Brief Training of Psychoneuroendocrinoimmunology-Based Meditation (PNEIMED) Reduces Stress Symptom Ratings and Improves Control on Salivary Cortisol Secretion Under Basal and Stimulated Conditions

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Context: Meditation is proposed as an anti-stress practice lowering allostatic load and promoting well-being, with brief formats providing some of the benefits of longer interventions.

Objectives: PsychoNeuroEndocrinoImmunology-based meditation (PNEIMED) combines the teaching of philosophy and practice of Buddhist meditation with a grounding in human physiology from a systemic and integrative perspective. We evaluated the effects of four-day PNEIMED training (30 h) on subjective and objective indices of stress in healthy adults.

Design: A non-randomized, controlled, before-and-after study was conducted. Participants ($n = 125$, mostly health practitioners) answered a questionnaire rating stress symptom before (T0) and after (Tf) a PNEIMED course. In an additional sample ($n = 40$; smokers, overweight persons, women taking contraceptives, and subjects with oral pathologies were excluded), divided into PNEIMED-attending (intervention, $n = 21$) and non-meditating (control, $n = 19$) groups, salivary cortisol was measured upon awakening and during a challenging mental task.

Results: Self-rated distress scores were highly reduced after the PNEIMED course. In the intervention group, improvement of psychological well-being was accompanied by decrease in cortisol levels at awakening. No T0-vs-Tf changes in distress scores and morning cortisol were found in controls. Based on baseline-to-peak increment of cortisol response at T0, 26 subjects ($n = 13$ for each group) were classified as task-responders. The amplitude and duration of the cortisol response decreased after PNEIMED, whereas no effects were found in controls.

Conclusions: Brief PNEIMED training yields immediate benefits, reducing distress symptoms and adrenocortical activity under basal and stimulated conditions. PNEIMED may represent an effective practice to manage stress and anxiety, particularly among subjects facing a multitude of job-related stressors, such as healthcare workers.

Key words: Stress management, mental stress, hypothalamic–pituitary–adrenal axis, healthcare workers, Symptom Rating Test

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INTRODUCTION

Repeated and/or protracted exposure to physical or psychosocial stress may result in wear-and-tear of body's regulatory systems and allostatic overload, thereby affecting well-being[1] and health trajectories.[2] Stress management interventions (INTs), including meditation, are regarded as effective strategies to reduce allostatic load and promote psychological and physical well-being. Indeed, several meditation practices have been found to improve cognitive performance, mood, and affective processes,[3–5] and have been associated with improvements in immune system functioning, emotional regulation, and behavioral stress.[6,7]

Though beneficial, meditation programs generally require considerable time and financial commitment. However, brief formats of mental training can provide some of the benefits that result from longer interventions. Zeidan et al.[8,9] showed

ISSN 1550-8307/$36.00

http://dx.doi.org/10.1016/j.explore.2014.02.002

that four days of mindfulness meditation reduces pain, fatigue, and anxiety scores, and improves cardiovascular regulation, mood, and executive functions. Using a different style of meditation, Tang et al.[10,11] reported that five days of integrative body–mind meditation practice improves attention and self-regulation; reduces depression, anxiety, and fatigue; and promotes a better regulation of the autonomic nervous system.

PsychoNeuroEndocrinoImmunology (PNEI)-based meditation (PNEIMED), as it was developed by Carosella and Bottaccioli,[12–14] combines the transmission of scientific knowledge on the systemic vision of the human organism, emphasizing the importance of the mind–body relationship and psychosomatic network,[15,16] with that of philosophical principles and meditative and stress-control practices of the Buddhist tradition *Mahayana*, integrated with elements from modern versions (Psychosynthesis according to Assagioli[17]).

The present study aims to investigate, in healthy adults, the effects of a brief (four and a half days) PNEIMED training course on subjective and objective indices of stress, i.e., psychological distress symptom ratings and hypothalamic–pituitary–adrenal (HPA) activity, indexed by measuring salivary cortisol levels. Our experimental hypothesis is that PNEIMED training may have a positive effect on stress-related psychological and biological variables, lowering perceived distress levels and reducing cortisol secretion under both basal (upon awakening) and stimulated conditions (in response to acute mental stress).

METHODS

PsychoNeuroEndocrinoImmunology-Based Meditation (PNEIMED): Theory and Practice

This method, based on a critical and non-religious approach, draws upon the classic Buddhist tradition and the teaching of Tenzin Gyatso, the current Dalai Lama. PNEIMED refers to the Buddhist *Mahayana* tradition (or "Great Vehicle"), which includes meditation techniques of the *Theravada* tradition (also called *Hinayana* or "Small Vehicle") emphasizing the ability of the individual to achieve a state of conscious well-being (enlightenment) through the constant practice of concentration (*samatha*) and deep vision (*vipassana*). In addition, this method underscores the role of knowledge and philosophical study, which are believed to make the practice of meditation more effective. Indeed, according to Tenzin Gyatso, the *Mahayana* tradition is "the Buddhism of knowledge, the 21st century Buddhism based on an extremely solid knowledge which includes new scientific progress."[18] Science is not seen as being in opposition to the meditative path, but rather as an essential tool to enhance awareness and favor the liberation of contemporary man.

