The Transcriptional Signature of a Runner's High

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ABSTRACT

HICKS, S. D., P. JACOB, O. PEREZ, M. BAFFUTO, Z. GAGNON, and F. A. MIDDLETON. The Transcriptional Signature of a Runner's High. *Med. Sci. Sports Exerc.*, Vol. 51, No. 5, pp. 970–978, 2019. **Introduction**: Endorphins, endocannabinoids, monoamines, and neurotrophins have all been implicated in the euphoric response to endurance running, known as a runner's high (RH). The epitranscriptional mechanisms regulating this effect have not been defined. Here, we investigate peripheral micro–ribonucleic acid (miRNA) changes unique to athletes experiencing postrun euphoria, yielding insights into gene networks that control an RH. **Methods**: A cohort study involving 25 collegiate runners (48% females, age = 20 ± 1 yr) examined salivary RNA levels before and after a long-distance run. Participants were divided into RH and nonrunner's high (NRH) groups based on surveys of four criteria (mood, lost sense of time, run quality, and euphoria). Physiological measures were also recorded (temperature, heart rate, blood pressure, pupillary dilatation, and salivary serotonin). Levels of miRNAs and their messenger RNA targets were compared across pre- and postrun samples from RH and NRH groups with two-way ANOVA. Representation of opioid, gamma-aminobutyic acid (GABA), endocannabinoid, neurotrophin, serotonergic, and dopaminergic pathways was assessed in DIANA miRPath. Pearson's correlation analyses examined relationships between miRNAs and RH indices. **Results**: RH participants (n = 13) demonstrated postrun mydriasis (P = 0.046) and hypothermia (P = 0.043) relative to NRH participants (n = 12) but had no difference in serotonin dynamics (P = 0.88). Six miRNAs (miR-194-5p, miR-4676-3p, miR-4254, miR-4425, miR-1273-3p, miR-6743-5p) exhibited significant effects (false discovery rate P value < 0.05) across pre- or postrun and RH/NRH groups. These miRNAs displayed target enrichment for opioid (P = 2.74E−06) and GABA (P = 0.00016) pathways. miR-1237-3p levels were related with lost sense of time (R = 0.40). Mitogen-activated protein kinase (*MAPK11*), an endocannabinoid target of miR-1273-3p, was nominally elevated in RH participants (false discovery rate P value = 0.11). **Conclusions**: Unique dynamics in miRNA concentration occur in athletes with subjective/objective evidence of RH, targeting genes implicated endorphin, endocannabinoid, and GABAergic signaling. **Key Words:** MICRORNA, SALIVA, GENOMICS, EXERCISE, ENDURANCE RUNNING

The term "runner's high" (RH) is commonly used to describe the feeling of euphoria experienced by athletes engaged in endurance running (1–3). This state is clinically characterized by four key components: 1) anxiolysis, 2) analgesia, 3) sedation, and 4) euphoria (4). Runners experiencing a postrun high subjectively report "relaxation," and many describe their running experience as effortless, with a "lost sense of time" (5). The intensity and the duration of exercise appear to be critical factors for RH achievement (6,7).

In this era of increasing depression rates and opioid addiction, advancing our understanding of the mechanisms underlying a natural RH could provide a simple adjunct to behavioral and pharmacological therapy (8,9). Indeed, distance running represents a relatively simple and inexpensive method for addressing disorders of both mood and pain (10,11). Exercise is associated with stress reduction, as well as alleviation of moderate anxiety and depression (12). It has been proposed that running contributes to these positive effects through stimulation of endorphins, endocannabinoids, and other brain-related neurotrophic factors (1–3,7,13).

The endorphin hypothesis suggests that an RH results from enhanced opioid signaling during exercise (12). Endorphins include endogenous neuropeptides formed in the central nervous system that preferentially bind μ-opioid receptors to relieve pain. The endorphin hypothesis is supported by the observation that levels of opioid polypeptides (e.g., β-endorphin) rise in both peripheral blood and cerebrospinal fluid after exertion (14,15). The administration of naloxone (a nonspecific opioid antagonist) has been shown to reverse exercise-induced pupillary miosis, pain perception, and subjective mood improvement (14,16). Recently, Boecker and colleagues (2) used positron emission tomography to demonstrate that opioidergic binding in the frontolimbic cortex of human athletes changes with running and correlates with levels of euphoria.

Impediments to endorphin transport across the blood–brain barrier have led to the alternative theory that endocannabinoid signaling could mediate an RH (4). Endocannabinoid levels increase in both humans and cursorial mammals after high-intensity endurance running but are not altered in either group

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after low-intensity walking (1). An elegant study by Fuss and colleagues (4) demonstrated that endocannabinoid signaling in mice is essential to running-induced anxiolysis and analgesia, and these effects are mediated by cannabinoid receptors on GABAergic neurons.

