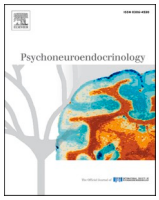
Psychoneuroendocrinology 158 (2023) 106407

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen



Human sweat contains oxytocin

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ARTICLE INFO

Keywords:
OT
Testosterone
Social condition
Skin
Physical activity
Sweat
Saliva
Extraction

ABSTRACT

Background: Oxytocin (OT) has been detected in various body fluids, including blood, urine, saliva, breastmilk, and spinal fluid. Consistent with models that regard skin as a social organ and in line with studies demonstrating that skin cells express both OT and its receptor, our study sought to examine the presence of OT in human sweat.
Methods: Overall, 553 individuals participated in a pilot study and three experiments. Firstly, 50 participants provided sweat after engaging in various sports for different durations. Secondly, 26 participants provided sweat from forehead, upper-chest, forearm, and underarm, including 11 in natural setting and 15 following OT administration and a 30-minute exercise. Thirdly, of 435 volunteers, 97 provided sufficient axillary sweat for assaying. Of these, 84 participated in a naturalistic experiment that involved saliva and sweat collection in response to physical activity in either solitary or social settings. OT and testosterone (TS) were assayed in sweat and saliva.
Results: Intense activity for at least 25 min was required to produce sufficient sweat for OT analysis. Highest OT levels were found in axillary sweat compared to sweat from the forehead, upper-chest, and forearm. Salivary OT and TS increased after both solitary and social physical activity; however, higher sweat OT was found after solitary sports. Post-hoc preliminary findings indicate that highly extroverted individuals exercising in solitary environments showed the highest sweat OT levels.
Conclusions: Findings demonstrate, for the first time, the presence of OT in human sweat and show the feasibility of its measurement. Much further research is required to illuminate how sweat OT is impacted by personality and social context and to uncover the role of the skin in OT production.

1. Introduction

Oxytocin (OT) is a unique molecule that plays a role in multiple aspects of our life. Known for its pro-social role, OT also functions as a chemical messenger in various brain regions and in different organs and tissues throughout the mammalian body. Mainly produced in the hypothalamus, OT acts as both a neurotransmitter in the nervous system and as a hormone when released by the posterior pituitary gland into the bloodstream. Apart from hypothalamic production, OT is produced in other regions of the body, including the uterus, placenta, ovaries, pancreas thymus, heart and vasculature (Burbach et al., 2006; Muscatelli et al., 2018; Uvnäs-Moberg et al., 2019). OT has been detected in various body fluids, such as blood, urine, saliva, CSF, and semen (Jurek and Neumann, 2018; MacLean et al., 2019; Goverde et al., 1998), as well as in fluid suctioned from skin blisters (Deing et al., 2013). Our laboratory has measured OT in a variety of body fluids, including blood (Feldman et al., 2007, 2011; Gordon et al., 2008,) urine, (Feldman et al., 2011; Pratt et al., 2015), saliva (Feldman et al., 2011; Weisman et al., 2012; Rassovsky et al., 2019; Djalovski et al., 2021), and breast milk (Yirmiya et al., 2023) and has demonstrated the associations between OT and prosocial and affiliative behavior and attitudes in infants, children, adolescents, and adults and in healthy and high-risk populations. In the current study, we report on the measurement of OT in human sweat. It is possible that such OT may be produced in skin tissues, given the reported presence of OT in skin tissue (Deing et al., 2013; Denda and Nakanishi, 2022).

The skin serves as a social organ via skin-brain interactions, and the central role social touch plays in processes of affiliation and communication attests to the skin's key social function (Morrison et al., 2010; Elias Abdus-Saboor, 2022). Feldman (2020) theorized that the long-term impact of mother-newborn skin-to-skin contact may relate to its impact on the infant's developing OT system. It has been shown that skin-to-skin contact between mothers and infants increases OT production (Cong et al., 2015; Hardin et al., 2020), maternal and paternal touch

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https://doi.org/10.1016/j.psyneuen.2023.106407
Received 28 May 2023; Received in revised form 20 August 2023; Accepted 23 September 2023
Available online 28 September 2023

of their infants during social interactions increased infant OT levels (Feldman et al., 2011), social dialogue that involves synchrony between couples or best friends enhanced OT production (Djalovski et al., 2021), and during the initiation of a romantic relationship affectionate touch is associated with greater OT levels (Schneiderman et al., 2012).