Hence, PNEIMED emphasizes the teaching of full awareness achieved by combining meditative methods with scientific knowledge of the organization and holistic functioning of the human body. PNEIMED practitioners are encouraged to explore their mind, biological processes, and the interaction between their biological and psychological systems. It is crucial for them to understand the stress system, and its relationship with emotions, cognition, and their biological correlates, and to observe their influences on behavior.

PNEIMED bears some similarities to the Mindfulness method[19] but also differs in other respects. Both methods aim to make the tradition of Eastern meditation techniques (especially Buddhist) accessible to the Westerners, although Mindfulness focuses mainly on the *Theravada* tradition. Both methods share several meditation techniques such as sitting meditation, body scan, and observing one's own thoughts, sensations, and images without holding or judging them. However, during PNEIMED sessions, other techniques are used to improve concentration and attention, e.g., the evocation of "emotional words" is used to recognize emotion and foster detachment from it.

PNEIMED course. Each PNEIMED course lasts four and a half days (30 h in total) and offers a balance of theory and practice including informative scientific lessons on the principles of PNEI and stress management techniques. Lessons are organized in alternate sessions (PNEI and stress management techniques) of one and a half hours each. PNEI teaching is tailored to the cultural background and educational level of the participants.

The scientific lessons of the first day provide an overview of mind–body relationships based on a historical reconstruction of modern theories spanning from Descartes to the birth of physiopathology to modern scientific genetic reductionism and the paradigm shift of epigenetic and psychoneuroendocrinoimmunology. The lessons of the second day focus on the neurophysiology of emotions and the relationship between emotions and consciousness. The lessons of the third day address both neurobiology and the psychology of stress, highlighting the pathological consequences of chronic stress on the brain and immune and metabolic systems. The lessons of the fourth day and the final half day focus on the available scientific evidence on the effects of anti-stress techniques and meditation, nutrition, and physical activity on the human organism, encouraging course participants to make a general change in their lifestyles.

Anti-stress techniques and meditation are based on stress relaxation exercises and guided imagery. Each participant is taught the exercises receiving considerable individual attention, since the class size does not exceed 20. The technique is based on the exercises included in the texts of Carosella and Bottaccioli.[12,13] The exercises are introduced with explanations of their origins, their philosophical foundations, the purposes for which the PNEIMED method applies them, and their use in daily life. Details are provided on the position to be assumed during the exercises and on how to start and finish the exercise. On the first day, course participants are taught a relaxation exercise which gradually relaxes the whole body, from head to toe, as well as the mind. At this initial stage, guided relaxation is not carried to particular depth to allow participants to get accustomed to the new physiological condition, with parasympathetic tone being highly predominant, which many have never experienced before. They are then taught the initial visualization exercises of geometric shapes (triangles, circles, and squares) and their possible combinations. On the second day, an intermediate-level relaxation exercise is taught, making use of preliminary visualization exercises whereby participants imagine writing and then deleting their own names on a black board. Basic breathing exercises are also used. On the third day, participants are taught to visualize words like "patience," "calm," "peace," "serenity," "courage," and "compassion," and to visualize their own bodies from the front, the side, above, and behind. They are then encouraged to practice this exercise at home using mirrors. On the fourth day and the final half day, a deep relaxation technique is taught using the visualization of complex scenes (e.g., a lake) and the observation of one's thoughts and of oneself.

PNEIMED Intervention

Subjects. Participants, enrolled after signing an informed consent form, were healthy adults who attended PNEIMED courses ($n = 125$, 31 males and 94 females, aged 18–60 years, mostly health practitioners). In various Italian cities, seven PNEIMED courses were held, maintaining the very same training module, over a period of five consecutive years (2006–2011).

Psychometric measures. A validated Italian version of the Symptom Rating Test (SRT; Italian validation by Fava et al.[20,21]) was administered before (T0) and after (Tf) the PNEIMED course. SRT is a 30-item questionnaire measuring four different scales of psychological distress (anxiety, depression, somatization, and inadequacy), mainly revealing aspects of personality status. The questionnaire is available in different time frames (i.e., last day, last week, last month, and last year), and here we used the "four-days-ago" period. The total SRT score is expressed as the sum of self-rated scores (using a 0–4 Likert scale) on individual items: "never" is worth 0 points; "only sometimes," 1 point; "often," 2 points; "very often or most of the time," 3 points; and "always," 4 points. The total score can range from 0 to 120. For each scale, data are presented as mean ± standard deviation (SD). T0-vs-Tf differences were assessed by paired *t*-test in all psychological scale ratings and total scores.

