level, namely: heart rate; respiratory harmony; mental calmness; neuronal activity; resilience; brain activation control capacity; an additional objective was to evaluate the efficiency of the two devices.

#### **4.2.3.2. Assessment methods (LOT3)**

The neuroelectrophysiological measurements were performed in an isolated, quiet space with natural daylight and a temperature of 24 Celsius degrees. Initially, subjects were assessed while in a relaxed mental state with a head-mounted Muse headband, located in the prefrontal area of the brain. The relaxation period was 5 minutes, with eyes closed, sitting in a chair, during which time data were collected using a tablet or phone connected to the Muse technology. (Muse's EEG technology) Subsequently, in the same room and under the same conditions, the subjects were fitted with the Mendi (The science behind Mendi), strip and given approximately 2 minute prior instruction on how the task was to be performed. The subjects focused for 5 minutes on a mental task from the Mendi application on their tablet or mobile phone.

#### **4.2.3.3. Results**

On the basis of the ECG signal (Muse), mental calmness, respiratory harmony and average heart rate (AVG-HR) were recorded during the 5 minutes of relaxation, both at T1 and T2. The results obtained and presented in Fig. 4.8. show a significant favourable evolution of the three parameters analysed.

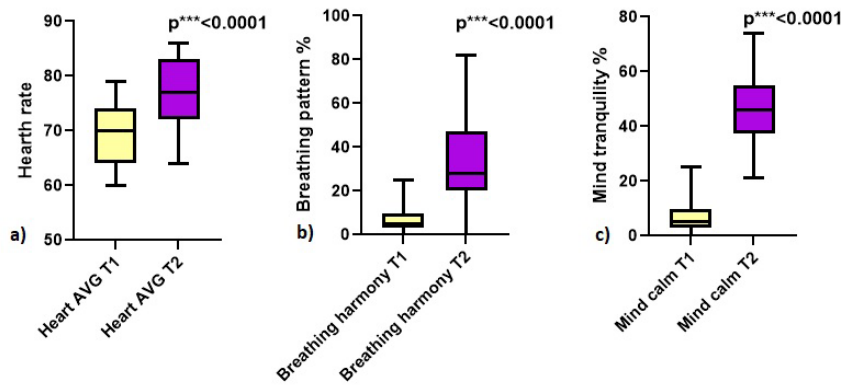


Fig. 4.8. - EEG-Muse parameters at T1/T2

a) Heart rate, b) Respiratory harmony and c) Mental calmness

Using the Mendi device, we tried to identify the existence of initial variables (T1) specific to a high level of perceived stress, as well as the presence of post-intervention changes (T2). The results obtained show a significant decrease ( $p = 0.0022$ ) in the level of neuronal activity in the A-PFC area in parallel with a significant decrease ( $p = 0.00463$ ) in resilience, while no significant changes were observed in the control parameter (Fig.4.9.).

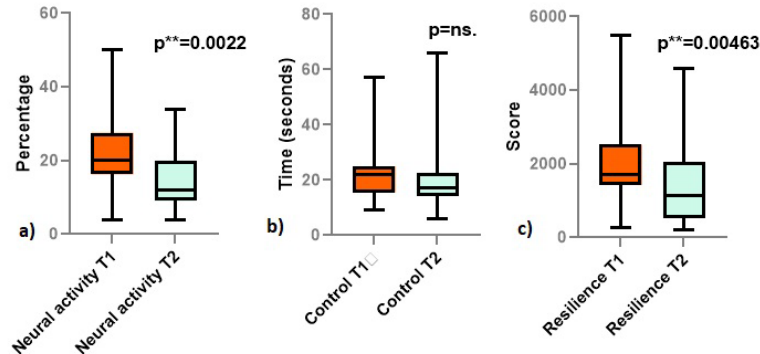


Fig. 4.9. Brain activity level at T1/T2 moments

a) Neuronal activity, b) Control and c) Resilience

The important aspect pursued by this evaluation was the brain response following the HWC intervention, as I aimed to produce functional and/or structural changes in the brain, since the brain has the capacity for neuroplasticity.