It is unlikely that a single neuropeptide or neurohormone acts independently in a sufficiently broad manner to facilitate all four components of an RH. A recent study of 11 male cyclists found significant correlations between postexercise levels of plasma anandamide and brain-derived neurotrophic factor, suggesting that brain-derived neurotrophic factor might mediate neuroplasticity and mood (7). Changes in dopamine, norepinephrine, and serotonin (5-HT) have also been associated with exercise in animals (13,17,18). Moreover, descending projections from the locus coeruleus and Raphe nucleus (sources of norepinephrine and serotonin, respectively) are believed to reduce sensitivity to nociceptive inputs, possibly through the action of opioid-expressing spinal cord interneurons, thus forming part of the basis for the effectiveness of selective serotonin or norepinephrine reuptake inhibitors (SNRIs) in treating chronic neuropathic pain (19).

Neurohormonal responses to running are determined by interindividual variation that can be partly explained by genetics (20). Therefore, defining the genomic response to running may provide insights into the neurohormonal cascades that trigger an RH. Micro–ribonucleic acids (miRNAs) are noncoding nucleic acids that regulate protein production through targeted binding of coding messenger RNAs (mRNAs [21]). miRNAs protected within extracellular vesicles can easily cross the blood–brain barrier, allowing the measurement of brain-related molecules in peripheral biofluids, such as blood or saliva (22–24). miRNAs are altered in skeletal muscle, blood, and saliva after aerobic running activity (25–27). Recently, we demonstrated that long-distance running led to alterations in saliva levels of miRNAs and downstream mRNAs involved in metabolism, fluid balance, and myosin regulation (27). Surprisingly, a significant number of exercise-related miRNA targets were implicated in brain-related processes, including GABAergic synapse function, and morphine addiction. These findings were consistent with several previous investigations that noted connections between saliva and brain RNA content (23,24,28,29). Such connections may result from exosomal miRNA signaling between the five cranial nerves that densely innervate the oral cavity, or from glymphatic drainage of the central nervous system, which occurs in proximity to the oropharynx.

Here, we examine the salivary transcriptome (including miRNAs and mRNAs) among trained distance runners to test the hypothesis that epigenetic networks regulating opioid, endocannabinoid, and monoamine signaling are "altered" in runners with subjective and objective evidence of an RH.

METHODS

This observational cohort study was approved by the Independent Review Board at Marist College. Written informed consent was obtained from all participants.

Participants. Participants included 13 male and 12 female collegiate distance runners, 18–23 yr of age. The sample size was determined from our previous experience measuring salivary RNA (24,27–29) and prior studies of RH physiology in humans (1,5–7,14). Using a *t*-statistic with a noncentrality parameter determined that the sample provides >80% power to detect an effect size (Cohen's *d*) of 0.584 across pre- and postrun variables with α set at 0.05. Participants were enrolled on the morning of their weekly "long run," which was defined by a run exceeding 55 min, and comprising ≥20% of weekly running distance, as we have previously reported (27). Individual run distances were tailored to average weekly mileage to control for interindividual variation in effort that might result from a single prescribed course or distance. Of note, all courses had identical net-elevation profiles and were completed at 70%–75% of maximum heart rate. Run intensity was monitored by athletes and their coaching staff, and athletic training personnel were available for monitoring. Exclusion criteria for all participants included illness (e.g., upper respiratory infection or gastrointestinal infection) or orthopedic injury in the past 7 d. Participants with a history of endocannabinoid or opioid use were not excluded, but interactions between substance use history and saliva RNA expression were explored *post hoc* as described below. Participants were divided into those with or without an RH, based on subjective report of four factors: 1) mood, 2) loss of sense of time, 3) run quality, and 4) RH symptoms. Participants assigned to the RH group met at least three of the following four criteria: 1) improved mood (≥1 point increase on a 10-point Likert face scale postrun), 2) lost sense of time (≥30 min without checking watch during the run), 3) above-average run quality (score >5 on a 10-point scale), and 4) endorsement of at least one RH symptom ("strong," "effortless," "relaxed," and "euphoric"). These criteria were assessed through participant surveys, administered 15 min after run completion.

Data and sample collection. Survey data and physiological measurements were collected for all participants 10–15 min before the run (~8:30 AM) and 10–15 min after the run (~10:00 AM). Demographic factors included age (yr), sex, and race (Table 1). Oropharyngeal factors relevant to saliva collection were also assessed: time since last tooth brushing (h), time since last meal (h), and dietary restrictions (e.g., gluten-free, dairy-free, vegetarian, and specific food allergies). Given the study's focus on opioid, endocannabinoid, and monoamine pathways (and downstream effects on anxiety and mood), information about substance use and mental health status was collected for each participant. History of alcohol, nicotine, cannabinoid, and opioid use was anonymously self-reported. Frequency and time since last use were also collected. Anxiety and depression symptoms were assessed prerun in all participants with validated screening tools (Generalized Anxiety Disorder 7-item Scale; Patient Health Questionnaire 9-item Scale). Use of prescription medication for disordered mood was recorded. Running and fitness details were recorded, including maximum heart rate (bpm), $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$), weekly running distance (km), distance run on the day of the study

TABLE 1. Participant characteristics.