Cortisol Tests

Subjects. A sub-sample of subjects was recruited for the second part of the study, in which psychometric analyses were combined with laboratory measures of salivary cortisol levels under basal and stimulated conditions. All subjects willing to register for PNEIMED course (years 2009–2011) were e-mailed an advertisement describing design and aims of the study and detailing experimental methods and procedures. Phone contacts were provided for prospective participants who desired additional information. Volunteers were enrolled after signing an informed consent form. Smokers, overweight persons [with body mass index (BMI) >25], premenopausal women who were menstruating, women taking oral contraceptives or other hormonal therapies, and subjects affected by oral or dental pathologies were excluded. Selected participants ($n = 40$) were divided in two groups: intervention group, composed of subjects who regularly attended a PNEIMED course (INT; $n = 21$; six males and 15 females; aged 38–59 years), and control (CTR) group, composed of subjects who volunteered to spend the same period of time in the same setting where PNEIMED course

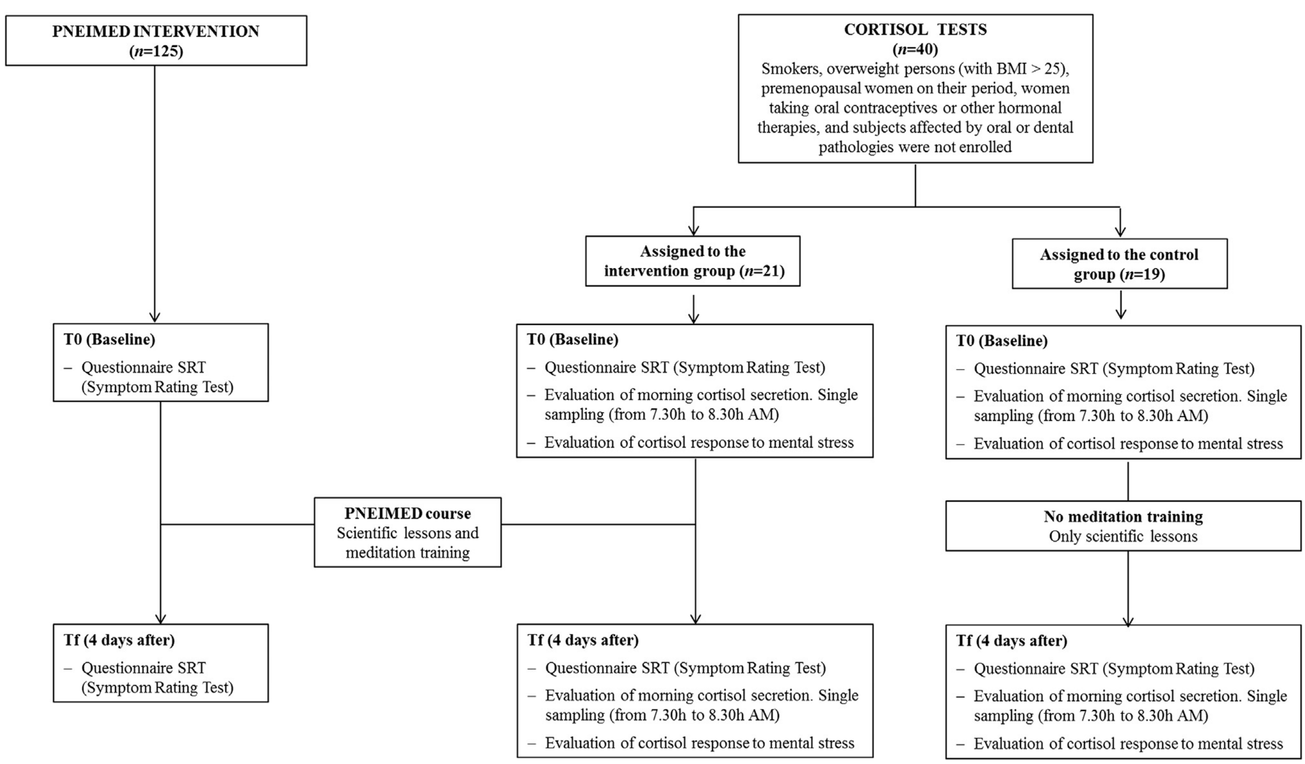

Figure 1. Flowchart of study participants.

was held, participating in scientific lectures and other daily activities but not meditation training (CTR; $n = 19$; nine males and 10 females; aged 25–58 years). Here, a non-random selection procedure was used. We thought this approach would be appropriate to evaluate the effectiveness of a holistic intervention in open-field settings, as it is well suited for considering the subject in his/her natural context. It is worth noting that non-randomized procedures are proposed as valid alternative methods in public health intervention studies.[22] Moreover, such procedures could be used in the context of complementary and alternative medicine to overcome eventual skepticism of participants.[23] A schematic flowchart of research participants is shown in Figure 1.

Salivary cortisol detection. Procedures were conducted as previously described.[24] At least 1 ml of saliva was collected in Salivette® (Sarstedt Aktiengesellschaft & Co., Nümbrecht, Germany). Subjects were instructed not to consume water or food (including candies or chewing gum) or brush their teeth within 30 min prior to sample collection. Saliva samples were centrifuged at 1000*g* for two minutes, and supernatant was collected and stored at −20°C. Determination of cortisol in saliva was performed using electrochemiluminescence immunoassay ("ECLIA") on Elecsys® 2010 (Roche Diagnostic, Manheim, Germany), according to manufacturer's instructions. Cortisol concentration was expressed as nanomoles per liter (nmol/l). All data are reported as mean ± standard error of means (SEM). The lower limit of detection for the assay was 0.5 nmol/l and the upper limit of the standard curve was 1750 nmol/l.