#### **4.2.3.4. Discussion**

Breathing is a vital function that can influence the level of stress in the body, and the effectiveness of HWC was demonstrated by a significant increase in respiratory harmony that paralleled statistically significant reductions in perceived stress, depression and anxiety. Furthermore, I could interpret that the evolution of the resting heart rate parameter is an optimized value obtained post HWC, and the state of mental calmness could be explained by the training of conscious breathing combined with the mental switch during the HWC sessions, being also in line with the reduction of psychoemotional affective states. The increase in mental calmness (Muse) overlapped with the reduction in neuronal activity (Mendi) in area A-PFC (BA10), both achieved post HWC intervention. Both the Muse and Mendi devices provided me with interesting information, but further studies are needed to draw a conclusion.

#### **4.2.4. Possible correlations between the parameters analysed pre and post HWC**

##### **4.2.4.1. Introduction – the working hypothesis and specific objectives**

I aimed to obtain a statistically significant model, a rather strong one, in order to be able to put together effective screening packages in order to identify a high level of stress, so that a timely and effective intervention with "anti-stress" measures would take place to reduce the risk of developing chronic non-communicable diseases. Therefore, at this stage, I have chosen as **a specific objective** the identification of effective screening packages in detecting the level of stress in the body, for the prophylaxis of chronic diseases.

##### **4.2.4.2. Methods of analysis**

In order to have stronger statistically significant results, I chose to introduce several parameters in the analysis. So, I started by correlating the two miRs with each other (miR-21 with miR-26) in order to increase the strength of significance, and I ended up with five parameters (miR-21 with miR-26, stress, QOL and depression), because I observed a higher increase in the strength of significance (about 60%). I used the same statistical analysis tools, what differed was only the number of variables.

#### **4.2.4.3. Results**

Model 3 with five variables was the strongest (60%), being obtained by correlating miR-21, miR-26, stress, and QOL with depression. At T1, a strong relationship between these predictors and depression was obtained. It was also observed that about 59.4% of the variability in depression could be explained by this model, suggesting that the selected predictors contribute significantly to explain the variation in depression scores, as well as that the model would have good generalizability for a larger population (52%). An F-value (8.054) and p-value ( $p < 0.001$ ) indicate that the regression model is statistically significant, suggesting that the selected predictor variables (miR-21, miR-26, Stress and QOL) contribute significantly to explaining the variability in T1 depression.

Next, I analysed the same parameters at T2, where it was observed that about 53.6% of the depression variability can be explained by this model, suggesting that the selected predictors contribute significantly to explaining the variation in depression scores, as well as that the model would have a relatively good generalizability for a larger population (45%). The F value = 6.360 and p value = 0.001 suggest that the regression model is statistically significant. This means that the predictor variables included in the model contribute significantly to explaining the variation in depression at T2.

#### **4.2.4.4. Discussion**

In model 3, by adding a new parameter (QOL) to miR-21, miR-26 and stress I reached an increase in the strength of significance of almost 60%, which explains the variability of depression at T1, while at T2 the variability is lower, but still sufficient (53.6%). The result is sound and indicates that the selected variables are relevant for understanding depression at both T1 and T2. I believe that Model 3 could be used successfully as it covers a wide range of subjective and objective parameters relevant for the level of stress. These parameters being significant denotes confidence in the model and the relevance of each of them associated with the state of depression, which is the most constant component state of the three emotional affective states which can influence the level of stress in the body.