Participant Characteristics	All Runners (N = 25)	RH (n = 13)	NRH (n = 12)	P
Demographic and medical features				
Age, yr	20 ± 1.3	20 ± 1	21 ± 2	0.084
Female, n (%)	12 (48)	5 (38)	7 (58)	0.34
Caucasian, n (%)	23 (92)	11 (85)	12 (100)	0.16
BMI, kg·m^{-2}	20.4 ± 1.7	20.5 ± 1.8	20.3 ± 1.6	0.79
Time since meal, h	2.8 ± 3.8	1.5 ± 1.9	4.2 ± 3.8	0.054
Time since toothbrush, h	2.0 ± 2.6	1.8 ± 2.4	2.2 ± 2.9	0.72
Diet restrictions present, n (%)	6 (24)	1 (7)	5 (41)	0.058
Medication use, n (%)	1 (4)	0 (0)	1 (9)	0.34
Substance use and psychiatric features				
Alcohol use, n (%)	22 (92)	11 (84)	11 (92)	0.16
Nicotine use, n (%)	1 (4)	0 (0)	1 (9)	0.34
Cannabinoid use, n (%)	9 (38)	6 (46)	3 (27)	0.35
Opioid use, n (%)	1 (4)	1 (7)	0 (0)	0.34
Anxiety, n (%)	3 (12)	0 (0)	3 (25)	0.081
Depression, n (%)	7 (28)	4 (31)	3 (25)	0.75
Running features				
$\dot{V}O_{2max}$, mL·kg^{-1}·min^{-1}	59 ± 9	60 ± 8	58 ± 9	0.66
Maximum heart rate, bpm	197 ± 2	198 ± 2	196 ± 2	0.08
Weekly distance, km	89 ± 16	91 ± 18	85 ± 14	0.39
Time run on collection day, min	83 ± 13	84 ± 15	83 ± 10	0.83
Distance on collection day, km	18 ± 2.5	18 ± 2.6	18 ± 2.4	0.68
Running pace, min·km^{-1}	4.6 ± 0.4	4.6 ± 0.3	4.7 ± 0.4	0.62

Data are presented as mean ± SD unless otherwise indicated. Medication use includes any prescription medications for disordered mood. The sole medication use reported here is a selective serotonin reuptake inhibitor. Maximum volume of oxygen consumption ($\dot{V}O_{2max}$) was estimated based on exercise and nonexercise equations, as described in the methods. All P values based on two-tailed Student's t-test.

(km), run duration on the day of the study (min), and run pace on the day of the study (min·km^{-1}). $\dot{V}O_{2max}$ was estimated using exercise (30) and nonexercise (31) measures validated in college-age cohorts by George and colleagues. The first estimate of $\dot{V}O_{2max}$ uses gender, body mass, running performance, and heart rate to estimate actual $\dot{V}O_{2max}$ (R = 0.90), whereas the second equation relies on gender, body mass index (BMI), and reports of physical activity and function (R = 0.86). Mean $\dot{V}O_{2max}$ was estimated for each participant by determining the average of the exercise and nonexercise estimates. Blood pressure (mm Hg), temperature (°C), heart rate (bpm), and pupil diameter (mm) were recorded before and after the run. Temperature was measured with a hospital-grade Exergen Temporal Artery Thermometer per manufacturer instructions, by passing the lens of the device from the center of the forehead horizontally to the hairline. For postrun measurements, attempts were made to pat excessive sweat dry to eliminate the effects of evaporative cooling on temperature detection. Weight (kg) and height (cm) were measured and used to determine BMI (kg·m^{-2}). After a water rinse, 2 mL of whole saliva was collected pre- and postrun through active expectoration into Oragene RE-100 saliva collection kits (DNA Genotek, Ottawa, Canada) for RNA isolation and Falcon 50 mL conical tubes (Fisher Scientific, Waltham, MA) for hormone measurements. We chose to interrogate saliva because of its ease of collection and our previous studies demonstrating its potential relevance to brain-related changes during endurance exercise (27). All saliva samples were stored at −20°C before processing.

Saliva processing. Saliva samples from the Falcon 50-mL conical tubes were used to assess pre- and postrun serotonin (5-hydroxyindoleacetic acid) concentrations with a Fast Track enzyme immunoassay kit (Rocky Mountain Diagnostics, Colorado Springs, CO), per manufacturer protocol. We chose to measure salivary serotonin because it has been correlated with validated measures of mood (32) and peripheral serotonin levels are altered by exercise (33). Briefly, 100 μL of acylated standards, controls, and samples were pipetted into respective wells of the serotonin 5-hydroxyindoleacetic acid microtiter strips, along with 25 μL of serotonin antiserum. The plates were covered and incubated for 20 h at 4°C. Well contents were then aspirated before a three-wash cycle and addition of 100 μL of enzyme conjugate. After a 30-min incubation at room temperature (600 rpm), well contents were discarded and washed. Next, 100 μL of substrate was added for 30 min at room temperature (600 rpm). Finally, 100 μL of stop solution was administered to each well, and absorbance was measured within 10 min at 450 nm. Mean absorbance levels of the standard samples (linear measures) were plotted against corresponding concentrations (logarithmic measures) to determine serotonin concentrations for three sample replicates (ng·mL^{-1}).