At the beginning (T0) and at the end (Tf) of the PNEIMED training, cortisol concentration was measured in a single salivary sample collected 30 min after wake-up (from 7.30 AM to 8.30 AM; awakening cortisol). Cortisol response to mental stress was investigated by applying the Raven's Advanced Progressive Matrices, a non-verbal, multiple-choice measure of reasoning and general intelligence for adult and adolescents[25]; in each test item, subjects were asked to identify the missing element to complete a pattern, and items became increasingly difficult as progress was made through the test. To increase the level of situational stress, the test was performed in front of an evaluative audience, and subjects were prompted to perform as fast and accurate as possible. Previous studies[26] have shown that similar types of mental task can induce an increment of cortisol levels compatible with that elicited by standard psychosocial stress protocols, such as Trier Social Stress Test.[27] Both at T0 and Tf, the test was done around noon, and the duration of the task was 10 min. Items were changed in the second test, though retaining the same level of difficulty. Salivary samples were collected at three time points across the task: five minutes before starting the test (baseline, sampling time 1, s1), 10 min after task completion (s2, cortisol peak response), and then after 30 min (s3, recovery).

Table 1. Demographic Characteristics of Samples Enrolled in PNEIMED Intervention ($n = 125$) and in Cortisol Tests ($n = 40$). Note the High Prevalence of Health Practitioners

			Cortisol Tests ($n = 40$)			
	PNEIMED Intervention ($n = 125$)		Intervention ($n = 21$)		Control ($n = 19$)	
Demographic Characteristics	Mean (SD)	*n* (%)	Mean (SD)	*N* (%)	Mean (SD)	*N* (%)
Age (years)	47.34 (4.09)		49.29 (10.59)		43.19 (10.09)	
Gender						
Male		31 (24.8)		6 (28.6)	9 (47.4)	
Female		94 (75.2)		15 (71.4)	10 (52.6)	
Education						
Secondary or high school		19 (15.2)		4 (19.1)	6 (31.6)	
Bachelor degree or higher		106 (84.8)		17 (80.9)	13 (68.4)	
Profession						
Psychologist, psychotherapist		44 (35.2)		5 (23.8)		3 (15.8)
Medical Doctors		19 (15.2)		8 (38.1)		4 (21.1)
Medical Nursing		13 (10.4)		–		1 (5.3)
Physiotherapists, Osteopaths		13 (10.4)		4 (19.1)		1 (5.3)
Pharmacists		4 (3.2)		–		–
Naturopaths		6 (4.8)		–		–
Sociologists, Health workers		6 (4.8)		3 (14.3)		2 (10.5)
Teachers		6 (4.8)		1 (4.7)		1 (5.2)
Employees		2 (1.6)		–		1 (5.2)
Students		2 (1.6)		–		2 (10.5)
Other		10 (8.0)		–		4 (21.1)

PNEIMED = psychoneuroendocrinoimmunology-based meditation; SD = standard deviation.

Data analyses. A Shapiro–Wilk test rejected normality of data distribution for raw cortisol values, and as a result cortisol data were log-transformed; however, the raw values are reported in the results section so as to be physiologically meaningful and are presented as mean ± standard error of the mean (SEM).

In both intervention (INT) and control (CTR) groups, T0-vs-Tf differences in awakening basal cortisol levels were assessed by paired *t*-test. Analysis of variance (ANOVA) testing was also used to evaluate before-vs-after within-group differences; effect size was calculated by Cohen's *d* statistic. Data were adjusted for age and gender as covariates by analysis of covariance (ANCOVA).

Acute cortisol response to mental stress was quantified by measuring (i) hormone concentration at the three sampling times (s1, s2, and s3; nmol/l) and (ii) the area under the curve of all measures (s1–s3) with respect to ground [area under the curve (AUC); nmol/l × time], an index that is assumed to reflect the total cortisol output across the task.[28] AUC was calculated using SPSS Statistics software (version 20.0). In accordance with previous literature,[29–31] subjects of both INT and CTR groups were subdivided in two categories, responders (R) and non-responders (NR), on the basis of the amplitude of their cortisol response to mental stress. In our study, a baseline-to-peak cortisol rise of 2.5 nmol/l was considered as cutoff value to separate R from NR; such an elevation corresponds to an increase of approximately 1 μg/dl total cortisol in serum, which is thought to reflect a cortisol secretory episode.[32] In responders, T0-vs-Tf differences in cortisol response indices were assessed with paired *t*-tests. Repeated-measures multivariate analysis of variance (MANOVA) test with time (T0-vs-Tf) as within-group factor was calculated for cortisol levels measured at the different sampling points (s1–s3), and for AUC. Cohen's *d* statistic was also calculated to determine effect size. Differences in AUC values were controlled for age and gender as covariates by ANCOVA testing.

All the ANOVA, MANOVA, and ANCOVA analyses were performed using the statistical software program STATA (version 9.0).