## 5. Conclusions and personal contributions

### 5.1. General conclusions

1. Stress, a major problem in modern society, is associated with various pathologies, especially with chronic non-communicable diseases, and the classical medical approach which considers stress as only an associated risk factor, and not a disease-causing one, has proved totally ineffective.
2. In recent times, therefore, there has been a need for a paradigm shift with regard to stress, which has brought to light various more or less effective long-term 'anti-stress' methods and therapies that consider stress as a factor in the development of these diseases.
3. Therefore, specializations and specialists have begun to appear who, through various "anti-stress" interventions such as massage, melotherapy, Bowen therapy, breathing exercises, yoga, mindfulness techniques, aromatherapy, coping strategies, supplements, etc., only partially cover the need of individuals to reduce their stress level.
4. This diversity of approaches, which is more akin to disorganization, is most likely due to a lack of understanding of the concept of stress, even though it has been studied for over 80 years.
5. Thus, the need to find effective 'anti-stress' intervention solutions that produce long-term results in order to prevent the onset of chronic diseases, which are so costly and devastating for health and the medical system, has become obvious.
6. In this thesis I have demonstrated the effectiveness of the HWC intervention in significantly reducing the level of stress, both of the perceived stress (stress, anxiety and depression) and of the physical stress itself, an effectiveness evidenced by the post HWC modification of two stress-associated epigenetic markers, miR-21 and miR-26.
7. Furthermore, HWC, by reducing the level of stress in the body, contributed to the individuals' improved quality of life and to more inspired life-related decisions, which proves that the intervention can be successfully applied in the prophylaxis of chronic diseases frequently associated with stress.

8. Using miR-21 and miR-26, I have linked psycho-emotional states (stress, anxiety and depression) to a certain level of stress in the body, thus conferring epigenetics the defining role of this link.
9. miR-21 and miR-26 are positively correlated, being associated with sympathetic activation and various forms of cellular stress, and according to the level of the activation they can lead to positive adaptive or negative dysfunctional changes, with a major impact on psycho-emotional and physical health.
10. miR-21 is the most widely studied miR, being associated with numerous medical conditions, although it is not specific to any disease, so it could more likely be considered 'stress-specific', which in turn is a non-specific process of the organism.
11. I confirmed, through significant changes in miR-21 post HWC, the findings of other studies regarding the possible involvement of miR-21 in the cellular stress response as a result of perceived psychological stress.
12. Most likely, the maintenance of minimal variations around positive values (between 0 and 1) of miR-21 could ensure a balance between cell proliferation and cell death processes, which would contribute to the optimal functioning of the immune system for a good psycho-emotional and physical health status of the individual.
13. miR-26b has been reported to be associated with psychological stress responses and the presence of psychiatric disorders. The predominantly negative miR-26 values could be due to a process of neuronal damage, even if subclinical. This area is apparently invisible, as subjects are compensated (without chronic diseases), but could become visible (clinical) if the intervention is not timely and effective.
14. The increase in miR-26 post HWC may explain the presence of a neuronal regeneration process, being associated with the processes of neurogenesis. Thus, I consider that miR-26 values between -0.5 and 0.5 could maintain a balance between reparative and degenerative processes, especially at the neuronal level, for a better adaptation in psycho-emotional-mental stressful situations.
15. DASS-21R and QOLI are effective instruments, both for the assessment of the initial state and for the assessment of the HWC intervention, with the only amendment that in DASS-21R the psychological construct of "stress" should be renamed.
16. CD could be a useful instrument, as the T2 assessments showed a qualitative increase in the decision-making capacity, probably due to a decrease in the anxiety



parameter, but to obtain significant changes a larger group of subjects is probably needed.