Saliva samples from the Oragene RE-100 kits were used to assess pre- and postrun concentrations of individual miRNAs and mRNAs via high throughput sequencing, as previously described (27). Briefly, RNA was extracted with a standard Trizol technique and RNeasy mini columns (Qiagen, Valencia, California). An Agilent Bioanalyzer (Agilent Technologies, Santa Clara, California) assessed RNA quality and yield before library construction with the Illumina TruSeq Small RNA protocol. RNA sequencing was performed on a NextSeq 500 Instrument (Illumina, San Diego, California) at 10 million single-end, 50-base reads per sample. Adapter trimming, QC analysis, and RNA read alignments were performed in Partek Flow Software (Partek, St. Louis, MO). Reads were aligned to build 38 of the human genome with the Shrimp2 aligner. The Refseq transcript database (version 82), miRBase precursor

microRNA database (version 21), and miRBase mature microRNA database (version 21) were used to quantify mRNAs, precursor miRNAs, and mature miRNAs, respectively. Of the 27,867 mRNAs and 4649 miRNAs contained in these databases, only those with robust concentrations (raw counts ≥10 in at least 20% of samples) were interrogated for between-group differences. Before differential expression analysis, mRNA and miRNA read counts underwent separate quantile normalization, mean centering, and *z*-score transformation. We have previously used this same RNA sequencing approach in numerous prior studies (24,27–29).

Statistical analysis. A two-tailed Student's *t*-test was used to compare demographic, medical, oropharyngeal, and run characteristics among RH and nonrunner's high (NRH) groups ($P < 0.05$). Postrun differences in blood pressure, heart rate, temperature, and pupil diameter were compared between RH and NRH groups ($P < 0.05$). For salivary serotonin data, a Dixon's *Q*-test was used to identify and remove outlier replicate measurements that exceeded a 90% confidence threshold ($n = 6$) (34). Salivary serotonin concentrations ($ng \cdot mL^{-1}$) were compared between all prerun and postrun samples using a two-tailed homoscedastic *t*-test ($P < 0.05$). Salivary serotonin concentrations were also compared between RH and NRH groups pre- and postrun.

We explored the influence of distance running and an RH on microRNA expression with a two-way (run status–subject) ANOVA. Samples were assigned prerun or postrun status, and RH or NRH status. The miRNAs with significant effects (false discovery rate *P* value < 0.05) of both running and RH were reported. Because miRNAs affected only by RH status (i.e., no run status effects) might not be unique to the specific euphoria resulting from distance running, we focused subsequent analyses solely on the miRNAs with both run status and RH effects. For these miRNAs of interest, putative mRNA targets were identified in DIANA miRPATH (35). The mRNAs with moderate target prediction evidence (microT-cds threshold >0.80, $P < 0.05$) were interrogated for overrepresentation among six KEGG pathways with a Fisher's exact test criterion of $P < 0.05$: morphine addiction (hsa05032), GABAergic synapse (hsa04727), retrograde endocannabinoid signaling (hsa04723), neurotrophin signaling (hsa04722), serotonergic synapse (hsa04726), and dopaminergic synapse (hsa04728). A two-way ANOVA was also used to identify coding transcripts (mRNAs) from the morphine addiction and retrograde endocannabinoid pathways affected by running or RH. Only coding transcripts (mRNAs) that demonstrated robust salivary expression and were targeted by the miRNAs of interest were included. Relationships between individual changes in salivary miRNA concentration and the four subjective measures of an RH (postrun mood change, run rating, time without checking watch, and number of RH symptoms) were assessed with Pearson correlation testing ($R > [0.4]$, $P < 0.05$). miRNA relationships with serotonin change, endocannabinoid use history, and postrun pupillary diameter were similarly assessed. Statistical analysis of RNA variables was performed in Metaboanalyst version 4.0.

RESULTS

Participants. Participants had a mean age of 20 ± 1.3 yr, a mean BMI of 20.4 ± 1.7 $kg \cdot m^{-2}$, and were mostly Caucasian (23/25, 92%; Table 1). The majority reported previous alcohol use (22/25, 92%). Nine participants reported prior recreational use of cannabinoids (38%). Few had previously used nicotine (1/25, 4%) or opioids (prescription or recreational; 1/25, 4%). Two participants had used cannabinoids and alcohol in the past 24 h. No other participants reported substance use of any kind in the past week. Only 3/25 (12%) participants met standardized screening criteria for mild–moderate anxiety, and 7/25 met criteria for mild depression (28%). One participant was taking a selective serotonin reuptake inhibitor. There were no differences between RH and NRH groups in age, sex, race, BMI, time since last meal, time since last toothbrush, dietary restrictions, substance use history, or rates of anxiety or depression (all $P \geq 0.05$).

