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Sex-dependent salivary microRNA expression profiles and psychophysiological responses to acute stress in healthy adults

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ABSTRACT

MicroRNAs (miRNAs) have attracted great interest as potential biomarkers in several research domains, including acute and chronic stress conditions. While most of these studies focused on circulating blood miRNA levels, the investigation on the feasibility and utility of miRNAs as biomarkers of stress in other biological fluids, such as saliva, has just started. The objective of the present study was to explore changes in the expression levels of candidate salivary miRNAs following acute stress in a sample (n = 111) of healthy participants, and to examine their potential associations with psychophysiological parameters and biological sex. Participants were exposed to an immersive multimodal environment stress test, i.e. the IMVEST protocol, which induced clear psychophysiological stress responses as evidenced by increased subjective perception of stress, elevated cortisol levels, increased heart rate, and reduced heart rate variability. Among the miRNAs that were reliably detectable in all participants, miR-21, miR-25, miR-26b, miR-29a, and miR-200a showed significant changes in their expression levels after stress exposure. Also, significant sex differences in expression levels and/or stress responses were found for miR-21, miR-23a, miR-26b, and miR-29a. These results suggest that these specific miRNAs may represent promising candidate markers associated with stress-related biological responses. On the other hand, other candidate miRNAs (miR-126, miR-144, and miR-183) showed significant changes after stress exposure and/or between sexes, but were detectable only in some participants, thus questioning their utility as reliable salivary biomarkers.

1. Introduction

It is widely recognized that psychosocial stress in humans exerts a significant impact on both physical and mental health (Slavich, 2016; Yaribeygi et al., 2017). Exposure to psychosocial stress primarily activates the sympathetic–adrenal–medullary system and hypothalamic–pituitary–adrenal axis (McEwen, 1998), which trigger a cascade of physiological changes, including increased levels of cortisol and epinephrine, elevated heart rate and blood pressure, and heightened activity of pro-inflammatory cytokines (McEwen, 1998; Allen and Dwivedi, 2020; O'Connor et al., 2021). While these responses are adaptive in the short-term, their prolonged activation contributes to systemic alterations across cardiovascular (Osborne et al., 2020; Hinterdobler et al., 2021), immune (Kemeny and Schedlowski, 2007), and psychophysiological systems (James et al., 2023), which, in turn, increase the risk of developing stress-related disorders in vulnerable individuals (Vogelzangs et al., 2010). Therefore, identifying individual patterns of maladaptive stress response to clarify pathways linking stress exposure to disease vulnerability remains a major goal in stress research.

Recent evidence has identified microRNAs (miRNAs) as key regulators of cellular function under various stress conditions (Du et al., 2019). miRNAs are small, non-coding RNA molecules of approximately 22–23 nucleotides in length that play a crucial role in post-transcriptional gene regulation by binding to messenger RNAs (mRNAs) and promoting their

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degradation, cleavage, or translational repression (Bartel., 2004, Bartel., 2009). Depending on the severity and duration of stress exposure, miRNAs seem to play important roles in helping cells restore homeostasis upon sudden environmental changes, or, in the long run, initiate a new gene expression program (Leung and Sharp., 2010). Beyond their regulatory role, miRNAs have also emerged as potential biomarkers of various processes of the organism, including stress responsivity (Du et al., 2019). Because of their stable presence in biological fluids, the study of these associations in human whole blood, plasma, serum, and saliva has grown exponentially over the last years (Turchinovich and Cho., 2014).

Relatedly, research has suggested that in humans both acute (Beech et al., 2014; Vaisvaser et al., 2016) and chronic (Katsuura et al., 2012; Honda et al., 2013) stress conditions are associated with changes in circulating blood levels of several miRNAs, suggesting their potential involvement in psychological stress reactions and stress-associated processes (Leung and Sharp., 2010). Intriguingly, while these studies have analysed miRNA presence and stress-related modulation in the whole blood (Wiegand et al., 2017), only one study, to the best of our knowledge, has investigated stress-induced miRNA changes in other biological fluids, specifically saliva (Wiegand et al., 2018). In this exploratory study conducted in 24 healthy humans, acute exposure to a laboratory psychosocial stress paradigm (i.e., the Trier Social Stress Test) induced changes in six salivary miRNAs, namely miR-16, miR-20b, miR-21, miR-26b, miR-29a, and miR-134. However, among these, only miR-20b, miR-21, and miR-26b exhibited statistically significant variations compared to baseline (Wiegand et al., 2018). Albeit limited by the small sample size, these results warrant a more in-depth characterization of stress-induced changes in salivary miRNA expression, which may represent a new, interesting avenue in this field of research, given the non-invasive nature and simplicity of saliva collection.

Thus, as only initial evidence is currently available, the primary objective of the present study was to explore the temporal dynamics of candidate salivary miRNAs in response to acute stress in a relatively large sample of healthy participants, with the aim of identifying potential stress-responsive miRNAs. Candidate miRNAs (miR-10a, miR-16, miR-19b, miR-20b, miR-23a, miR-25, miR-26b, miR-29a, miR-126, miR-134, miR-144, miR-183, and miR-200a) were selected based on previous preliminary studies investigating stress-related miRNA expression in saliva and other biological fluids, as well as review articles summarizing miRNAs implicated in acute and chronic stress responses (Hunsberger et al., 2009; Wiegand et al., 2017, 2018; Sárközy et al., 2018; Holliday et al., 2025). Furthermore, none of the existing studies have examined the potential influence of biological sex on miRNA expression under either acute or chronic stress conditions. Therefore, the second objective of the present study was to investigate whether stress-induced changes in salivary miRNA expression differ between women and men. Lastly, we conducted exploratory analyses to examine whether stress-related miRNA changes were associated with key psychophysiological indices of the stress response. To address our objectives, we exposed participants to an ecological, multimodal virtual psychosocial stress test, i.e. the IMVEST test (Rodrigues et al., 2021). IMVEST has recently been validated as an effective protocol for inducing acute stress in laboratory settings. This paradigm incorporates core elements of stress elicitation, including perceived threat to physical self, social-evaluative threat, negative feedback, uncontrollability, and unpredictability (Rodrigues et al., 2021). Relatedly, IMVEST has been shown to reliably induce significant psychophysiological stress responses in healthy individuals (Rodrigues et al., 2021).

2. Material and methods

2.1. Ethics approval and participants

The study was approved by the local Ethics Committee (Comitato Etico dell'Area Vasta Emilia Nord, Emilia-Romagna Region, Italy, under the ID 523/2023/SPER/UNIPR) and all procedures were performed in accordance with the Declaration of Helsinki. Participants were primarily recruited on the university campus and surrounding community, and therefore likely had relatively similar educational backgrounds. All participants provided written informed consent. To be eligible for the study, participants had to be at least 18 years old and report good physical and psychological health. Exclusion criteria included: previous history of psychiatric or cardiovascular disorders, use of medications during the last week, and presence of oral inflammation/infection that could affect salivary analyses. A sample size calculation was performed using G*Power 3.1.9.2 for Windows (Kang, 2021), based on the small effect size found in the previous exploratory study for salivary miR-29a expression changes following acute stress (Wiegand et al., 2018) and the literature suggesting a possible correlation between miR-29a blood levels and parameters of acute psychophysiological response to stress (Vaisvaser et al., 2016). Considering a small effect size ($f = 0.11$), $\alpha = 0.05$, $\beta = 0.20$, the estimated sample size for a repeated measures design including time as a within-subject factor and sex as a between-subject factor was $n = 104$. In this study, we recruited 122 participants, and after exclusion of 11 participants because of missing physiological data, the final sample consisted of 111 healthy subjects (Table 1).

2.2. Procedure

Prior to attending the laboratory session, which took place between 2:00 and 5:00 pm to minimize circadian effects on cortisol levels, participants were asked to abstain from the following activities: drinking alcohol and engaging in vigorous physical exercise for 12 h, smoking and drinking caffeine for 4 h, consuming food or any other drink (except water) for 2 h, and brushing their teeth for 2 h. The study design is illustrated in Fig. 1. Upon arrival, participants completed a series of socio-demographic, lifestyle, and psychometric questionnaires (see 2.4). Then, they were asked to report how stressed they felt in that moment on a Visual Analogue Scale (VAS) ranging from 0 to 100. Subsequently, the first saliva sample was collected using the Passive Drool method (Salimetrics, Cambridge, UK). Participants were then fitted with the Firstbeat Bodyguard 2 device (Firstbeat Technologies, Finland) for continuous recording of R–R intervals throughout the entire protocol. During the first 10 min, participants sat quietly in a comfortable chair while reading simple instructions about the use of virtual reality (VR) controllers. Then, participants were equipped with an Oculus Quest 2 headset and instructed about the upcoming task. After a 2.5-min VR training session, participants underwent a 10-min VR stress exposure (see IMVEST procedure (2.3) for details). Immediately after the VR task, participants provided the second saliva sample and completed the VAS scale measuring perceived stress. Then, participants were given 40 min of

Table 1
Participants' characteristics, overall and stratified by sex.