17. The Muse and Mendi instruments used for neuroelectroencephalographic investigations were particularly useful at T2, confirming significant favorable changes in the parameters: mental calmness, respiratory harmony, heart rate, neuronal activity and resilience, in close agreement with the reduction in stress level, but to be able to detect the level of stress in the body further studies are needed in order to draw a conclusion.
18. The dynamic relationships between respiratory, cardiovascular and sympathetic variables may explain the favorable response to HWC, following the adoption of an autonomic optimized breathing model, an integral part of the HWC, as it is known that dysfunctional cardiorespiratory coupling is correlated with pathological states, as well as altered affective states.
19. In order to be able to intervene in a timely "anti-stress" manner, appropriate screening packages are needed to detect the level of stress in the body, and the model (3) proposed in this paper could be a promising start, which deserves to be tested in the near future, as it contains both subjective and objective indicators relevant to the level of stress.
20. Particularly in the last two years, findings in the field of stress and psychoemotional impairment have started to appear in the literature, as well as eloquent results of saliva-based epigenetic assessments, which I could use to validate the conclusions in this paper.
21. Prophylaxis is a growing area, and the study of the relationships between environmental factors and genetics in the development of disease has begun to provide new evidence. In recent decades there has been a great deal of emphasis on controlling the external factors which may cause disease, and little or almost no emphasis on controlling or regulating the internal, psycho-emotional factors. I therefore believe that there is a pressing need for this psycho-emotional component to be taken into account in any causality analysis relating to an individual's state of illness.

## **5.2. Personal contributions**

HWC is a science in its infancy, which has required a huge personal effort to establish specific standards and approaches, with no universally validated application procedures existing either internationally, let alone nationally, or locally. I believe that this is among the first, if not the first research in the literature to link the change in stress level obtained after modeling the psycho-emotional component through a HWC intervention with changes in salivary miR-21 and miR-26.

However, I managed to design an online intervention, starting from a single case, and then recalibrated it in such a way that I could establish some essential procedures and steps in the process. I designed the first version of HWC for a subject who presented an altered quality of life, and I calibrated it in such a way that it could be effective in increasing the quality of life by improving the following indicators: self-esteem, level of communication and socialization, general well-being, level of physical energy and muscle tone, as well as psycho-emotional state. Thus, I came up with an effective variant consisting of 12 sessions. Initially I only used psychometric assessments.

Later I recalibrated the intervention by increasing the number of subjects and the number of instruments and investigations used, with the aim of reducing stress levels, evidenced by an increased quality of life and epigenetic markers. Thus, I used standardized psychometric tests, to which I added epigenetic (miR-21 and miR-26) and neuroelectrophysiological investigations to increase the relevance and accuracy in assessing stress levels. In the end I arrived at an efficient version consisting of 8 sessions in which each individual goes through a specific number of steps in this process.

I also contributed to a deeper understanding of the concept of stress, making a clear differentiation between psycho-emotional impairments (stress, anxiety and depression) and somatic stress with the help of the instruments and investigations used, while establishing the relationship between these components.

I believe that through this work I have contributed to highlighting possible non-invasive methods of investigating stress, with the help of the instruments used.

Another gain of this research would be the discovery of possible screening models for detecting stress levels in the body by combining subjective (psychometric) and objective (epigenetic) tests, in order to provide rapid and effective "anti-stress" interventions. Model

3 could be successfully used, and, as it is relatively strong (60%), it could represent a good starting point worth further investigation.

Last but not least, for this research I set up a multidisciplinary team, including physiologists, psychologists, psychoneurophysiologists, biologists, geneticists, computer scientists, statisticians, which gave me the possibility to gain a deeper understanding of the concept of stress, and gave an extra touch of professionalism and originality to this research.

I appreciate that I made an important personal contribution by studying these stress-related miRs, as miR research is still a field that is too little addressed and explained. Therefore, the beauty of this research was to uncover the variation and orientation of miR-21 and miR 26, as well as their versatility to stress, managing to capture the increasing trend in their levels. Even though I encountered many difficulties in selecting subjects at the beginning, they responded positively and significantly to the intervention, which brought a great gain both for the understanding of stress and for the scientific certification of the HWC intervention. Further studies are needed as miRs are very versatile, with each miR controlling numerous genes. The analysis of these miRs in relation to stress level is more eloquent and insightful compared to the analysis based on psychometric tests, providing valuable additional data about a possible risk of disease if timely and effective intervention is not taken.

The data from this research offers the possibility of developing an interdisciplinary system for chronic disease prophylaxis, which could represent a new perspective on disease that could help to relieve the burden on the medical health system while providing solutions for the prophylaxis of chronic non-communicable diseases, including outside a formal medical setting.

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