Run characteristics. Among all participating runners, the mean estimated $\dot{V}O_{2max}$ was 59 ± 9 $mL \cdot kg^{-1} \cdot min^{-1}$ (males, 62 ± 9 $mL \cdot kg^{-1} \cdot min^{-1}$; females, 56 ± 7 $mL \cdot kg^{-1} \cdot min^{-1}$), and the average maximal heart rate was 197 ± 2 bpm. Participants reported an average weekly running distance of 89 ± 16 km and covered 18 ± 2.5 km on the day of collection at an average speed of 4.6 ± 0.4 $min \cdot km^{-1}$ (Table 1). There was no difference between RH and NRH groups in $\dot{V}O_{2max}$, maximum heart rate, weekly distance, distance run on collection day, time run on collection day, or run speed on collection day (all $P \geq 0.05$).

Measures of an RH. The RH group displayed improved postrun mood (+2 points on a 10-point Likert scale, $P = 0.036$) relative to NRH participants (+0 points postrun on a 10-point Likert scale) and reported higher run satisfaction scores (7/10, $P = 1.8E-05$) than the NRH group (4/10; Table 2). The RH group endorsed more RH symptoms (2 ± 1; $P = 0.0004$) than the NRH group (0 ± 1). A greater number of RH participants also experienced lost sense of time (11/13, 85%; $P = 0.0084$) than NRH participants (4/12). There was no difference in postrun blood pressure (systolic $P = 0.24$; diastolic $P = 0.95$) or heart rate ($P = 0.12$) between RH and NRH groups. The RH group demonstrated reduced postrun temperature (36.0°C; $P = 0.043$) and increased postrun pupil diameter (4.8 mm; $P = 0.046$) relative to NRH group temperature (36.8°C) and pupil diameter (3.5 mm).

Salivary serotonin. For all participants, prerun levels of salivary serotonin (1190 $ng \cdot mL^{-1}$) were higher than postrun levels (742 $ng \cdot mL^{-1}$; $P = 0.033$). There was no difference between RH and NRH groups in prerun serotonin levels (RH = 1230 $ng \cdot mL^{-1}$; NRH = 1353 $ng \cdot mL^{-1}$; $P = 0.75$), postrun serotonin levels (RH = 663 $ng \cdot mL^{-1}$; NRH = 845 $ng \cdot mL^{-1}$; $P = 0.61$), or the postrun change in salivary serotonin concentration (RH = −566 $ng \cdot mL^{-1}$; NRH = −507 $ng \cdot mL^{-1}$; $P = 0.88$; Fig. 1).

TABLE 2. Subjective and objective elements of RH.

	All Participants	RH (n = 13)	NRH (n = 12)	P
Subjective				
Mood change, −10 to +10	+1 ± 2	+2 ± 1	0 ± 2	0.0036
RH symptoms endorsed, n	1 ± 1	2 ± 1	0 ± 1	0.0004
Loss of time, n (%)	15 (60)	11 (85)	4 (33)	0.0084
Run quality, 0–10	5 ± 2	7 ± 1	4 ± 2	1.8E−05
Objective				
Postrun SBP, mm Hg	125 ± 8	123 ± 4	127 ± 10	0.24
Postrun DBP, mm Hg	77 ± 6	77 ± 5	77 ± 6	0.95
Postrun HR, bpm	89 ± 9	86 ± 6	92 ± 11	0.12
Postrun temperature, °C	36.4 ± 0.9	36.0 ± 0.9	36.8 ± 0.6	0.043
Postrun pupil diameter, mm	4.2 ± 1.5	4.8 ± 1.7	3.5 ± 1.1	0.046

Data are presented as mean ± SD unless otherwise indicated. P values reflect statistical differences between the RH group and the NRH group on a two-tailed Student's t-test. Summary of subjective measures: Mood change reflects a participant's postrun change in self-reported mood on a validated 10-point Likert face scale. Four RH "symptoms" were selected to query positive neurobiologic and psychiatric responses to exercise. These included 'euphoric,' 'relaxed,' 'strong,' and 'effortless.' The number of symptoms endorsed by each group is displayed. Loss of time was defined by a self-reported period of ≥30 min without checking a stopwatch during the run. Run quality was self-rated on a 0–10 Likert scale, where 10 was defined as a 'complete RH' and 0 was defined as 'the most difficult, grueling run imaginable.'
SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

Salivary miRNA. There were 460 miRNAs with robust concentrations in pre- or postrun saliva (counts ≥10 in ≥20% of samples). Of these, 80 miRNAs demonstrated a pre- and postrun effect (false discovery rate P value < 0.05) and 22 had an RH effect on two-way ANOVA (see Table, Supplemental Digital Content 1, miRNAs with significant effect of run or RH on saliva expression, http://links.lww.com/MSS/B476). Six miRNAs were influenced by both run status and RH status (Fig. 2). We focused further analyses on these six miRNAs because of their dynamic relationship with both running and euphoric state. Three of the six miRNAs trended up postrun only in RH participants (miR-1237-3p, miR-6743-5p, and miR-4254). Two of the miRNAs demonstrated an upward trend postrun only in the NRH group (miR-194-5p and miR-4676-3p). One miRNA (miR-4425) displayed a downward trend postrun only in the RH group.