RESULTS

PNEIMED Reduces Psychological Stress Symptoms

All subjects enrolled in the first part of the study ($n = 125$; 31 males and 94 females, aged 18–60 years; mostly health practioners; Table 1) answered the SRT questionnaire, both at the beginning and at the end of a PNEIMED training. Results showed that self-rated psychological distress was reduced after PNEIMED, with all psychometric dimensions showing significantly lower scores at Tf than at T0 (Table 2).

The SRT questionnaire was administered also in the smaller sample of subjects ($n = 40$) who underwent salivary cortisol measures; demographic characteristics of both INT ($n = 21$) and CTR ($n = 19$) groups are reported in Table 1. At T0, total SRT scores was similar in the two groups (19.71 ± 12.32 and 15.63 ± 12.96 for INT and CTR, respectively; *t*-test, $P = .31$). At Tf, PNEIMED-attending subjects reported significant reduction in all psychological scores, whereas only slight, non-significant, decrease of symptom ratings was found in controls (Table 2).

PNEIMED Reduces Basal Cortisol Secretion

In PNEIMED-attending subjects, paired *t*-test revealed significant T0-vs-Tf variations in the cortisol levels at awakening, with morning hormone concentrations being markedly reduced after the training (13.92 ± 2.64 and 7.66 ± 1.21 nmol/l at T0 and Tf, respectively; $P < .005$; Figure 2). In contrast, no before-vs-after differences were found in control group (13.54 ± 3.69 and 16.08 ± 2.38 nmol/l at T0 and Tf, respectively; $P = .31$; Figure 2). ANOVA testing (time as factor) confirmed within-subjects effect in the intervention group [F (1,20) = 3.26, $P >$ .05, Cohen's $d = 0.022$]. ANCOVA analysis controlling for age

Table 2. Before-vs-After Comparison Between SRT Scores at T0 and Tf. Data Are Collected in Samples Enrolled in PNEIMED Intervention ($n = 125$) And in Cortisol Tests ($n = 40$). All Dimensions of Psychological Distress Are Highly Reduced after PNEIMED Course, But Not in Control Subjects

			Cortisol Tests ($n = 40$)			
	PNEIMED Intervention ($n = 125$)		Intervention ($n = 21$)		Control ($n = 19$)	
	Baseline (T0)	Four days after (Tf)[a]	Baseline (T0)	Four days after (Tf)[a]	Baseline (T0)	Four days after (Tf)[a]
Symptoms	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Anxiety	5.81 (4.22)	2.01 (2.69)[b]	6.28 (3.16)	1.67 (1.43)[b]	4.37 (4.09)	3.89 (3.84)[c]
Depression	4.42 (3.99)	1.64 (2.03)[b]	4.62 (2.99)	2.10 (2.14)[b]	3.68 (3.99)	3.53 (3.60)[c]
Somatization	3.53 (3.31)	1.41 (1.82)[b]	4.14 (3.64)	1.48 (2.23)[b]	3.89 (3.10)	3.47 (2.97)[c]
Inadequacy	4.39 (3.52)	1.29 (1.78)[b]	4.19 (3.46)	1.62 (2.89)[b]	3.68 (3.13)	3.42 (2.65)[c]
Total SRT score	18.09 (12.09)	6.17 (6.65)[b]	19.71 (12.32)	6.86 (7.61)[b]	15.63 (12.96)	14.32 (11.69)[c]

SRT = Symptom Rating Test.

[a]Compared with baseline values (T0).

[b]$P < .05$.

[c]Not significant.

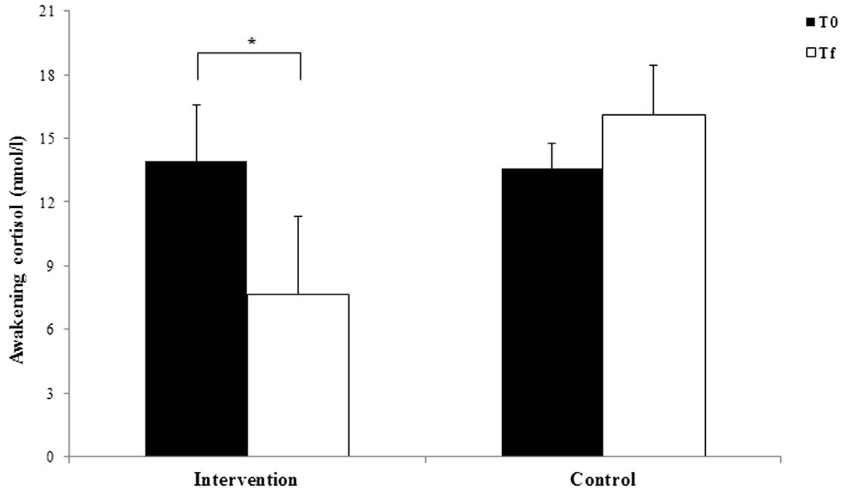

Figure 2. Awakening cortisol levels measured at the beginning (T0) and at the end (Tf) of PNEIMED course. Mean concentration (± SEM) is significantly reduced at Tf in the intervention group (13.92 ± 2.64 and 7.66 ± 1.21 nmol/l at T0 and TF, respectively; $n = 21$), but not in control group (13.54 ± 3.69 and 16.08 ± 2.38 nmol/l at T0 and Tf, respectively; $n = 19$). Paired *t*-test, $^{*}P < .05$. PNEIMED = psychoneuroendocrinoimmunology-based meditation; SEM = standard error of means.

and gender as covariates furthermore confirmed the effect [F (1,39) = 6.24, $P < .05$, Cohen's $d = 0.36$].