In addition to its involvement in the social-regulatory functions of affiliative touch, evidence for OT production in peripheral tissues points to a possible role for OT in wound healing and skin regeneration (Poutahidis, 2013), suggesting that OT may function as a natural medicine for pain relief (Carter et al., 2020). Grinevich and Charlet (2017) proposed three mechanisms by which OT mediates peripheral analgesia: spinal cord, pituitary, and local skin release. OT has been shown to ameliorate skin damage (İşeri et al., 2008), accelerate wound healing (Poutahidis et al., 2013), and prevent skin aging (Cho et al., 2019). These mechanisms may be induced by skin-based stimulation that have been shown to induce OT production, such as skin-to-skin interaction (Cong et al., 2015), gentle touch, or nociceptive stimulation (Grinevich and Charlet, 2017). OT in sweat may be part of this general role of OT in mediating analgesia in the skin. For example, it has been shown that OT elicits electrophysiological responses in cultured sweat gland cells (Ring and Mörk, 1997).

Research continues to uncover new OT production and receptor sites. Of special interest for the current study is the expression of OT and OT receptors (OTR) in skin tissue (Denda et al., 2012; Deing et al., 2013). Notably, Denda considers the epidermis as the "third brain" (2015). He claims that epidermal keratinocytes express a variety of functional environmental sensors of temperature, mechanical stress, and chemical stimuli as well as a series of neurotransmitters and their receptors, including OT, that play crucial roles in the brain and influence whole-body state and emotions (Denda, 2015; Elias and Abdus-Saboor, 2022). Furthermore, it has been suggested that OT, which is produced in healthy skin in epidermal keratinocytes, may influence mental states (Denda et al., 2013). It is thus plausible that OT in the skin plays an important role in human emotions, cognitions, and physical and mental health.

Studies on the social function of OT have mainly assessed OT before and after social interactions or in response to socially related cues when the participant is alone. However, few studies compared changes in OT levels when the same activity is carried out when an individual is alone versus when it is conducted while an individual is in a social context. Studies assessing social interactions have shown that OT levels in saliva, urine, and plasma increase following dyadic interactions between mothers and children, among couples, between best friends, among members of a social group, and even among strangers (Gordon et al., 2008; Pratt et al., 2015; Djalovski et al., 2021; Influs et al., 2018; Rassovsky et al., 2019). We have previously shown that close physical contact induced greater salivary OT increase after high-intensity martial arts training with ground grappling as compared to “punch-kick” sparring (Rassovsky et al., 2019). In contrast, a study that compared choir versus solo singing showed differential effects on salivary OT concentrations and found that OT showed a mild increase following solo singing, whereas after choir singing OT levels were significantly reduced, (Schladt et al., 2017). These findings highlight the social context as an important feature to consider when assessing OT response and should be taken into consideration when measuring OT in sweat.

To examine OT in sweat, we induced sweating through physical activity that stimulates both eccrine and apocrine glands. Eccrine glands, found throughout the body, secrete odorless sweat to regulate body temperature and to dispose of waste (Wilke et al., 2007; Baker, 2019). Apocrine glands, found in specific locations like the underarm and pubic and anal regions, secrete proteins and lipophilic chemicals with physiological functions that help attract the opposite sex. Apocrine glands release is controlled by the limbic system (Folk and Semken, 1991; Hu, 2018) and is secreted via hair follicles while experiencing sexual excitement or emotional distress, such as anxiety, pain, and fear (Hu et al., 2018). We chose to induce sweat through physical activity, as it stimulates both types of glands. Other methods would have mainly stimulated eccrine glands, and thus merely produce perspiration for thermoregulation.