	Total sample	Men (M)	Women (W)	P value	D-Cohen
N	111	56	55		
Age (years)	24.7 ± 4.6	24.3 ± 5.2	25.1 ± 3.9	0.186	0.17
BMI (kg/m^2)	22.1 ± 3.3	23.2 ± 4.2	21.0 ± 2.4	< .01	0.64
Smokers (%)	31.5	28.6	34.5	0.498	
Self-reported ethnicity	Caucasian (100%)				
PSS (score)	18.1 ± 7.3	17.0 ± 7.3	19.2 ± 7.2	0.054	0.31
STAI-T (score)	45.2 ± 10.8	44.3 ± 10.3	46.0 ± 11.2	0.208	0.16
CES-D (score)	17.5 ± 10.8	17.5 ± 11.9	17.4 ± 11.0	0.483	0.00

Note. Data are reported as means ± standard deviation. Abbreviations: BMI = Body Mass Index; PSS = Perceived Stress Scale, STAI = State-Trait Anxiety Inventory (STAI-T: Trait version), CES-D = Center for Epidemiological Studies Depression Scale.

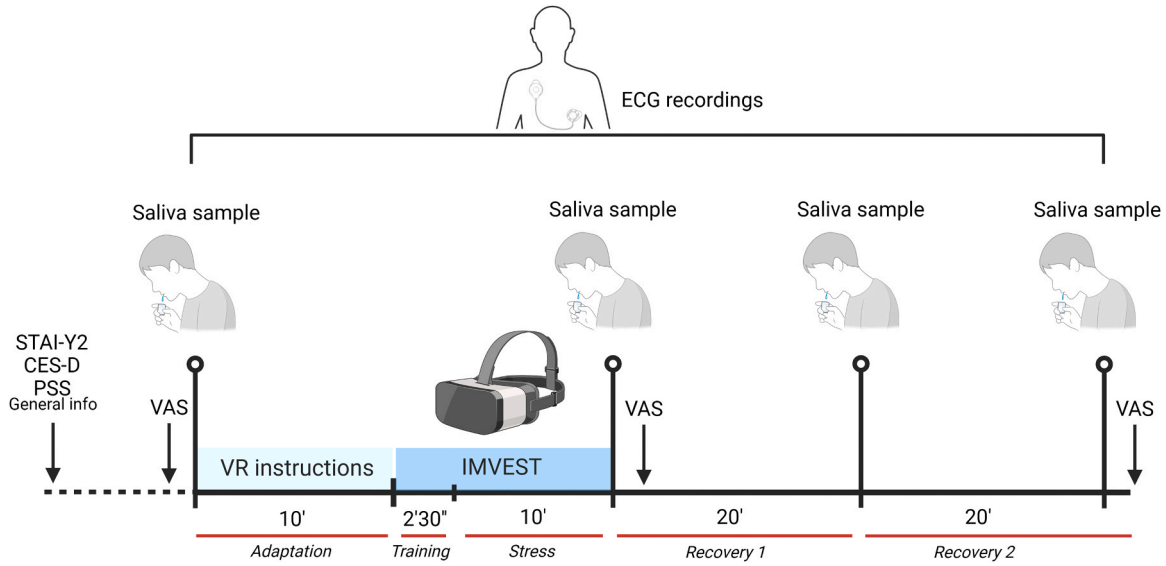


Fig. 1. Timeline of the experimental procedures. Abbreviations: CES-D = Center for Epidemiological Studies Depression Scale; HR = Heart Rate, HRV = Heart Rate Variability; IMVEST = Immersive Multimodal Virtual Environment Stress Test; PSS = Perceived Stress Scale; VAS = Visual Analogue Scale measuring the level of perceived stress from 0 to 100; VR = Virtual Reality. Created in BioRender (https://BioRender.com/zw77mmq).

relaxed sitting to recover. Additional saliva samples were collected after 20 min and at the end of the recovery session. Lastly, participants completed the VAS scale to measure perceived stress and were debriefed.

2.3. IMVEST paradigm

The IMVEST paradigm followed the procedure described in detail by the original study (Rodrigues et al., 2021). Briefly, participants were immersed in a 15 m^2 virtual room with square floor tiles. Initially, a training session was conducted to allow participants to become familiar with the virtual environment, the controller interface, and the arithmetic task. Specifically, they were presented with a series of arithmetic calculations (addition or subtraction) and had to indicate the correctness of the solution using the controller. After this 2.5 min training session, the stress task was initiated. Sonata for 2 Pianos and Percussion (Assai lento) by Béla Bartók was played during the stress induction condition to create a restless atmosphere due to its high arousal and negative valence properties (Russo et al., 2013). In addition, participants had 2.5 s to indicate the correctness of the displayed solution for each arithmetic calculation. The difficulty of the arithmetic task was continuously and dynamically adjusted to the individual's ongoing performance to increase the level of difficulty of the task. Incorrect answers or failure to respond in time could result in the "explosion" of a floor tile, accompanied by a variety of alarming auditory and visual cues. Social-evaluative threat was induced by showing participants an artificial "average participant score" that was always higher than their own. To introduce an element of unpredictability, there was a 5% chance that a correct answer would be evaluated as incorrect. The stress-inducing scenario was divided into four 2.5-min segments. Following each segment, an information message was displayed stating that their performance was below the average, and they were encouraged to enhance their efforts. All these different elements of the IMVEST procedure are designed to induce cognitive, social, sensory and physical stress, as multimodal stressors have more efficacy compared to unimodal ones (Maras et al., 2014).

2.4. Psychometric questionnaires

The trait version of the State-Trait Anxiety Inventory (STAI-T, trait version) (Spielberger, 1983) was used to assess the severity of trait anxiety. The STAI-T is a 4-point Likert scale consisting of 20 items assessing how individuals generally feel, independent of situational factors (e.g., "I feel secure," I feel troubled"). In this study, responses showed excellent internal consistency (Cronbach's $\alpha = 0.92$).

The Perceived Stress Scale (PSS) was used for measuring the perception of stress over the last month (Cohen et al., 1983). Items ask how unpredictable, uncontrollable, and overloaded respondents have found their lives during the last month. Scores ranging from 14 to 26 are considered "moderate perceived stress", those ranging from 27 to 40 are considered "high perceived stress" (Cohen et al., 1983). In this study, responses showed excellent internal consistency (Cronbach's $\alpha = 0.88$).

The Center for Epidemiological Studies Depression Scale (CES-D) is a 20-item self-report scale designed to measure depressive symptomatology during the last week in the general population (Radloff, 1977). Standard cutoffs are > 16 for mild depression and > 23 for clinical depression. In this study, responses showed excellent internal consistency (Cronbach's $\alpha = 0.92$).

2.5. Heart rate and heart rate variability analysis

Raw R-R intervals obtained with the Firstbeat Bodyguard 2 device were analyzed with the Kubios HRV software (Tarvainen et al., 2014) using a medium filter for threshold-based artefact correction (Alcantara et al., 2020). Separate estimates of heart rate (HR, reported in beats per minute) and heart rate variability (HRV) were generated. The root mean square of successive beat-to-beat interval differences (RMSSD, ms) was used as a vagally-mediated index of HRV (Laborde et al., 2017). RMSSD is less susceptible to respiratory and movement artifacts compared to the alternative frequency-domain high-frequency component (Penttilä et al., 2001). The entire recordings were categorized into four phases: Adaptation (10 min), Stress (10 min), Recovery 1 (20 min), and Recovery 2 (20 min) (Fig. 1).

2.6. Salivary analysis

To obtain sufficient material for cortisol and miRNA analyses, 0.8 ml of saliva were collected by passive drool at each assessment point (Fig. 1). Subsequently, two aliquots were prepared: the first, containing 0.2 ml of saliva, for cortisol analysis, and the second, containing 0.6 ml of saliva, for miRNA analysis.

2.6.1. Cortisol analysis

Saliva samples for cortisol analysis were frozen at −20°C. On the day of the analysis, samples were thawed, brought to room temperature and centrifuged (1500 g × 10 min), resulting in a clear supernatant of low viscosity. Salivary cortisol levels were determined by enzyme-linked immunosorbent assay (High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit: Salimetrics LLC, State College, PA). Samples were assayed in duplicates following kit instructions with a 96-well plate, using the BioTek 800 TS absorbance reader and Gen5 software (BioTek Instruments Inc., Vermont, USA). The inter-assay and intra-assay coefficients of variability were 5.9 and 8.4, respectively.