PNEIMED Attenuates Cortisol Response to Mental Stress

In both intervention ($n = 21$) and control ($n = 19$) groups, cortisol response to cognitive challenge was evaluated at the beginning and at the end of the course. Both at T0 and Tf, mental task elicited a significant increase in cortisol peak (s2) with respect to baseline (s1), thus indicating that the task was indeed stressful and able to activate HPA axis (INT, at T0: s1 = 4.68 ± 0.92 and s2 = 13.88 ± 3.31 nmol/l, and at Tf: s1 = 4.30 ± 0.91 and s2 = 10.11 ± 1.79 nmol/l; CTR, at T0: s1 = 7.48 ± 1.76 and s2 = 27.10 ± 3.28 nmol/l, and at Tf: s1 = 5.25 ± 1.27 and s2 = 20.84 ± 3.60 nmol/l; all $P < .005$). Pre-stress, baseline cortisol levels (s1) were similar in the two groups, both at T0 ($P = .66$) and Tf ($P = .71$).

Based on the amplitude of their cortisol responses at T0, indexed as baseline-to-peak increment, subjects were divided into two groups: non-responders, showing cortisol rises of less than 2.5 nmol/l (NR; $n = 8$ and $n = 6$ for INT and CTR, respectively), and responders, with cortisol increases larger than 2.5 nmol/l (R; $n = 13$ for INT and CTR). In accordance with previous reports,[27,28] subjects who did not respond to the stressor at T0 were excluded from further analysis.

In the intervention group, indices of stress-induced cortisol response were all remarkably lower at Tf compared with T0. In particular, paired *t*-test revealed significant before-vs-after reduction in cortisol peak (s2: 20.22 ± 4.52 and 11.46 ± 2.15 nmol/l at T0 and Tf, respectively; $P < .05$; Figure 3A) and AUC (407.94 ± 91.72 and 245.52 ± 51.50 nmol × time at T0 and Tf, respectively; $P < .05$; Figure 3B), thus pointing to a marked decrease of HPA reactivity to mental stress after the PNEIMED training. In contrast, the same indices showed no T0-vs-Tf differences in control group (s2: 28.22 ± 2.61 vs 25.91 ± 4.43 nmol/l, $P = .26$; AUC: 480.66 ± 72.39 vs 417.93 ± 143.25 nmol × time, $P = .33$; Figure 3A and B). In the intervention group, repeated-measures MANOVA test with time as factor (T0-vs-Tf) confirmed within-subjects effect for cortisol levels measured at the different sampling points [(F 1,25) = 9.25, $P < .05$, Cohen's $d = -0.35$] and for AUC [(F 1,25) = 7.64, $P < .05$, Cohen's $d = -0.41$]. Before-vs-after reduction in AUC was confirmed by ANCOVA analysis after controlling for age and gender as covariates [(F 1,25) = 3.63, $P < .05$, Cohen's $d = 0.37$]. No significant effect was found in control subjects.

Paired *t*-test comparison between cortisol values measured at baseline (s1) and at 30 min post-peak (recovery, s3) was used as an index of response duration. At T0, hormone levels at s3 were higher than at s1 (INT: s3, 9.32 ± 1.76 vs s1, 6.18 ± 1.28 nmol/l; CTR: s3, 16.13 ± 1.87 vs s1, 5.84 ± 1.71 nmol/l; $P < .005$), indicating that salivary cortisol concentration was not yet returned to pre-stress, baseline levels. In the intervention group, but not in controls, this difference was abolished at Tf, when s3 values became similar to s1 (INT: s3, 6.06 ± 1.11 vs s1, 5.22 nmol/l; $P = .18$; CTR: s3, 15.64 ± 4.81 vs s1, 4.41 ± 1.11 nmol/l; $P < .05$), thus pointing to a shorter duration of cortisol response, with faster return to pre-stress levels.