mRNA targets. Together, the six miRNAs of interest had 2530 putative mRNA targets. These targets demonstrated enrichment for KEGG pathways related to morphine addiction (15/24 mRNAs; P = 2.74E−06) and GABAergic synapse (16/31 mRNAs; P = 0.00016; Table 3). Retrograde endocannabinoid signaling (16/33 mRNAs; P = 0.063), neurotrophin signaling (13/77 mRNAs; P = 0.47), serotonergic synapse (16/50 mRNAs; P = 0.30), and dopaminergic synapse (18/52 mRNAs; P = 0.33) did not display miRNA target enrichment. Of the 57 mRNAs involved in morphine addiction (opioid signaling) and retrograde endocannabinoid signaling, 31 mRNAs were targeted by the 6 miRNAs of interest (54%), and 11 had robust concentrations in saliva (counts ≥10 in ≥20% of samples). Mitogen-activated protein kinase 11 (*MAPK11*), a target of miR-1237-3p, displayed a nominal difference (raw P = 0.010; false discovery rate P value = 0.11) between RH and NRH groups on two-way ANOVA. Levels of *MAPK11* were relatively lower in NRH participants (see Figure, Supplemental Digital Content 2, Two-way ANOVA of opioid and endocannabinoid mRNAs in a runner's high, http://links.lww.com/MSS/B477), particularly postrun.

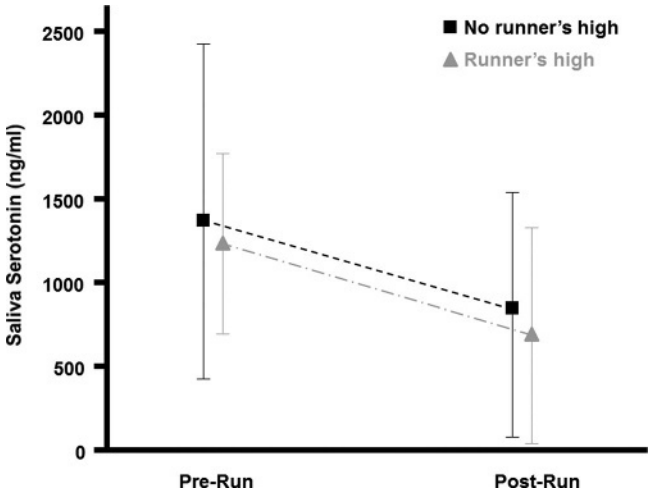

FIGURE 1—Salivary serotonin changes postrun regardless of RH status. Salivary concentrations of serotonin, measured 15 min before and after a long-distance run, decreased postrun (P = 0.033) regardless of whether an RH was reported. There was no difference in prerun levels (P = 0.75), postrun levels (P = 0.61), or the postrun change (P = 0.88) in serotonin between RH and NRH groups.

Transcriptional correlations. Relationships between the postrun concentration changes in the six miRNAs of interest and the RH variables were explored with Pearson correlation analyses (Fig. 3). Salivary levels of miR-4425 displayed significant inverse relationships with the number of RH symptoms endorsed (R = −0.42, P = 0.037) and pupil diameter (R = −0.42, P = 0.045). Salivary levels of miR-1237-3p were directly related with time without checking a stopwatch (R = 0.40, P = 0.049). There was no correlation between any of the six miRNAs of interest and history of marijuana use (P > 0.05). There were also no relationships detected between salivary miRNA levels and mood change, run quality, or change in serotonin levels. The 11 salivary mRNAs involved in endocannabinoid or opioid signaling were examined for correlations with the six miRNAs of interest. GABA type A receptor beta1 subunit (*GABRB2*), GABA type A receptor alpha subunit (*GABRA1*), and *MAPK11* displayed significant (P < 0.05) correlations with miRNAs of interest (see Figure, Supplemental Digital Content 3, Postrun changes in the saliva miRNAs altered by an RH correlate with postrun fluctuations in cannabinoid/opioid mRNAs, http://links.lww.com/MSS/B478).