2.6.2. miRNA analysis

Saliva samples for miRNAs analysis were collected without the addition of RNA stabilizers, following procedures used in previous salivary miRNA studies (e.g., Wiegand et al., 2018). Immediately after collection, samples were frozen at −80°C until biochemical analyses to minimize RNA degradation. For miRNAs analysis, samples were thawed at room temperature and total RNA was extracted from 200 μL of saliva using the miRNeasy Serum/Plasma Advanced Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. Total RNA, including miRNA, was eluted from the RNeasy UCP MinElute spin column using 20 μL of RNase-free water. The synthetic spike-in miRNAs UniSp2 and UniSp4 were included in the RNA isolation procedure. The miRCURY LNA Reverse Transcription Kit (QIAGEN) was immediately used for the cDNA synthesis, and the obtained cDNA was stored at −20 °C until further analysis. Following RNA extraction, samples were not refrozen, as reverse transcription to cDNA was performed immediately. The expression levels of candidate miRNAs (miR-10a, miR-16, miR-19b, miR-20b, miR-23a, miR-25, miR-26b, miR-29a, miR-126, miR-134, miR-144, miR-183, and miR-200a) were quantified by RT-qPCR using the miRCURY LNA miRNA Custom PCR Panels (QIAGEN), with the synthetic miRNAs UniSp6 and UniSp-39–3p used as retrotranscription and RT-qPCR positive controls. Next, 4 μL of cDNA template (diluted 1:40), 0.95 μL of RNase-free water and 5.05 μL of 2X miRCURY SYBR Green Master Mix, supplemented with the ROX Reference Dye, were added into the PCR plate for each sample. Real-time PCR was performed on the QuantStudio3 system (Applied Biosystem, Thermo Fisher Scientific, USA), with the following amplification conditions: 95 °C for 2 min, followed by 40 amplification cycles at 95 °C for 10 s and 56 °C for 1 min.

To normalize expression levels, miR-16 was selected as the housekeeping control based on literature suggesting that it may be the most suitable internal control miRNA for saliva-based studies (Sullivan et al., 2022). This was confirmed by our analysis using GeneGlobe software (QIAGEN, Hilden, Germany), which revealed that it was the most stable miRNA across all time points among our panel of miRNAs (Stability factor = 0.072). Samples with low expression levels [Ct > 35] were excluded. Subsequently, the relative miRNA expression was quantified using the $2^{-\Delta Ct}$ method (Zalewski and Bogucka-Kocka., 2025). For each sample, ΔCt values were obtained by subtracting the Ct of the housekeeping miR-16 from the Ct of the target miRNA, and the relative expression was calculated as $2^{-\Delta Ct}$. In this way, higher $2^{-\Delta Ct}$ values correspond to higher relative miRNA expression.

2.7. Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 29.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p < 0.05$. The normality of distributions was assessed using the Kolmogorov–Smirnov test. Sex differences in the general characteristics of the participants were assessed using t or χ^2 tests. Because the study sample consisted of relatively homogeneous young adults with normal weight and a narrow age range, for the main analyses we used repeated measures ANOVAs with "time" as the within-subject factor (four levels: adaptation, post-stress, recovery 1, recovery 2) and "sex" as the between-subject factor for physiological variables (cortisol, HR and HRV), VAS scores, and miRNA levels. Pre-planned contrasts using *t*-tests were conducted to examine differences between each post-stress assessment point and the adaptation phase, as well as sex differences at each assessment point. Because these contrasts were defined a priori within the repeated-measures design, no formal adjustment for multiple comparisons was applied in order to avoid increasing the risk of Type II errors (Rothman, 1990). For all parameters, stress responses were also evaluated by calculating the area under the curve with respect to baseline (i.e., adaptation, AUC_b), and sex differences in AUC_b values were assessed using *t*-tests. Lastly, Pearson's correlation coefficients were computed between AUC_b values and psychological characteristics. Additional analyses were conducted including age and body mass index (BMI) as covariates, and the overall pattern of results remained unchanged.

3. Results

3.1. Participant characteristics and psychometric scores

There were no discrepancies between self-reported biological sex and gender identity among the participants of this study. Therefore, current results are interpreted in terms of sex differences. Men and women of this sample had similar mean age and men had significantly greater BMI (Table 1). Women reported marginally significant greater levels of stress than men (PSS scores), with a small effect size. Moreover, no significant sex differences emerged in terms of trait anxiety (STAI scores) and depressive symptomatology (CES-D scores) (please refer to Table 1 for statistical details).

3.2. Psychophysiological responses to IMVEST

3.2.1. Subjective stress (VAS scale)

Repeated-measures ANOVA revealed a significant main effect of "time" ($F_{2218} = 89.3$, $p < 0.001$) and a significant "time × sex" interaction ($F_{2218} = 5.1$, $p = 0.007$). Specifically, participants reported significantly greater levels of stress just after the end of the IMVEST protocol compared to adaptation and recovery ($p < 0.05$ for all) (Fig. 2A, left panel). Moreover, women reported higher levels of perceived stress at each assessment point compared to men (adaptation: $p = 0.023$; post-stress: $p < 0.001$; recovery: $p = 0.006$) (Fig. 2A, middle panel). Also, women exhibited significantly greater AUC_b values compared with men, with a medium effect size ($t = 2.59$, $p = 0.005$, $d = 0.492$) (Fig. 2A, right panel).

3.2.2. Salivary cortisol

Repeated-measures ANOVA revealed a significant main effect of "time" ($F_{3327} = 6.3$, $p < 0.001$). Specifically, cortisol levels were significantly higher during recovery 1 (i.e., 20 min after the end of the stress, where peak levels of cortisol were expected) compared with the adaptation phase ($p = 0.048$). No significant sex differences emerged either in temporal changes across the protocol (Fig. 2B, middle panel) or in total cortisol variation (AUC_b; $t = –1.19$, $p = 0.119$, $d = –0.225$; Fig. 2B, right panel).

3.2.3. Heart rate

Repeated-measures ANOVA revealed a significant main effect of "time" ($F_{3309} = 304.7$, $p < 0.001$). As expected, HR was significantly higher during the stress phase compared with the adaptation period ($p < 0.001$) (Fig. 2C left panel). No significant sex differences emerged either in the temporal changes across the protocol (Fig. 2C, middle panel) or in total HR variation (AUC_b; $t = –1.19$, $p = 0.119$, $d = –0.225$; Fig. 2C, right panel).

3.2.4. Heart rate variability

Repeated-measures ANOVA revealed a significant main effect of "time" ($F_{3309} = 121.3$, $p < 0.001$). As expected, vagally-mediated HRV