Physical activity is well-known for its beneficial outcomes. Performing physical activity during the COVID lockdown in Spain was associated with better mood and perceived health (Reigal et al., 2021). Individuals who performed strong and persistent physical activity during the pandemic in China reported better emotional well-being compared to those performing light physical activity (Qin et al., 2020). OT has been reported to be involved in oxidative stress (Buemann and Uvnäs-Moberg, 2020; Jurek and Neumann, 2018). Physical activity causes oxidative stress (Buemann and Uvnäs-Moberg, 2020). Salivary OT increases while performing physical activity (Rassovsky et al., 2019; Jong et al., 2015). Physical activity is associated with empathy in young adults with specific gene variations of both OT and vasopressin receptors (Shima et al., 2022). In light of the above, it is tempting to tie OT to physical activity and behavior. High levels of OT are usually associated with greater trust, generosity, and positive social perceptions (Human et al., 2016). Extroversion involves greater sociability, dominance, excitement seeking, and positive effect. Underlying these somewhat distinct features of extroversion appears to be a heightened reward sensitivity and motivation toward pleasant stimuli, which may engender greater social engagement and affiliative motivations (Human et al., 2016). To illuminate the associations between OT, physical activity, and personality, we measured OT response to physical activity in sweat in relation to the "big five" personality traits with no specific a-priori hypotheses.

In contrast to OT in human sweat, which has not been previously studied, the study of Testosterone (TS) release in sweat is well-established (Zouboulis et al., 2006; Elliott et al., 2017). As such, the present study, which is admittedly new, was designed to measure TS as an analogous marker. Testosterone is secreted by sweat in both males and females (Zouboulis et al., 2006) and is secreted in saliva in response to physical activity (Sokoloff et al., 2016; D’Andrea et al., 2020). Moreover, levels of TS in saliva and sweat are responsive to the social environment as well as to social interactions with members of the opposite sex, as seen in studies of saliva (Djalovski et al., 2021; Roney et al., 2007) and sweat (Elliott et al., 2017). Thus, to provide a comparison with a social hormone that is known to be present in human sweat, we designed our study to investigate both OT and TS and to assess their inter-relatedness. TS measurements were used as a parameter quality control for our results, since expected measurements of TS are based on well-established studies in the literature.

To our knowledge, this is the first study that aims to assess the presence of OT in human sweat. Our study is, therefore, exploratory and has three goals related to the release of OT during physical activity: 1) to determine and validate whether OT is present in human sweat by way of elucidating the body regions where it can be most readily studied, 2) to examine whether training environments (solo or social) impact upon OT levels in saliva and sweat and 3) to explore the associations between OT and TS levels in sweat and saliva.

2. Methods

2.1. Participants and Procedure

Our project comprises four inter-related studies to verify the presence of OT in sweat; one pilot study, and three experiments, the last of which is a naturalistic study in two phases (Time 1 and Time 2). Participants in all studies were healthy volunteers aged 16–64 years (M=24.6, SD= 10.2). In all, there were 553 participants, including 293 (53%) men and 260 (47.0%) women, for details see Table 1.

Participants were included if they exercised regularly at least twice a week for at least 30 min, were physically healthy, and had no known psychological issues. Procedures of all studies were approved by the Reichman University Ethics Committee. Participants were provided

Table 1
Participant's Age and Gender.

Experiment	Men			Women		
	n	M,yr	SD	n	M,yr	SD
Pilot study	31	24.5	4.8	19	22.8	3.5
Experiment 1	33	23.1	2.2	45	23.8	1.4
Experiment 2[a]	8	24.1	5.7	7	23.4	4.3
Experiment 3						
T1	244	27.2	8.4	191	25.1	7.3
T2[b]	49	27.6	8.3	35	25.4	7.2

[a] - Participant of experiment 2 took part in the pilot study.
[b] - Participant of T2 took part in T1

with a summary of the research and its goals before participating and signing informed and health consent forms.

2.1.1. Pilot study

The goal of the pilot study was to determine the type and length of physical activity that is best suited to provide an analyzable amount of sweat. Fifty subjects, students and volunteers, who were recruited through friends, family, and social media, took part in the pilot study, including 31 men and 19 women (Table 1). They were asked to provide saliva at baseline and post activity, to perform their regular physical activity in their naturalistic environment, and to try to collect axillary sweat throughout the workout. Besides the biological samples, the participants' gender, age, type of workout performed, its duration and the perceived intensity of the workout, were documented.