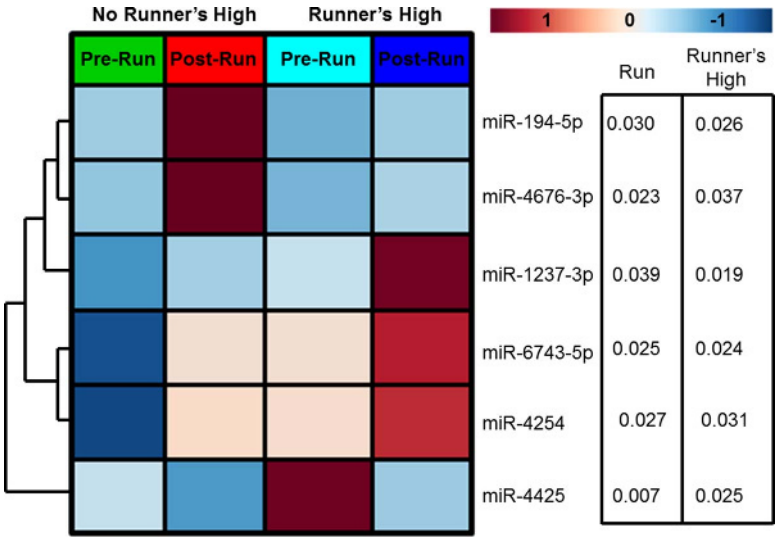

FIGURE 2—Six salivary miRNAs with effect of running and RH. The heatmap displays quantile normalized mean group expression for the six salivary miRNAs significantly affected by both run status (pre- and postrun) and RH status on two-way ANOVA. Upregulated miRNAs are displayed in *red* and downregulated miRNAs are in *blue*. A complete hierarchical clustering technique using Pearson's correlation analysis was used for dendritic grouping of the six miRNAs. A two-way ANOVA was used to determine effects of run status and RH status on each miRNA (*P* values displayed).

The strongest association was observed between miR-194-5p and *GABRA1* ($R = -0.51$, $P = 0.00014$). Notably, *GABRA2*, but not *GABRA1*, is a putative target of miR-194-5p (microT-cds = 0.96). However, associations between miRNA/mRNA target pairs were observed for both miR-1237-3p/*MAPK11* ($R = -0.28$, $P = 0.046$, microT-cds = 0.89) and miR-1237-3p/GABRA1 ($R = 0.39$, $P = 0.046$, microT-cds = 0.97).

DISCUSSION

This cohort study of 25 collegiate distance runners identified a novel epitranscriptional network, including six miRNAs, which may regulate the genomic response to an RH. The six miRNAs targeted gene networks implicated in opioid signaling and GABAergic synapse pathways. Endocannabinoid- and serotonin-related mRNAs were not significantly targeted by the six miRNAs, and salivary serotonin levels did not differ between RH and NRH groups. One of the six miRNAs, miR-1237-3p, could regulate local expression of *MAPK11*, whose protein product mediates signal transduction through cannabinoid receptor 1 (CNR1 [36]). Notably, levels of miR-1237-3p and miR-4425 were correlated with subjective and objective indices of an RH, and miR-1237-3p was inversely correlated with *MAPK11* levels.

Many studies have previously identified opioid, GABAergic, and endocannabinoid signaling changes in the physiological response to endurance exercise (1–3,7). To our knowledge, the present study is among the first to describe how epitranscriptional mechanisms may mediate this response. Past investigations have typically relied on comparisons of pre- and postexercise samples to ascertain the influence of endurance training on mood and pain regulation. Anecdotally, endurance athletes report that not every workout results in euphoria, analgesia, or anxiolysis. Therefore, to amplify transcriptional signals specific to an RH, we divided runners based on subjective measures of the four RH components into RH and NRH groups. Remarkably, although RH and NRH groups did not differ in any medical, demographic, or run-related features, RH participants displayed significant hypothermia and mydriasis relative to NRH participants. These physiological changes can also be observed with the administration of benzodiazepines, a class of drugs that cause anxiolysis, muscle relaxation, and sedation through inhibition of GABA (37). RH participants report similar symptoms, undergo parallel physiological changes, and display alterations in the levels of six miRNAs that inhibit GABAergic transcripts.

Previously, Fuss and colleagues (4) have also reported that ablation of the endocannabinoid receptor on GABA neurons prevents RH in mice. Although we did not find that miRNAs associated with RH overtargeted endocannabinoid transcripts, we did detect peripheral changes in the levels of *MAPK11*, which participates in endocannabinoid signal

TABLE 3. Pathways targeted by the six miRNAs of interest.

KEGG Pathway	*P*	mRNAs Targeted, *n* (%)	miRNAs Involved, *n*
Morphine addiction	2.74E−06	15 (63)	4
GABAergic synapse	0.00016	16 (52)	5
Retrograde endocannabinoid signaling	0.063	16 (48)	5
Neurotrophin signaling	0.47	13 (17)	5
Serotonergic synapse	0.30	16 (32)	6
Dopaminergic synapse	0.33	18 (35)	6

P values reflect the likelihood of mRNA target enrichment on Fisher's exact test. KEGG, Kyoto Encyclopedia of Genes and Genomes.