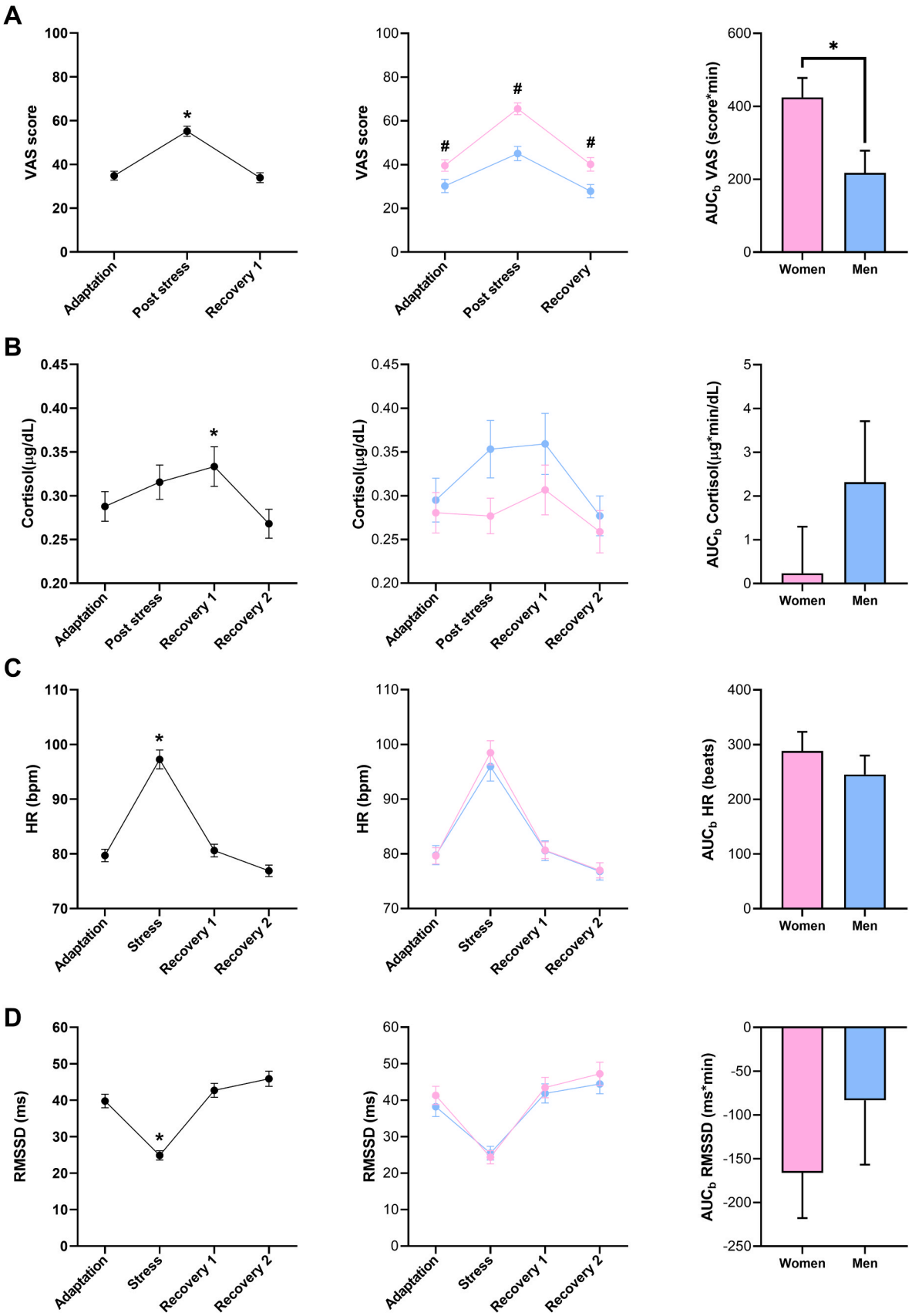


Fig. 2. Psychophysiological responses to the IMVEST paradigm in the whole population (left panels), stratified by sex (middle panels), and expressed as area under the curve with respect to adaptation (AUC_b, right panels). Abbreviations: IMVEST = Immersive Multimodal Virtual Environment Stress Test; HR = Heart Rate; RMSSD = Root Mean Square of Successive beat-to-beat Differences; VAS = Visual Analogue Scale. Data are expressed as means ± standard errors. * $p < 0.05$ vs adaptation; # $p < 0.05$ men vs women.

(RMSSD index) was significantly lower during the stress phase compared with the adaptation period ($p < 0.001$, Fig. 2D, left panel). No sex differences were observed in the temporal changes across the protocol (Fig. 2D, middle panel) or overall vagally-mediated HRV reduction (AUC_b: $t = -0.931$, $p = 0.177$, $d = -0.180$, Fig. 2D, right panel).

3.3. Detection of salivary miRNAs

Table 2 reports the percentage of individuals in whom salivary candidate miRNAs were detectable. Several candidate miRNAs were detectable in the whole population (miR-16, miR-19b, miR-21, miR-23a, miR-25, miR-26b, and miR-29a; miR-200a), some (miR-126, miR-144, and miR-183) were detectable only in a subset of participants, while others (miR-10a, miR-20, and miR-134) were not detectable.

3.4. Changes in miRNA expression across the IMVEST protocol

Results of repeated measures ANOVAs are summarized in Table 3 (effect of "time") and Table 4 (effect of "sex"). For each miRNA investigated, we did not find any significant "time x sex" interaction.

3.4.1. Changes over time and between sexes in miRNAs detectable in all individuals

miR-21 expression levels showed a significant decrease at all three post-stress time points compared to the adaptation phase (Fig. 3 A, left panel). Pre-planned analyses revealed that men had significantly higher miR-21 expression levels during the adaptation phase compared to women (Fig. 3 A, middle panel). Consistently, men showed a significantly larger reduction of miR-21 expression after stress compared with women, with a small-to-medium effect size (AUC_b: $t = 1.84$, $p = 0.035$, $d = 0.35$; Fig. 3 A, right panel).

miR-29a expression levels were significantly lower at the post-stress and recovery 2 assessments compared to the adaptation phase (Fig. 3B, left panel). Also, men showed significantly higher miR-29a expression levels than women during the adaptation phase, recovery 1, and recovery 2 (Fig. 3B, middle panel), but no significant sex differences were found in the overall reduction of miR-29a expression levels (AUC_b: $t = 0.68$, $p = 0.25$, $d = 0.13$; Fig. 3B, right panel).

miR-200a expression levels also decreased significantly at the post-stress and recovery 2 assessments relative to the adaptation phase (Fig. 3 C, left panel), with no sex differences (AUC_b: $t = 1.27$, $p = 0.104$, $d = 0.33$; Fig. 3 C, middle and right panels).

miR-26b expression levels were significantly reduced at recovery 2 compared to adaptation (Fig. 4B, right panel). However, when we stratified by sex, we observed a significant overall reduction in miR-26b expression levels only in men (AUC_b: $t = 2.06$, $p = 0.021$, $d = 0.39$ vs women; Fig. 4B, right panel).

Table 2
Percentage of individuals in whom salivary target miRNAs were detectable in the current sample.

Target miRNA	designation	(% of participants)
miR-16*	hsa-miR-16–5p	100%
miR-19b	hsa-miR-19b-3p	100%
miR-21	hsa-miR-21–5p	100%
miR-23a	hsa-miR-23a-3p	100%
miR-25	hsa-miR-25–3p	100%
miR-26b	hsa-miR-26b-5p	100%
miR-29a	hsa-miR-29a-3p	100%
miR-200a	hsa-miR-200a-3p	100%
miR-126	hsa-miR-126–3p	50%
miR-144	hsa-miR-144–3p	38%
miR-183	hsa-miR-183–5p	56%
miR-10a	hsa-miR-10a-5p	0%
miR-20	hsa-miR-20b-5p	0%
miR-134	hsa-miR-134–5p	0%

Note. * miR-16 was used as housekeeping miRNA.

Table 3
Main effect of "time" for repeated measure ANOVA and pre-planned comparisons for candidate miRNAs.

	Main effect of time	Stress vs adapt.	Rec1 vs adapt.	Rec2 vs adapt.
miR-19b	$F_{(3330)} = 0.9$, $p = 0.432$	$p = 1$	$p = 1$	$p = 1$
miR-21	$F_{(3330)} = 9.1$, $p < 0.001$	$p = 0.002$	$p = 0.009$	$p = 0.004$
miR-23a	$F_{(3330)} = 0.5$, $p = 0.653$	$p = 1$	$p = 1$	$p = 1$
miR-25	$F_{(3330)} = 6.5$, $p < 0.001$	$p = 0.583$	$p = 0.781$	$p < 0.001$
miR-26b	$F_{(3330)} = 2.4$, $p = 0.071$	$p = 1$	$p = 1$	$p = 0.005$
miR-29a	$F_{(3330)} = 6.1$, $p < 0.001$	$p < 0.001$	$p = 0.083$	$p = 0.017$
miR-126	$F_{(3162)} = 0.1$, $p = 0.96$	$p = 0.65$	$p = 0.613$	$p = 0.836$
miR-144	$F_{(3120)} = 3.1$, $p = 0.03$	$p = 1$	$p = 1$	$p = 0.040$
miR-183	$F_{(3183)} = 3.9$, $p = 0.01$	$p = 0.003$	$p = 0.028$	$p = 0.010$
miR-200a	$F_{(3330)} = 5.0$, $p = 0.002$	$p < 0.001$	$p = 0.324$	$p = 0.025$

miR-25 expression levels increased significantly at recovery 2 compared to the adaptation phase (Fig. 4 A, left panel), with no sex differences (AUC_b: $t = 0.41$, $p = 0.34$, $d = -0.08$; Fig. 4 A, middle and right panels).

miR-19b expression levels showed no significant variation across phases (Fig. 4 C, left panel) or between sexes (Fig. 4 C, middle and right panels).

miR-23a expression levels showed no significant variation across phases (Fig. 4D, left panel), but were consistently higher in men during adaptation, post-stress, and recovery 2 compared to women (Fig. 4D, middle panel). However, no significant differences emerged in overall miR-23a expression variation from adaptation (AUC_b: $t = -0.57$, $p = 0.28$, $d = -0.10$; Fig. 4D, right panel).

3.4.2. Changes over time and between sexes in miRNAs that were not detectable in all individuals

miR-126 expression levels did not show significant variations over time in those participants in whom it was detectable ($n = 55$) (Fig. 5 A, left panel). However, men ($n = 32$) exhibited significantly higher expression levels during adaptation compared with women ($n = 23$) (Fig. 5 A, middle panel). Consequently, AUC_b values were significantly lower in men compared with women ($t = 1.76$, $p = 0.042$, $d = 0.48$; Fig. 5 A, right panel).

miR-144 expression levels displayed a significant decrease at recovery 2 compared to adaptation in those participants in whom it was detectable ($n = 42$) (Table 3; Fig. 5B, left panel), without any sex differences.

miR-183 expression levels exhibited a significant reduction at all assessment points compared with adaptation in those participants in whom it was detectable ($n = 62$) (Fig. 5 C, left panel). Also, men ($n = 26$) showed significantly greater miR-183 expression levels than women ($n = 36$) during adaptation, post-stress, and recovery 2 (Table 4), and a significantly larger reduction in miR-183 expression levels after stress (AUC_b: $t = 2.15$, $p = 0.018$, $d = 0.55$; Fig. 5 C, right panel).

3.5. Partial correlations between stress-related miRNA and psychophysiological changes and psychological characteristics

No significant partial correlations (correcting for age and BMI) were found between stress-related miRNA changes (AUC_b values) and psychometric characteristics in the overall population or after stratification

Table 4
Main effect of "sex" for repeated measure ANOVA and pre-planned comparisons for candidate miRNAs.