From this pilot study we learned that by performing intense aerobic activity, participants were able to provide axillary sweat after 25 min of workout. Out of the first fifty volunteers, eleven individuals, including 7 men and 4 women, provided axillary sweat samples post-activity as well as saliva samples at both baseline and post-activity. Those subjects who provided both sweat and saliva samples were included in the OT analysis.

2.1.2. Experiment 1: OT is detected in sweat collected from various body regions

Here we aimed to explore the possibility of collecting sweat from various body regions and to study the expression of OT in these regions. In this controlled experiment, we asked trainees, who cycled indoors, to volunteer by providing sweat from various body regions: underarm, forehead, upper chest, and forearm throughout their workout. Indoor cycling was chosen for this portion of the study since, being stationary, it allowed the trainees to access many test tubes adjacent to their training position. Trainees were encouraged to do their best to provide sweat. Out of the 76 individuals (Table 1) who took part in this experiment, 14 men (M=24.0, SD=1.4 years) managed to provide enough volume of sweat from all regions. In contrast, none of the women provided sufficient amounts of sweat from all regions. Altogether 80% of the men and 56% of the women did provide axillary sweat sufficient for measuring OT.

This part of the study taught us that the easiest region to collect sweat from is the underarm. When training outdoors, the trainees are limited in the number of tubes they can carry throughout their workout. Therefore, research should focus on the most relevant sweat regions. We focused on underarm sweat since in most of the cases in which trainees managed to provide sufficient sweat for analysis, it was from the underarm.

2.1.3. Experiment 2: OT in sweat in response to intranasal OT administration

Fifteen subjects, who previously provided at least 0.5 ml of axillary sweat participated in the OT administration study, in total 8 men and 7 women. Prior to administration, participants provided a baseline saliva sample. They then self-administered a single 24IU dose of intranasal OT, three sprays to each nostril. Fifteen minutes after administration they provided a second saliva sample and then started their regular solitary aerobic training, running, cycling, stepping (on a stepper) and indoor skying (by elliptical training machine). Axillary, forehead, chest, and forearm sweat were collected after 40 min of workout. At that time, a third saliva sample was provided. Participants received compensation of 150 NIS.

2.1.4. Experiment 3: Naturalistic Study

2.1.4.1. Time I (screening). The purpose of the naturalistic study at Time 1 (T1) was to screen for those individuals who could provide sufficient volume of axillary sweat in the main study at Time 2 (T2). These individuals were recruited through personal contacts and the media both in and out of the university. Four hundred and thirty-five volunteers participated in the T1 screening phase, including 244 men and 191 women (Table 1). Trainees were instructed to perform their regular physical activity session wherever they chose and to provide baseline and post activity saliva samples as well as axillary sweat samples. When the experimenters received the biological samples, they also interviewed the participants about their physical activity, the presence of others while training, as well as subject's gender and age.

Of the original 435 subjects, only 98 individuals provided an adequate (minimum 300 microL) volume of sweat for hormonal measurement. The remaining participants could not provide enough sweat for OT measurement even after 40 min of workout and were therefore excluded from participating in the T2 phase of the experiment.

From this phase of the study (T1) we learned that some aerobic activities, for instance; jogging, cycling, or spinning, CrossFit, stepping on a stepper, skying -by elliptical training machine, or other similar devices - indoor or outdoor, and ball games, can induce production of adequate axillary sweat, while others, such as dancing, hot yoga, Pilates, weightlifting, and walking, do not.

2.1.4.2. Time II (main study). Of the 98 individuals who took part in the screening phase of the experiment (T1) and provided sufficient sweat, 94 agreed to participate in T2. Prior to physical activity, participants were instructed to repeat the T1 protocol and to follow strict instructions about eating and drinking. They also completed a short survey that included basic demographics and their planned workout and signed a consent form. In the case that subjects provided axillary sweat, they were asked to fill, post activity, a set of questionnaires. Those who provided axillary sweat, and thus took part in T2, were compensated by either payment of 150 NIS or by academic credits.

The full anonymous survey included, extensive demographic coverage, habits, health, fitness, physical activities, and personality traits, as well as questions regarding the specific physical activities they performed and whether or not they did so in company. All questionnaires were accessible via "Qualtrics" and could be filled out online through a link sent to their phones.