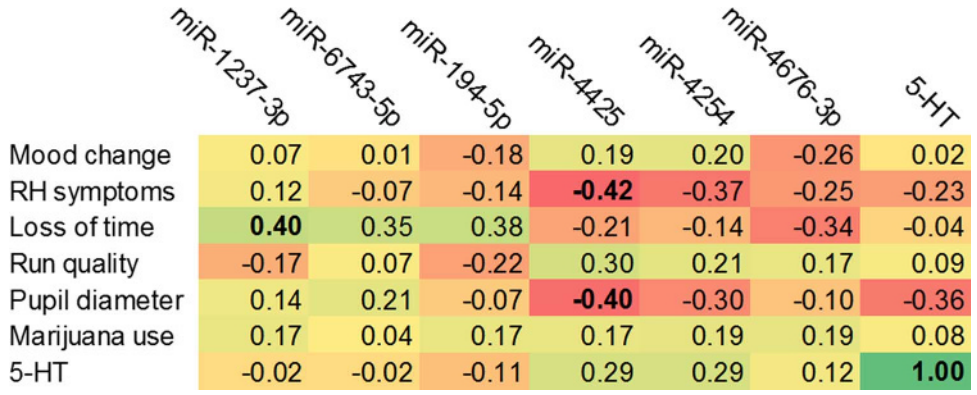

FIGURE 3—Relationships between salivary miRNAs and RH indices. Pearson correlation analyses were used to determine correlations between changes in the six salivary miRNAs pre- and postrun and six indices of an RH. Relationships between miRNAs of interest and marijuana use are also reported given the potential for confounding interactions. Color scales indicate strength of association (*R*-value) and significant relationships ($R \geq [0.40]$, $P < 0.05$) are displayed in bold. Note that miR-4425 levels were inversely related to both the number of RH symptoms endorsed and the postrun pupil diameter. Levels of miR-1237-3p were directly related to lost sense of time (quantified as number of minutes without checking a stopwatch). No miRNAs were associated with history of marijuana use. 5-HT, serotonin.

transduction (36). As previous studies have noted, there is an important distinction between the peripheral and the central physiological effects of an RH (1–4,15). This distinction highlights the relevance of salivary miRNA in humans. Although human studies generally prohibit direct measure of brain-related molecules, we have previously shown that exosomal miRNA content in saliva mirrors that in cerebrospinal fluid (24), and that salivary miRNA levels are altered in the pathophysiological disorders of the nervous system (28,29). The extracellular miRNAs measured in saliva may represent brain-related signaling molecules emerging directly from the cranial nerves that densely innervate the oropharynx. Such a role is consistent with *in situ* studies of human neuroblasts, showing that exosomal miRNA release is influenced by depolarization activity, associated with synaptic plasticity, and packaged with microtubule associated protein 1b (MAP1b [38]). Just as stimulation of neuroblast signaling can affect the concentration of miRNAs associated with MAP1b *in situ*, environmental influences (such as an RH) might trigger synaptic changes in cranial nerves that result in measurable miRNA/MAPK11 changes in saliva.

Consistent with previous studies that have identified reductions in serotonin immediately after exercise (39), we found decreased salivary serotonin in all participants postrun (Fig. 1). This trend did not differ between RH and NRH groups, and the miRNAs that changed in RH participants did not target serotonin signaling (Table 3). Furthermore, serotonin levels did not correlate with miRNA levels or RH measures (Fig. 3). These findings suggest that serotonin signaling may have a limited role in the immediate mood changes associated with an RH. These findings also highlight the importance of distinguishing athletes with subjective/objective symptoms of an RH, from those who have participated in endurance exercise, but lack subjective reports of euphoria.

Although the extensive characterization of medical, demographic, and subjective traits of this study's participants constitutes a relative strength, there are several limitations to the study design that should be considered when interpreting the results. Because this was primarily an investigation of transcriptional mechanisms, no measurements of downstream endocannabinoid or endorphin ligands are available. Pairing these measurements with transcriptional profiling in the central nervous system of running animals might provide additional insights, particularly given that some participants reported a history of marijuana use. This study found no association between marijuana use and transcriptional targets of an RH, but it is conceivable that downstream endocannabinoid ligands might be affected. An approach using animal models would control for this factor, as well as interindividual variation in genetics, behavior, and fitness that predominates human investigations (although such a study would sacrifice critical subjective symptom reports). Notably, in a study of longitudinal miRNA expression in macaque striatum after chronic exposure to Δ9-tetrahydrocannabinol (or a vehicle control), the six miRNAs identified in this manuscript showed no responsivity to cannabinoid administration (40).

This study attempts to control for many aspects of interindividual variation. Importantly, there were no differences between RH and NRH groups in age, sex, or race, and patterns of substance use were consistent across groups. All participants had similar fitness levels (estimated $\dot{V}O_{2max}$ and maximum heart rate), and there was no difference in run distance or pace between groups. Although participants completed the assigned course at approximately 70%–75% of maximum heart rate, variation in run intensity was not measured in the form of interrun heart rate. This could have influenced perception of an RH. There were statistically insignificant differences in time since last meal between RH and NRH groups, which could also have contributed to miRNA differences between the groups. However, we have previously investigated relationships between meal status and miRNA expression in runners and found only two salivary miRNAs (miR-4671-5p and miR-3917) associated with prerun meal timing (28). Finally, reported $\dot{V}O_{2max}$ levels are based on validated estimate scales and should be interpreted with caution.

In conclusion, this study demonstrates that unique peripheral changes in miRNA concentrations occur in both male and

female distance runners with symptoms of an RH. The six miRNAs target endorphin and GABAergic gene networks and display expression patterns that correlate with both subjective symptoms and objective physiological changes (pupil diameter). These findings support the idea that epitranscriptional molecules may regulate the euphoric experience reported by some endurance athletes.

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The results of the present study do not constitute endorsement by the American College of Sports Medicine.

The authors certify that the results of the study are presented honestly and without fabrication, falsification, or inappropriate data manipulation.

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