DISCUSSION

As the main outcome of the present study, we show that a brief PNEIMED training, in subjects with little or no prior experience of meditation, is able to induce a strong reduction of self-rated psychological symptoms of anxiety, depression, and stress. These findings confirm and extend previous evidence showing that brief meditation trainings (three to four days) can provide some of the benefits that result from longer interventions, improving attention and self-regulation,[10] lowering fatigue and anxiety scores,[9,10] and reducing pain ratings in association with functional changes in brain neurocircuitry activation and autonomic regulation.[8,11] As

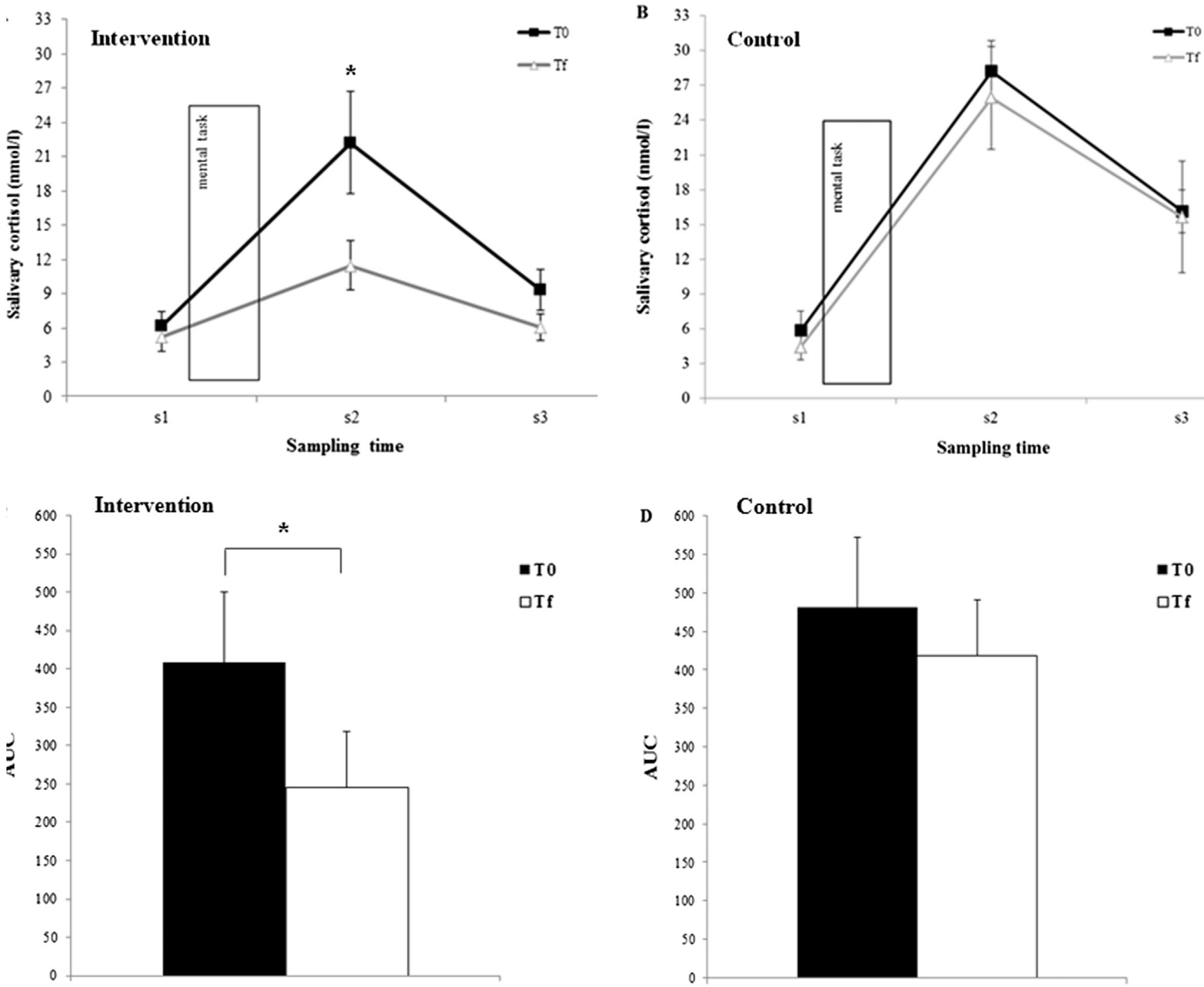

Figure 3. Acute cortisol response in task-responders is down-regulated by PNEIMED course. Mean cortisol levels (± SEM) at three sampling points (s1, baseline; s2, peak; and s3, recovery) in intervention ($n = 13$) and control ($n = 13$) groups are depicted (A and B). Mean values of AUC at the beginning (T0) and at the end (Tf) of PNEIMED course in intervention (C) and control subjects (D) are also depicted. In PNEIMED-attending subjects, significant before-vs-after reduction are found in cortisol peak (s2: 20.22 ± 4.52 and 11.46 ± 2.15 nmol/l at T0 and Tf, respectively) and AUC (407.94 ± 91.72 and 245.52 ± 51.50 nmol × time at T0 and Tf, respectively). In contrast, no differences are found in control group for the same indices (s2: 22 ± 2.61 vs 25.91 ± 4.43 nmol/l and AUC: 480.66 ± 72.39 vs 417.93 ± 143.25 nmol × time at T0 and Tf, respectively). Paired *t*-test, $^{*}P < .05$.

observed in a smaller group of subjects, in which psychometric analyses were combined with salivary cortisol measures, the reduction in self-rated psychological distress was accompanied by an improvement of adrenocortical activity, with decreased cortisol levels upon awakening and blunted cortisol response to a stressful cognitive task. In contrast, no before-vs-after differences in stress/anxiety ratings and cortisol secretion levels were found in control subjects, who shared the same relaxing and culturally stimulating setting without attending meditation training.