All questionnaires were bi-lingual (Hebrew and English) and subjects completed them according to their preference. Experiment T2 included 84 respondents, those who had provided adequate biological samples. Of these, 58% (n = 49) were men and 42% (n = 35) women (Table 1). The age range for men was 19–64 years and for women 19–62 years. The sample was comprised mainly of students with an average of 13.0 (SD = 2.1) years of education. The average body mass index for men was 24.5 (SD = 2.5) and for women 22.3 (SD = 2.7). Approximately half reported being in a relationship (52%), 41% were single, and a few (6%) were married. Only 6% of the respondents had children (two on average). Additionally, 91% of the participants reported no health issues. Respondents exercised on an average of 2.1 days a week (SD = 0.84, range: 1–4). In terms of medication, 78% did not take any medication (excluding birth control), while 35% of the female respondents (n = 12) did take birth control. 80% were nonsmokers. Amongst smokers, the

average number of cigarettes per day was 1.56 (SD = 0.86, range: 1–3). The percentage of smokers was higher for men (26%) than women.(6%).

2.2. Personality EPQR Questionnaire

Personality traits in the study were measured using the Revised Eysenck Personality Questionnaire - EPQR (Eysenck et al., 1985). The Hebrew version (Katz and Francis, 2000) is a 100-item questionnaire. Of these, 47 items were included in the study: the entire extroversion scale (23 items) and the entire neuroticism scale (24 items). Items are worded in the form of statements for which subjects indicate the degree of agreement on a 5-point scale (1 = do not agree at all to 5 = strongly agree). According to the questionnaire protocol, some items are reversed before participant's score were calculated by averaging. Both the extroversion and neuroticism scales have shown high reliability and validity. Internal consistency for the current sample was, for extroversion Cronbach α = 0.87 and for neuroticism Cronbach α = 0.94.

2.3. Sampling of Saliva and Sweat

Participants were asked to give two saliva samples baseline (pre) and post physical activity by passively drooling into clean 5 ml barcoded tubes and were asked to collect sweat after 20–40 min of exercising by sliding a 7 ml wide barcoded tube over the skin of the required region, mainly the axillary (underarm) region. They were instructed not to apply deodorants or antiperspirants on the day of collection. In addition, participants were instructed to avoid eating 60 min prior to their physical activity and to avoid drinking 20 min prior to saliva sampling.

2.3.1. Samples preparation

All samples were stored at − 25 °C prior to handling and in between stages.

Saliva samples underwent three freeze-thaw cycles (freezing at −80 °C and thawing at 4 °C) to precipitate mucus proteins. The liquid samples, of saliva (after the cycles) and sweat, were centrifuged twice at 4500 x g (4000 rpm) for 30 min, to remove any debris and small particles. The supernatant was transferred into clean Eppendorf barcoded tubes.

In cases where sweat samples were not sufficiently clear, those samples underwent two additional centrifugations, the first at 4500 x g for 20 min, and the second at 10,000 x g for 15 min. The supernatant was then transferred into a clean Eppendorf tube. All samples were stored at − 25 °C until assayed.

2.3.2. Extraction Protocol

Oxytocin extraction from relevant samples was conducted according to the recommended protocol by Oasis and others. We conducted similar protocols in our previous studies (Kor et al., 2022; Feldman et al., 2011), with several modifications. Samples were extracted on Oasis HLB cartridges 3cc/60 mg (Waters Oasis, MA, USA) which serve as a universal, reversed-phase sorbent for acidic compounds.

Cartridges were placed on a manifold, conditioned with methanol, and washed with water by introducing vacuum. Samples were acidified with 0.1% trifluoroacetic acid (TFA), ratio 1:1, centrifuged and the supernatant was loaded on cartridge, allowing gravimetry flow. Following this they were washed twice with 0.1% TFA and 10% acetonitrile solution, and eluted with 0.1% TFA and 80% acetonitrile, lyophilized, and kept at − 20 °C until assayed. Extractions were performed in duplicates. Efficacy was controlled with spiked samples and vacant samples. Lyophilized samples were reconstructed in the assay buffer immediately before analysis.

For recovery, spiked samples were prepared from ENZO EIA kit 10,000 pg/ml standard and diluted to the relevant concentration by the assay buffer.