		Men vs Women			
	Main effect of sex	Adaptation	Stress	Recovery 1	Recovery 2
miR-19b	$F_{(1109)} = 1.2$, $p = 0.278$	$p = 0.63$	$p = 0.11$	$p = 0.43$	$p = 0.59$
miR-21	$F_{(1109)} = 4.1$, $p < 0.001$	$p = 0.033$	$p = 0.280$	$p = 0.240$	$p = 0.067$
miR-23a	$F_{(1109)} = 8.9$, $p = 0.004$	$p = 0.016$	$p = 0.006$	$p = 0.160$	$p = 0.003$
miR-25	$F_{(1109)} = 0.2$, $p = 0.624$	$p = 0.658$	$p = 0.929$	$p = 0.927$	$p = 0.391$
miR-26b	$F_{(1109)} = 0.1$, $p = 0.804$	$p = 0.893$	$p = 0.956$	$p = 0.367$	$p = 0.966$
miR-29a	$F_{(1109)} = 5.8$, $p = 0.017$	$p = 0.033$	$p = 0.128$	$p = 0.017$	$p = 0.004$
miR-126	$F_{(1,53)} = 0.2$, $p = 0.635$	$p = 0.042$	$p = 0.725$	$p = 0.695$	$p = 0.494$
miR-144	$F_{(1,40)} = 5.9$, $p = 0.019$	$p = 0.070$	$p = 0.058$	$p = 0.103$	$p = 0.192$
miR-183	$F_{(1,60)} = 3.9$, $p = 0.002$	$p < 0.001$	$p = 0.005$	$p = 0.063$	$p = 0.020$
miR-200a	$F_{(1109)} = 0.2$, $p = 0.893$	$p = 0.472$	$p = 0.514$	$p = 0.713$	$p = 0.843$

by sex. Also, we did not find significant partial correlations between stress-related miRNA changes (AUC_b values) and psychophysiological (perceived stress, cortisol levels, HR and HRV) responses to IMVEST (AUC_b values).

4. Discussion

In this study, we were able to identify several miRNAs that were reliably detectable from the saliva of healthy adults of both sexes. Among these, miR-21, miR-25, miR-26b, miR-29a, miR-200a, showed significant changes in their expression levels after exposure to an immersive virtual acute stress paradigm, which induced clear psychophysiological stress responses. Also, significant sex differences in expression levels and/or stress responses were found for miR-21, miR-23a, miR-26b, and miR-29a. These results suggest that these miRNAs may represent promising candidate salivary markers associated with stress-related biological responses, with potential sex-related differences. On the other hand, other candidate miRNAs (miR-126, miR-144, and miR-183) showed significant changes after stress exposure and/or between sexes, but were detectable only in some participants, thus questioning their utility as reliable salivary biomarkers.

In this study, we exposed participants to an immersive acute stress protocol, i.e. the IMVEST task (Rodrigues et al., 2021). Our results confirmed previous results showing that the IMVEST protocol effectively elicites a psychophysiological stress response (Rodrigues et al., 2021), evidenced in this sample by increased subjective perception of stress (to a larger extent in women), elevated heart rate and reduced vagally-mediated HRV during stress exposure, as well as elevation in salivary cortisol levels 20 min after the end of the stress protocol. Although the stress-induced increase in cortisol levels was relatively modest, it was consistent with the expected temporal dynamics of cortisol responses to acute stress (Pattini et al., 2019; Carnevali et al., 2020). Moreover, testing sessions were conducted in the afternoon, when cortisol reactivity is typically attenuated compared with morning sessions due to circadian regulation. Therefore, the magnitude of the cortisol response observed here is in line with previous studies using moderate psychosocial stress paradigms in controlled laboratory settings (Pattini et al., 2019; Carnevali et al., 2020). Interestingly, although female participants reported higher levels of perceived stress on the VAS scales at the subjective level, this was not reflected at the cardiac and neuroendocrine level.

Encouraged by these results, the main objectives of the current study were to investigate the time course of changes in the expression levels of salivary miRNAs in response to an acute stressor and to explore potential sex differences. We selected a panel of candidate miRNAs (miR-10a, miR-16, miR-19b, miR-20b, miR-23a, miR-25, miR-26b, miR-29a, miR-126, miR-134, miR-144, miR-183, and miR-200a) based on previous studies in saliva and other biological fluids, and their previous association with acute and chronic stress responses (Hunsberger et al., 2009; Wiegand et al., 2017, 2018; Sárközy et al., 2018; Holliday et al., 2025).

Among the salivary miRNAs that were detectable in all participants in the present exploratory study, miR-21 emerged as one of the most promising candidate stress-responsive miRNAs. Specifically, we found a significant decrease in miR-21 expression levels immediately after stress exposure and throughout the recovery phase. This reduction was larger in men, likely due to the fact that men showed higher expression levels prior to stress exposure. Our findings are consistent with studies reporting reduced salivary (Wiegand et al., 2018) and circulating (Gidron et al., 2010) miR-21 expression levels following psychological stress exposure in healthy participants. Conversely, another study found increased miR-21 expression levels in whole blood after acute stress induction (Beech et al., 2014), but the sample consisted exclusively of heavy drinkers and individuals with major depressive disorder, which may explain the inconsistency with the results obtained from salivary samples of healthy and non-alcoholic participants. Interestingly, a study found that adrenaline can downregulate miR-21 expression, suggesting a direct link between stress hormones and miR-21 downregulation (Chen and Wang, 2017). To our knowledge, our study is the first to report that this reduction may indeed be sex specific. Although there is limited evidence on sex differences in miR-21, one study found that estradiol upregulates miR-21 via ERα-dependent mechanisms, suggesting that hormonal status may influence miR-21 expression levels (Bhat-Nakshatri et al., 2009). Interestingly, miR-21, one of the most abundant and highly conserved miRNAs, has been frequently claimed to be a specific predictive or prognostic biomarker of diseases such as cancer and heart disease in bodily fluid-based miRNA studies (Jenike and Halushka, 2021). In a recent review, the authors claimed that ". *miR-21 cannot be considered a specific biomarker for any disease if it is a biomarker of many diseases.*" (page 7) and that "*While its levels may genuinely vary across bodily fluids in disease states, these variations have no specificity.*" (page 7) (Jenike and Halushka, 2021). We agree with the points raised in this review; however, we propose that patients affected by these diverse conditions may share a common underlying factor, that is elevated stress levels. Therefore, our findings support the idea that miR-21 may represent one of the most promising candidate markers associated with stress-related biological processes.

Similar to miR-21, the expression levels of miR-29a and miR-200a were significantly reduced immediately after stress exposure and, with less consistency, during the recovery period. This reduction was similar between men and women, even though men showed higher expression levels of miR-29a across the experimental protocol. Previous literature on changes in miR-29a expression in response to stress is inconsistent: a previous study reported no significant changes in saliva (Wiegand et al., 2018), but this study was conducted in a small human sample ($n = 24$), while another study observed increased miR-29a levels in whole blood one-to-two hours after stress induction (Vaisvaser et al., 2016). miR-200a has been linked to early-life stress, cardiovascular disorders, and psychiatric disorders such as major depressive disorder and schizophrenia (Magenta et al., 2017; Allen and Dwivedi, 2020), and our study is the first to document changes in its expression levels in saliva in response to an acute stressor.