Cortisol is regarded as a valuable biological marker to assess the effectiveness of interventions intended to reduce stress, as it is secreted by the adrenal glands in response to stress, is considered to be a reliable marker of HPA activity, and has generally been found to be responsive to anti-stress practices, including meditation.[33] Long-term practice of mindfulness-based meditation was reported to lower morning and afternoon plasma cortisol levels in early-stage breast and prostate cancer patients,[6,34–36] and to reduce awakening salivary cortisol in substance abusers[37]; participating in a three-month self-care-promoting course including elements of mindfulness-based meditation protected medical students from the increase of morning and evening cortisol levels during stressful examination periods.[38] Here, we found that in

PNEIMED-attending subjects, morning salivary cortisol levels were significantly reduced after only four and a half days of practice, thus revealing that beneficial regulatory effects on basal cortisol secretion can be obtained with brief formats of training. This finding appears in line with recent reports by Vendana et al.,[39] showing that healthy subjects practicing Integrated Amrita Meditation, a technique combining breathing and muscle relaxation exercises with deep meditation, display a significant reduction of morning plasma cortisol after 48 h of training. Morning cortisol peak is thought to be determined mainly by situational factors, such as work overload or social stress,[40,41] and by anticipation of upcoming demands.[42] The reduction of morning cortisol after the PNEIMED training may thus help to ameliorate the situation-dependent adaptation to daily stress. Present observations gain particular relevance from recent clinical and epidemiological evidence showing that higher morning cortisol levels are associated with anxiety disorders[43] and to increased risk of mortality, hypertension, and diabetes in the older population.[44]

Major changes were observed in stress-elicited cortisol response after the PNEIMED training. In high-responder subjects, all indices appeared markedly decreased at Tf, notably peak cortisol response and AUC. Moreover, the cortisol response became less protracted after the course, with faster return of hormone secretion to pre-stress levels. No before-vs-after variations in cortisol response were instead observed in control subjects. Collectively, these observations suggest that PNEIMED training was able to reduce the amplitude and duration of cortisol response to mental stress in the group of subjects that were initially more responsive to the challenge. Previous studies have reported reductions in cortisol response to acute psychological and metabolic stressors after long-term meditation practice,[45–47] but the effects of briefer training were so far poorly explored. In agreement with present results, Tang et al.[10] documented a significant decrease in cortisol response to three-minute mental arithmetic task in a group of Chinese students receiving five days of 20-min integrative meditation training, with respect to a control group receiving only relaxation training.

Some limitations of present study need to be pointed out. First, the limited number of subjects enrolled admittedly precludes drawing definitive conclusions. Second, habituation could partly contribute to explain the observed reduction of acute stress response after PNEIMED. However, such contribution seems minor, since (i) in the second test session, the mental task was renewed, without altering its structure and level of difficulty. It is worth noting that in control subjects, all indices of cortisol response to mental task remained unchanged in the second session. (ii) Previous studies have shown that healthy, high-responder subjects exhibit persistently high cortisol responses to repeated psychological stressors, virtually unaffected by habituation.[47,48] Third, a single salivary sample was used to investigate basal morning cortisol secretion, although we are aware that multiple measures could yield a more complete picture. In most healthy people, morning awakening is associated with a brisk increase of cortisol secretion by about 40–80% of plasma awakening level, reaching its peak around half hour after wake-up and being relatively unaffected by gender, age, sleep duration, and time of awakening.[49,50] To avoid major biases in before-vs-after within-subjects comparisons, salivary samples were collected rigorously 30 min after awakening. In addition, the fact that subjects enrolled in our study shared the same setting and followed similar rhythms of diurnal activities plausibly minimized any bias due to various potential confounding factors unrelated to the intervention, e.g., individual differences in physical exercise, work load, lifestyles, and environmental stress.

In conclusion, present findings show that a brief PNEIMED training yields immediate benefits in healthy adults, since it markedly reduces self-rated symptoms of anxiety, depression, and distress, accompanied by an improvement of adrenocortical activity, with decreased morning cortisol levels and blunted cortisol response to stressful cognitive task. If the benefits of PNEIMED can be experienced after such a brief training regimen, then individuals may feel more inclined to continue practice, which can lead in the long run to better health outcomes; this suggests the potential of PNEIMED as an effective mental and physical health promotion strategy. It is worth noting that our sample was composed mainly by health practitioners (over 85% of the sample). Healthcare providers face a multitude of job-related daily stressors, which can alter their interactions with patients and colleagues[51]; controlling distress in these subjects is therefore of paramount importance for their coping capabilities and for avoiding potentially tragic errors. We believe that PNEIMED may represent an effective practice to reduce stress and anxiety among healthcare workers, thus providing an important tool to promote health practitioners' care resources and well-being.

Acknowledgments

Authors gratefully thank Drs. Katy Fowler and Timothy Bloom for reviewing the English throughout the article. Sources of funding for this work came from the Università Politecnica delle Marche (to M.M.D.'E.) and from the SIPNEI (to F.B. and A.C.).

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