2.4. Measuring Hormones

2.4.1. Measuring OT and TS in saliva and sweat

The concentration of OT and TS in saliva and in sweat was measured by using commercial Enzyme- Linked Immunosorbent Assay (ELISA) kits as we performed previously (Feldman et al., 2011; Gordon et al., 2017; Rassovsky et al., 2019; Weisman et al., 2012). OT was measured by three kits. Two were by ENZO (NY, USA). The first was ADI 901–153 (not available since 2013) and the second ADI 901–153 A. The third kit was by Cayman (Cayman Chemical, Ann Arbor, MI USA). TS was detected by a Salimetrics high sensitivity EIA Kit (CA, USA).

Measurements were conducted in duplicates, by following the instructions of the relevant kit. Three in-house controls were included in each OT plate (averaged: 27.0, 145, 250 pg/ml). In addition to the manufacturer's low and high TS controls (20.5 + 8.2 and 215.6 + 50, pg/ml), two in-house controls were included for each TS plate (85, 91 pg/ml). This action was taken to correlate between different plates that were measured at different periods. The concentration of OT or TS in the samples was calculated by using MAGELLAN software according to the relevant standard curves. When levels of OT or TS exceeded the calibration curve, samples were diluted by 1 to 100 or even by 1 to 1000 in the assay's buffer. OT and TS concentrations are expressed as picograms per milliliter – pg/ml.

The intra and inter assay Coefficients, of OT samples and controls, were less than 12.22% and 16.6% respectively. The intra and inter assay Coefficients, of TS samples and controls, were less than 5.70% and 7.43% respectively.

2.4.2. Validating the detection of OT

2.4.2.1. Comparison of OT concentration measured by ENZO and Cayman chemical EIA kits. Due to our awareness that the present study is preliminary, and to ensure that our finding of OT in sweat was not a mere artifact, we took the extra precaution of assaying an additional ten samples parallelly using the Cayman Kit. We were gratified to find that similar results were obtained by both kits. Averaged OT was 319.7 pg/ml for Cayman's kit, and 397.0 pg/ml for Enzo Kit., $r = 0.991$ $p < 0.001$.

2.4.3. Use of Extraction in the assaying of OT

The necessity of extraction was examined by comparing extracted samples with non- extracted samples, as can be seen in Table 2. We tested the buffer and spiked samples with a known concentration of OT (Table 2A); saliva samples (Table 2B); and sweat samples (Table 2C). These samples were pooled to get enough volume for extraction (at least 1.4 ml) as well as for detecting without extraction. We present the result of extraction of two sets of blood samples (Table 2D) tested by ENZO Kit ADI 900–153 A, and (Table 2E), measured during research done in the year 2011, by ENZO Kit ADI 900–153. These blood samples were collected as described in our previous studies (Kor et al., 2021; Schneiderman et al., 2012, 2014).

For all these, recovery was expressed as the yield of extraction, which is the ratio between extracted and non-extracted OT concentrations, as expressed as a percentage.

2.5. Data Analysis

Statistical analyses were conducted by using IBM SPSS 22.0 Statistics software. For the statistical analysis to be done with the use of normal distribution assumptions, the concentrations of OT and TS were log transformed. To test the relationship between hormone levels of OT and TS in saliva and sweat (during exercise) and personality characteristics (emphasizing extroversion as measured by the EPQR questionnaires), we calculated a bivariate zero-order (Pearson) correlations test and paired T-Test. Since TS manifests differently depending on gender, peripheral hormonal measurements were compared across subjects and

Table 2
Extraction and recovery of Spike, Saliva, Sweat and Bloods samples.