Another salivary miRNA that was detectable in all participants and

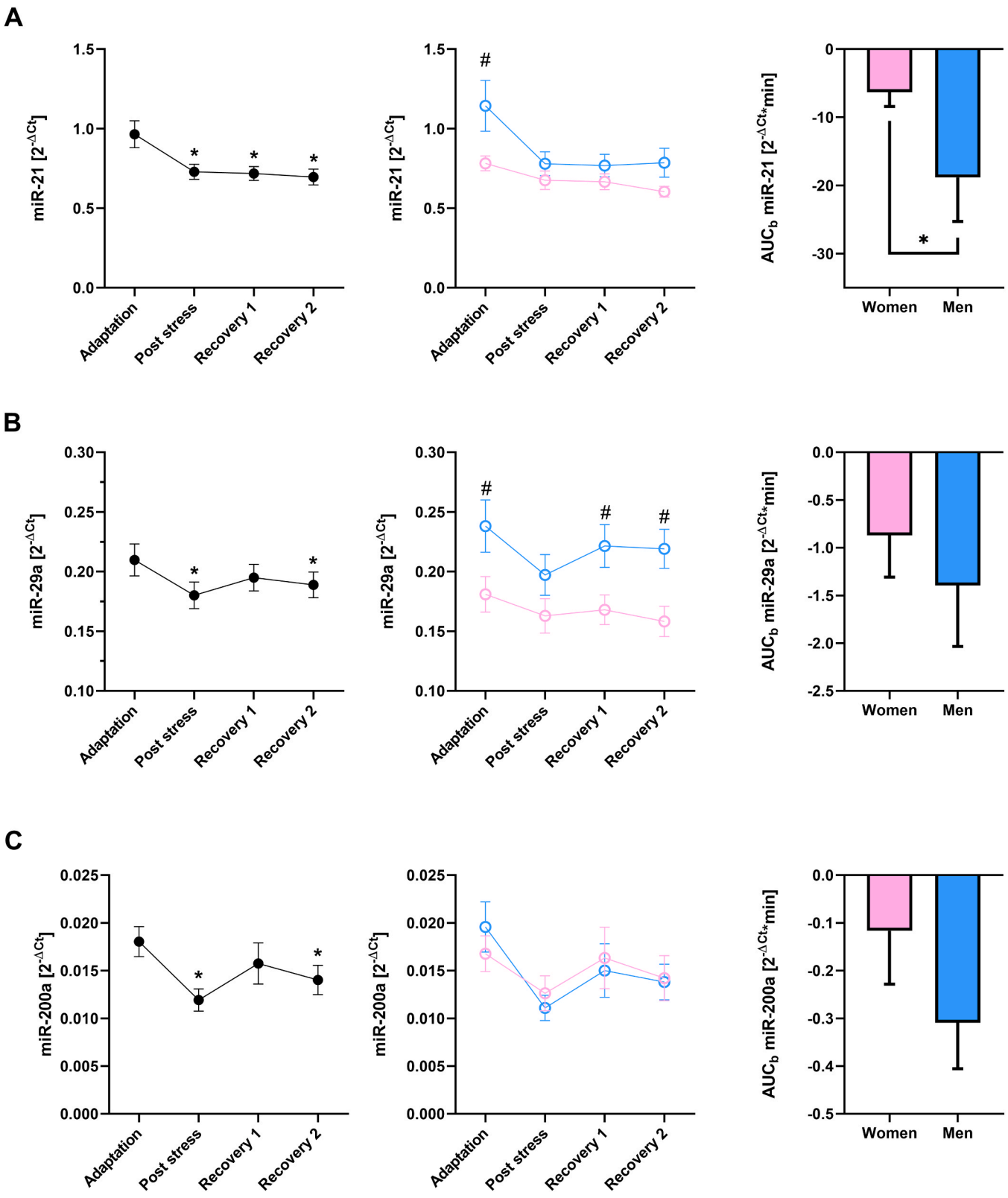


Fig. 3. miRNAs detectable in all individuals (part 1). Changes in miRNA expression levels over time in the whole population (left panels), stratified by sex (middle panels), and expressed as area under the curve with respect to adaptation (AUC_b, right panels). Data are expressed as means ± standard errors. * $p < 0.05$ vs adaptation; # $p < 0.05$ men vs women.

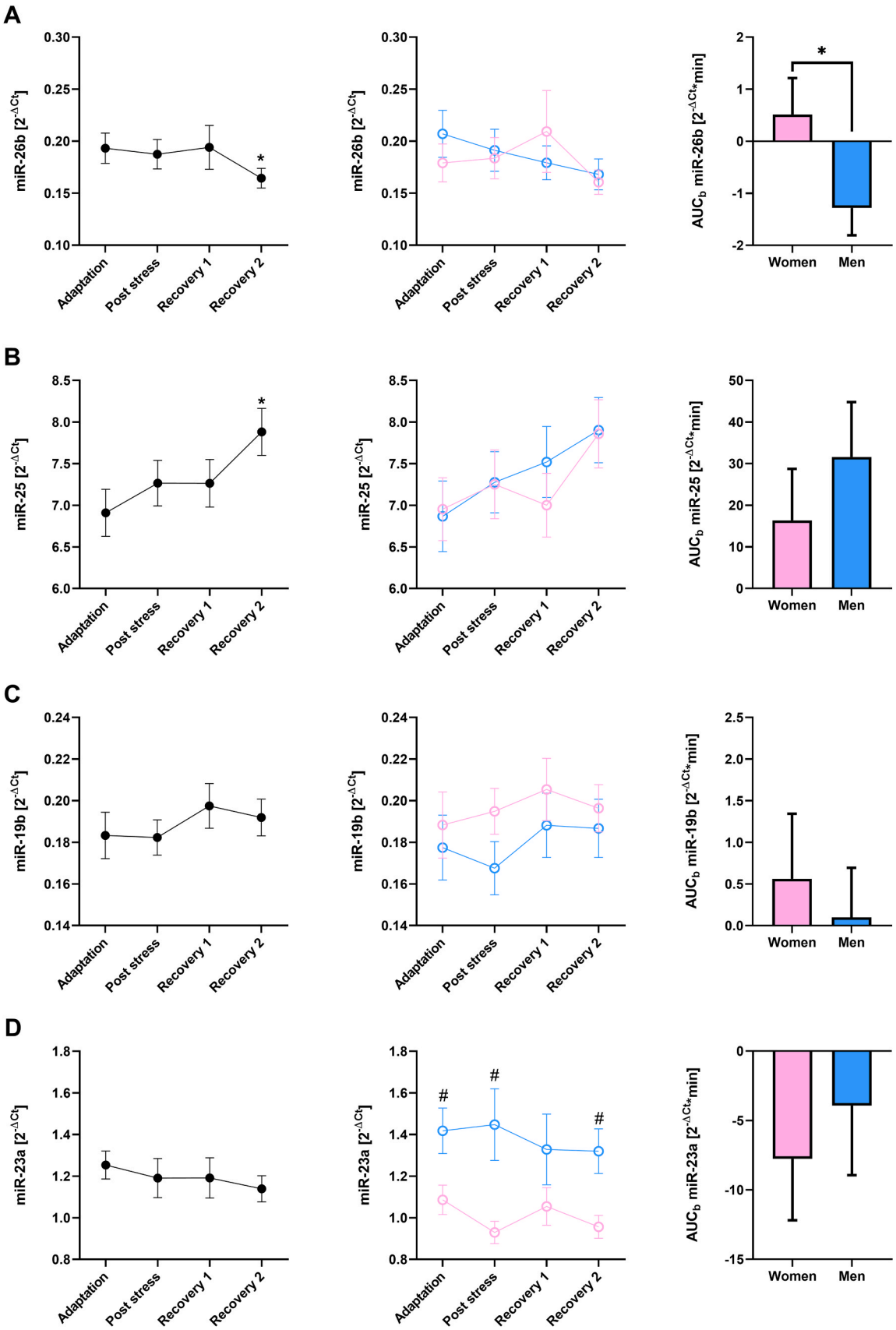


Fig. 4. miRNAs detectable in all individuals (part 2). Changes in miRNA expression levels over time in the whole population (left panels), stratified by sex (middle panels), and expressed as area under the curve with respect to adaptation (AUC_b, right panels). Data are expressed as means ± standard errors. * $p < 0.05$ vs adaptation; # $p < 0.05$ men vs women.