A	OT, pg/ml		
Sample	Target	Extracted	Recovery, %
Eluant (ACN 80%)	Control	<Min	
Assay Buffer	Control	<Min	
Assay Buffer	Control	<Min	
Spike 100 pg/ml	Expected 100	90	90
Spike 150 pg/ml	Expected 150	103	68.7
Spike 200 pg/ml	Expected 200	193	96.5
Spike 200 pg/ml	Expected 250	202	80.8
Spike 250 pg/ml	Expected 250	227	90.8
Spike 250 pg/ml	Expected 250	244	97.6
Spike 250 pg/ml	Expected 250	187	74.8
Spike 250 pg/ml	Expected 250	189	75.6
Spike 400 pg/ml	Expected 400	396	99
Spike 400 pg/ml	Expected 400	371	92.8
Spike 500 pg/ml	Expected 500	461	92.2
Spike 500 pg/ml	Expected 500	403	80.6
Spike- Recovery			**86.60%**
B	**OT, pg/ml**		
Sample	**Direct measurment**	**Extracted**	**Recovery, %**
Saliva-01	547	512.8	93.7
Saliva-02	505	467.2	92.5
Saliva-03	81.2	67.9	83.5
Saliva-04	77.7	63.6	81.8
Saliva- Recovery			**87.90%**
C	**OT, pg/ml**		
Sample	**Direct measurment**	**Extracted**	**Recovery, %**
Body Sweat Pool-11	66	60.9	92.3
Body Sweat Pool-12	20	<Min	
Body Sweat Pool-13	22.9	<Min	
Body Sweat Pool-14	20	<Min	
Body Sweat Pool-15	22.1	<Min	
Body Sweat Pool-16	19.1	<Min	
Axillary Sweat Pool-21	154	133.2	86.5
Axillary Sweat Pool-22	133	120.8	90.8
Axillary Sweat Pool-23	36.7	25.6	68.1
Axillary Sweat Pool-24	50.4	35.8	70.2
Axillary Sweat Pool-25	57.4	48.2	84
Axillary Sweat Pool-26	55.1	45.7	82.9
Axillary Sweat Recovery			**82.5%**
D	**OT, pg/ml**		
Sample	**Direct measurment**	**Extracted**	**Recovery, %**
Plasma-31	4127.5	367.3	8.9
Plasma-32	2858.2	592	20.7
Plasma-33	3256.3	457.9	14.1
Plasma-34	1678	539.1	32.1
Plasma Recovery			**19.0%**
E	**OT, pg/ml by ADI900–153 Kit**		
Sample	**Direct measurment**	**Extracted**	**Recovery, %**
Plasma-41	369.1	431.3	116.9
Plasma-42	356.2	327.95	92.1
Plasma-43	270.3	266.8	98.7
Plasma-44	568.3	415.7	73.1
Plasma Recovery			**92.5%**

Panels A, B. C, D measured by ENZO ADI900-153A Kit, whereas E measured by once available ENZO ADI900-153 Kit, which is not available since 2013.

were then compared separately for males and females. One-Way ANOVA examined the effects of high and low extroversion (using the median split) and solitary versus social exercise on levels of hormones.

3. Results

3.1. Detection of OT in axillary sweat

Since this is the first attempt to measure OT in human sweat, we began with a pilot study to test whether physical activity can elicit sufficient sweat for OT measurement in human axillary sweat and, if so, what type of physical activity are required to do so. We recruited 50 participants, who performed physical activity of their choice, and collected axillary sweat every 10 min. We also collected their saliva before and after the physical activity. Of the fifty participants, 11 provided a substantial amount of axillary sweat after 25 min of aerobic activity. The average sweat OT levels, in pg/ml, were M = 124.9, SE = 22.9 significantly higher than saliva samples, Table 3.

3.2. Extraction- Is it a prerequisite?

To examine whether or not extraction of samples is necessary prior to

Table 3
Statistical values and analysis for all studies.