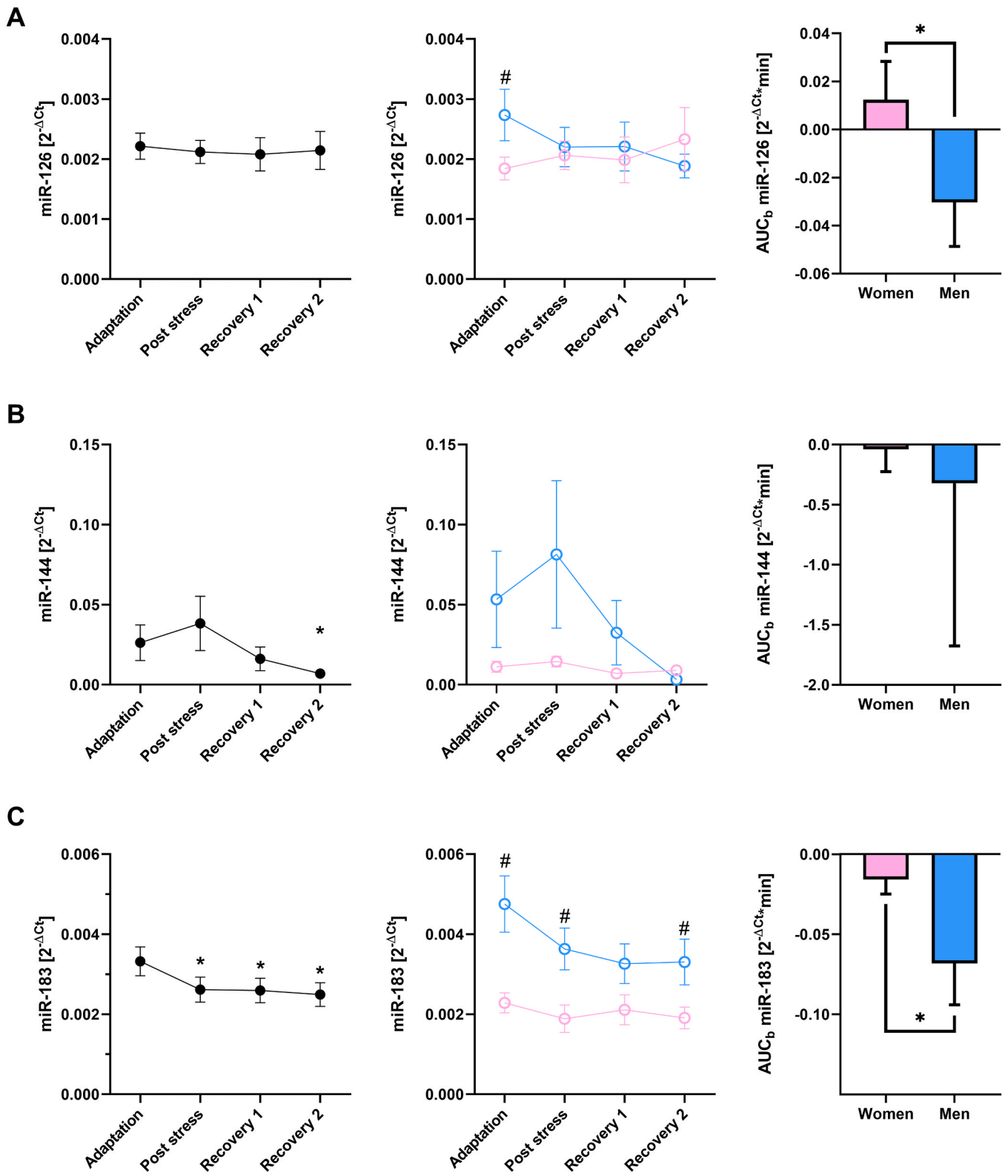


Fig. 5. miRNAs not detectable in all individuals. Changes in miRNA expression levels over time in the whole population (left panels), stratified by sex (middle panels), and expressed as area under the curve with respect to adaptation (AUC_b, right panels). Data are expressed as means ± standard errors. * $p < 0.05$ vs adaptation; # $p < 0.05$ men vs women.

showed significantly increased expression 40 min after stress exposure was miR-25. miR-25 plays a key role in the development of cardiac hypertrophy and heart failure under pathophysiological conditions (Sárközy et al., 2018). Under normal conditions, many miR-25 target mRNAs are involved in biological processes such as DNA damage response, cell cycle regulation, proliferation, and differentiation. Our results are the first to suggest that salivary miR-25 expression in healthy individuals might be influenced by acute stress exposure.

A similar temporal dynamic but in a different direction (i.e., reduction) was observed for changes in miR-26b expression levels after stress exposure in the whole population. Interestingly, when we stratified by sex, we found an overall increased expression across the protocol in women and an overall reduction in men, suggesting a possible sex-specific regulation. A previous study in a small group of healthy men and women (n = 24) reported an increase in miR-26b salivary expression immediately after stress, followed by a decline during recovery (Wiegand et al., 2018). Another study in a small sample of male and female medical students (n = 10) found higher miR-26b levels in whole blood before examination stress (Katsuura et al., 2012). However, these studies did not explore potential sex differences. Thus, our results warrant further investigation into sex differences in salivary miR-26b expression changes as a function of stress exposure.

Two miRNAs (miR-19b, miR-23a) were detectable from the saliva of all participants but did not show significant changes during the experimental protocol. Previous studies found reduced salivary miR-19b expression levels in a small (n = 4) group of individuals categorized as having high stress compared with low-stress individuals (Holliday et al., 2025), and associated miR-19b expression with PTSD onset in women but not in men (Linnstaedt et al., 2020).

In the current study, three candidate miRNAs (miR-126, miR-144, and miR-183) were not detectable in all participants, confirming previous preliminary results (Wiegand et al., 2018). Although changes over time and/or sex differences were found, current and past results suggest that these miRNAs are either absent or expressed at very low levels in saliva, making their utility as candidate salivary biomarkers of stress questionable.

4.1. Limitations

Some limitations should be acknowledged. First, we did not observe significant associations between miRNA expression levels and participants' psychological characteristics or physiological responses to stress. In addition, we did not assess participants' history of early life stress or lifetime trauma exposure, factors that may influence individual responses to acute stress. However, the study was conducted in a sample of healthy young adults with relatively low levels of stress, anxiety, and depressive symptoms. It is also possible that the use of area-under-the-curve measures, which reflect overall stress responses, did not capture stress-induced peak changes that may follow different temporal dynamics. Future studies using phase-specific or time-lagged analyses may better elucidate potential associations between miRNA expression changes and physiological stress responses. Also, saliva samples were collected without the use of RNA stabilizers, which may represent a potential source of variability in salivary miRNA measurements. Additionally, the interpretation of sex-related differences should be approached with caution, and future studies should incorporate direct measures of sex hormones and menstrual cycle phase to clarify hormonal modulation of stress-related miRNA expression. Lastly, the precise biological origin and functional significance of salivary miRNAs are poorly understood. Salivary miRNAs may derive from multiple sources, including passive diffusion or active transport from the circulation, secretion by salivary glands, release from exosomes, and contributions from local epithelial and immune cells within the oral cavity (Park et al., 2009; Michael et al., 2010). Importantly, participants with oral inflammation or infection were excluded from the present study, reducing the likelihood that the observed stress-related miRNA changes primarily reflect local inflammatory processes. Instead, the concomitant activation of autonomic and neuroendocrine stress systems supports the interpretation that these salivary miRNA changes may index biological responses to acute stress.

5. Conclusion

By capitalizing on a multimodal virtual acute stress paradigm to induce a psychophysiological stress response in healthy adults, the present study provides exploratory evidence that the expression of several salivary miRNAs is dynamically modulated following acute stress exposure. Among the miRNAs that were reliably detectable in saliva, miR-21, miR-25, miR-26b, miR-29a, and miR-200a emerged as promising candidate markers of stress-related biological responses, with some showing potential sex-related differences in baseline expression or stress reactivity. These findings highlight saliva as a feasible and non-invasive biological matrix for investigating molecular responses to psychosocial stress in humans. At the same time, the exploratory nature of the present study warrants cautious interpretation, and future research will be needed to replicate these findings in larger and more diverse samples and determine their potential relevance for understanding individual variability in stress responses and stress-related disease vulnerability.

CRediT authorship contribution statement

Luca Carnevali: Writing – review & editing, Funding acquisition, Conceptualization. **Cristina Dallabona:** Writing – review & editing, Data curation. **Andrea Sgoifo:** Writing – review & editing, Funding acquisition. **Giuseppina Porciello:** Writing – review & editing, Software, Data curation. **Luca Provenzano:** Writing – review & editing, Software. **Andrea Salaris:** Writing – review & editing, Software, Data curation. **Margherita Barbetti:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Maria Carla Gerra:** Writing – review & editing, Data curation. **Sebastiano Ravenda:** Writing – original draft, Investigation, Formal analysis, Data curation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Alcantara, J.M.A., Plaza-Florido, A., Amaro-Gahete, F.J., et al., 2020. Impact of using different levels of threshold-based artefact correction on the quantification of heart rate variability in three independent human cohorts. J. Clin. Med 9, 325. https://doi.org/10.3390/jcm9020325.

Allen, L., Dwivedi, Y., 2020. MicroRNA mediators of early life stress vulnerability to depression and suicidal behavior. Mol. Psychiatry 25, 308–320. https://doi.org/10.1038/s41380-019-0597-8.

Bartel, D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116, 281–297. https://doi.org/10.1016/S0092-8674(04)00045-5.

Bartel, D.P., 2009. MicroRNAs: target recognition and regulatory functions. Cell 136, 215–233. https://doi.org/10.1016/j.cell.2009.01.002.

Beech, R.D., Leffert, J.J., Lin, A., et al., 2014. Stress-related alcohol consumption in heavy drinkers correlates with expression of miR-10a, miR-21, and components of the TAR-RNA -binding protein-associated complex. Alcohol Clin. Exp. Res 38, 2743–2753. https://doi.org/10.1111/acer.12549.

Bhat-Nakshatri, P., Wang, G., Collins, N.R., et al., 2009. Estradiol-regulated microRNAs control estradiol response in breast cancer cells. Nucleic Acids Res 37, 4850–4861. https://doi.org/10.1093/nar/gkp500.

Carnevali, L., Pattini, E., Sgoifo, A., Ottaviani, C., 2020. Effects of prefrontal transcranial direct current stimulation on autonomic and neuroendocrine responses to psychosocial stress in healthy humans. Stress 23, 26–36. https://doi.org/10.1080/10253890.2019.1625884.

Chen, D., Wang, Z., 2017. Adrenaline inhibits osteogenesis via repressing miR-21 expression. Cell Biol. Int 41, 8–15. https://doi.org/10.1002/cbin.10685.

Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. J. Health Soc. Behav. 24, 385–396.

Du, J., Li, M., Huang, Q., et al., 2019. The critical role of microRNAs in stress response: Therapeutic prospect and limitation. Pharm. Res 142, 294–302. https://doi.org/10.1016/j.phrs.2018.12.007.
Gidron, Y., De Zwaan, M., Quint, K., Ocker, M., 2010. Influence of stress and health-behaviour on miRNA expression. Mol. Med Rep. 3. https://doi.org/10.3892/mmr_00000279.
Hinterdobler, J., Schunkert, H., Kessler, T., Sager, H.B., 2021. Impact of Acute and Chronic Psychosocial Stress on Vascular Inflammation. Antioxid. Redox Signal 35, 1531–1550. https://doi.org/10.1089/ars.2021.0153.
Holliday, E., Bagasra, A., Bagasra, O., Pandey, P., 2025. Assessing the feasibility of using salivary microRNAs as biomarkers to distinguish between chronic stress and childhood trauma in African American young women in an exploratory pilot study. Front Psychiatry 16. https://doi.org/10.3389/fpsyt.2025.1507064.
Honda, M., Kuwano, Y., Katsuura-Kamano, S., et al., 2013. Chronic Academic Stress Increases a Group of microRNAs in Peripheral Blood. PLoS One 8, e75960. https://doi.org/10.1371/journal.pone.0075960.
Hunsberger, J.G., Austin, D.R., Chen, G., Manji, H.K., 2009. MicroRNAs in mental health: from biological underpinnings to potential therapies. Neuromolecular Med 11, 173–182. https://doi.org/10.1007/s12017-009-8070-5.
James, K.A., Stromin, J.I., Steenkamp, N., Combrinck, M.I., 2023. Understanding the relationships between physiological and psychosocial stress, cortisol and cognition. Front Endocrinol. (Lausanne) 14. https://doi.org/10.3389/fendo.2023.1085950.
Jenike, A.E., Halushka, M.K., 2021. miR-21: a non-specific biomarker of all maladies. Biomark. Res 9, 18. https://doi.org/10.1186/s40364-021-00272-1.
Kang, H., 2021. Sample size determination and power analysis using the G*Power software. J. Educ. Eval. Health Prof. 18, 17. https://doi.org/10.3352/jeehp.2021.18.17.
Katsuura, S., Kuwano, Y., Yamagishi, N., et al., 2012. MicroRNAs miR-144/144* and miR-16 in peripheral blood are potential biomarkers for naturalistic stress in healthy Japanese medical students. Neurosci. Lett. 516, 79–84. https://doi.org/10.1016/j.neulet.2012.03.062.
Kemeny, M.E., Schedlowski, M., 2007. Understanding the interaction between psychosocial stress and immune-related diseases: A stepwise progression. Brain Behav. Immun. 21, 1009–1018. https://doi.org/10.1016/j.bbi.2007.07.010.
Laborde, S., Mosley, E., Thayer, J.F., 2017. Heart Rate Variability and Cardiac Vagal Tone in Psychophysiological Research - Recommendations for Experiment Planning, Data Analysis, and Data Reporting. Front Psychol. 8213. https://doi.org/10.3389/fpsyg.2017.00213.
Leung, A.K.L., Sharp, P.A., 2010. MicroRNA Functions in Stress Responses. Mol. Cell 40, 205–215. https://doi.org/10.1016/j.molcel.2010.09.027.
Linnstaedt, S.D., Rueckeis, C.A., Riker, K.D., et al., 2020. MicroRNA-19b predicts widespread pain and posttraumatic stress symptom risk in a sex-dependent manner following trauma exposure. Pain 161, 47–60. https://doi.org/10.1097/j.pain.0000000000001709.
Magenta, A., Ciarapica, R., Capogrossi, M.C., 2017. The Emerging Role of miR-200 Family in Cardiovascular Diseases. Circ. Res 120, 1399–1402. https://doi.org/10.1161/CIRCRESAHA.116.310274.
Maras, P.M., Molet, J., Chen, Y., et al., 2014. Preferential loss of dorsal-hippocampus synapses underlies memory impairments provoked by short, multimodal stress. Mol. Psychiatry 19, 811–822. https://doi.org/10.1038/mp.2014.12.
McEwen, B.S., 1998. Protective and Damaging Effects of Stress Mediators. N. Engl. J. Med 338, 171–179. https://doi.org/10.1056/NEJM199801153380307.
Michael, A., Bajracharya, S.D., Yuen, P.S., Zhou, H., Star, R.A., Illei, G.G., Alevizos, I., 2010. Exosomes from human saliva as a source of microRNA biomarkers. Oral. Dis. 16 (1), 34–38. https://doi.org/10.1111/j.1601-0825.2009.01604.x.
O'Connor, D.B., Thayer, J.F., Vedhara, K., 2021. Stress and Health: A Review of Psychobiological Processes. Annu Rev. Psychol. 72, 663–688. https://doi.org/10.1146/annurev-psych-062520-122331.
Osborne, M.T., Shin, L.M., Mehta, N.N., et al., 2020. Disentangling the Links Between Psychosocial Stress and Cardiovascular Disease. Circ. Cardiovasc Imaging 13. https://doi.org/10.1161/CIRCIMAGING.120.010931.
Park, N.J., Zhou, H., Elashoff, D., Henson, B.S., Kastratovic, D.A., Abemayor, E., Wong, D.T., 2009. Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. Clin. Cancer Res 15 (17), 5473–5477. https://doi.org/10.1158/1078-0432.CCR-09-0736.
Pattini, E., Carnevali, L., Troisi, A., et al., 2019. Psychological characteristics and physiological reactivity to acute stress in mothers of children with autism spectrum disorder. Stress Health 35, 421–431. https://doi.org/10.1002/smi.2870.
Penttilä, J., Helminen, A., Jartti, T., et al., 2001. Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. Clin. Physiol. 21, 365–376. https://doi.org/10.1046/j.1365-2281.2001.00337.x.
Radloff, L.S., 1977. The CES-D Scale. Appl. Psychol. Meas. 1, 385–401. https://doi.org/10.1177/014662167700100306.
Rodrigues, J., Studer, E., Streuber, S., Sandi, C., 2021. IMVEST, an immersive multimodal virtual environment stress test for humans that adjusts challenge to individual's performance. Neurobiol. Stress 15, 100382. https://doi.org/10.1016/j.ynstr.2021.100382.
Rothman, K.J., 1990. No adjustments are needed for multiple comparisons. Epidemiology 1, 43–46. https://doi.org/10.1097/00001648-199001000-00010.
Russo, F.A., Vempala, N.N., Sandstrom, G.M., 2013. Predicting musically induced emotions from physiological inputs: linear and neural network models. Front Psychol. 4. https://doi.org/10.3389/fpsyg.2013.00468.
Sárközy, M., Kahán, Z., Csont, T., 2018. A myriad of roles of miR-25 in health and disease. Oncotarget 9, 21580–21612. https://doi.org/10.18632/oncotarget.24662.
Slavich, G.M., 2016. Life Stress and Health. Teach. Psychol. 43, 346–355. https://doi.org/10.1177/0098628316662768.
Spielberger, C.D., 1983. Manual for the State-trait Anxiety Inventory (form Y) ("self-evaluation Questionnaire"). Consulting Psychologists Press.
Sullivan, R., Montgomery, A., Scipioni, A., et al., 2022. Confounding factors impacting microRNA expression in human saliva: methodological and biological considerations. Genes (Basel) 13, 1874. https://doi.org/10.3390/genes13101874.
Tarvainen, M.P., Niskanen, J.-P., Lipponen, J.A., et al., 2014. Kubios HRV – Heart rate variability analysis software. Comput. Methods Prog. Biomed. 113, 210–220. https://doi.org/10.1016/j.cmpb.2013.07.024.
Turchinovich, A., Cho, W.C., 2014. The origin, function and diagnostic potential of extracellular microRNA in human body fluids. Front Genet 5. https://doi.org/10.3389/fgene.2014.00030.
Vaisvaser, S., Modai, S., Farberov, L., et al., 2016. Neuro-epigenetic indications of acute stress response in humans: the case of MicroRNA-29c. PLoS One 11, e0146236. https://doi.org/10.1371/journal.pone.0146236.
Vogelzangs, N., Seldenrijk, A., Beekman, A.T.F., et al., 2010. Cardiovascular disease in persons with depressive and anxiety disorders. J. Affect Disord. 125, 241–248. https://doi.org/10.1016/j.jad.2010.02.112.
Wiegand, C., Heusser, P., Klinger, C., et al., 2018. Stress-associated changes in salivary microRNAs can be detected in response to the Trier Social Stress Test: An exploratory study. Sci. Rep. 8, 7112. https://doi.org/10.1038/s41598-018-25554-x.
Wiegand, C., Savelsbergh, A., Heusser, P., 2017. MicroRNAs in psychological stress reactions and their use as stress-associated biomarkers, especially in human saliva. Biomed. Hub. 2, 1–15. https://doi.org/10.1159/000481126.
Yaribeygi, H., Panahi, Y., Sahraei, H., et al., 2017. The impact of stress on body function: A review. EXCLI J. 1057–1072. https://doi.org/10.17179/excli2017-480.
Zalewski, D., Bogucka-Kocka, A., 2025. RQdeltaCT: an open-source R package for relative quantification of gene expression using delta Ct methods. Sci. Rep. 15, 29762. https://doi.org/10.1038/s41598-025-11822-0.