OT levels in saliva and sweat, pg/ml					Statistical Analysis		
Study	**Samples**	**n**	**M**	**SEM**	**Correlates**	**Pearson**	**T- Test**
Pilot Study							
	Saliva Baseline	11	35.7	7.5	Sweat & Saliva Baseline	r = .570, p = .067	$t_{(11)}$= 7.113, $p < 0.001$, Cohen's d= 2.145
	Saliva Post	10	46.1	8.9	Sweat & Saliva Post	r = .432, p = .213	$t_{(10)}$= 5.037 $p < 0.001$, Cohen's d= 1.593
	Axillary Sweat	11	124.9	22.0			
Experiment 1: Body Regions					**Correlation to axillary sweat**		
	Axillary Sweat	14	116.1	14.6			
	Forearm Sweat	14	54.5	8.0	Forearm Sweat	r = .501, p = .041	$t_{(13)}$= 5.177 $p < 0.001$, Cohen's d= 1.436
	Forehead Sweat	14	62.6	11.5	Forehead Sweat	r = .365, p = .100	$t_{(13)}$= 3.643 $p = 0.001$, Cohen's d= .974
	Upper-chest Sweat	14	45.6	6.9	Upper-chest Sweat	r = −.030, p = .461	$t_{(13)}$= 4.595 $p < 0.001$, Cohen's d= 1.193
Experiment 2: OT Administration							
	Saliva Baseline	15	71.6	9.7			
	Saliva 15 min	15	39033.9	27541.5			
	Saliva Post	15	10246.7	8989.7			
					Correlation to axillary sweat:		
	Axillary Sweat	12	26487.7	15382.0			
	Forehead Sweat	10	6395.1	4075.6	Forehead Sweat (9)	r = .719, p < .01	$t_{(9)}$= 2.69 $p < 0.05$, Cohen's d= .851
	Upper-chest Sweat	10	4176.4	2077.7	Upper-chest Sweat(10)	r = .541, p = .066	$t_{(8)}$= 2.684$p < 0.05$, Cohen's d= .895
Experiment 3: Naturalistic Study							
T1	Saliva Baseline	79	56.65	7.15			
	Saliva Post	79	102.9	14.96	Saliva Post & Baseline	r = .564, p < .001	$t_{(78)}$= 5.64 $p < 0.001$, Cohen's d= .634
	Axillary Sweat	68	134.4	31.72	Sweat & Saliva Post	r = .373, p = .746	$t_{(78)}$= 1.652 $p = 0.103$
T2	Saliva Baseline	83	65.6	7.31			
	Saliva Post	83	91.23	93.51	Saliva post & Baseline	r = .564, p < .001	$t_{(82)}$= 2.992 $p < 0.001$, Cohen's d= .328
	Axillary Sweat	78	169	43.24	Sweat & Saliva Post	r = .406, p = .812	$t_{(78)}$= .215 $p = 0.831$
					Inter- experiments Correlations:		
					Saliva Baseline T1& T2	r(79) = .599, p < .001	$t_{(78)}$= 2.59, p = 0.034
					Saliva Post T1 & T2	r (80) = .519, p < .001	$t_{(79)}$= 0.523, p = 0.602
					Sweat T1 & T2	r(67) = .396, p < .001	$t_{(66)}$ = 0.437, p = 0.664
TS levels in saliva and sweat, pg/ml							
Experiment 2: OT Administration							
	Axillary Sweat	7	2111.0	287.0			
	Forearm Sweat	7	935	70.9			
	Forehead Sweat	7	329.4	18.5			
	Upper-chest Sweat	7	529.5	96.3			
Experiment 3: Naturalistic Study							
T2	**Women**						
	Saliva Baseline	35	121.2	10.44			
	Saliva Post	35	165.3	10.79	Saliva post & Baseline	r = .593, p < .001	$t_{(34)}$= 4.519 $p < 0.001$, Cohen's d= .764
	Axillary Sweat	35	1488	10.44	Saliva & Sweat post	r = .397, p < .05	$t_{(34)}$= 17.170 $p < 0.001$, Cohen's d= 2.903
T2	**Men**						
	Saliva Baseline	48	232	12.37			
	Saliva Post	48	342.1	16.92	Saliva post & Baseline	r = .342, p < .05	$t_{(48)}$= 6.79 $p < 0.01$, Cohen's d= .980
	Axillary Sweat	48	4862	1277	Saliva & Sweat post	r = .363, p < .05	$t_{(34)}$= 9.780 $p < 0.01$, Cohen's d= 1.411
					OT to TS samples correlations		
					Saliva Baseline	r(83) = .122, p = .183	
					Saliva Post	r(78) = .007, p = .951	
					Axillary Sweat	r(78) = .122, p = .288	

Log transform, of hormones concentration, implemented for statistical analysis

the detection of OT by an EIA kit, we compared extracted saliva and sweat samples with non-extracted samples. To achieve an adequate volume for sweat analysis, we created a pool of sweat samples taken from several individuals. Table 2 demonstrates that when the concentration of samples is higher than 22 pg/ml, the recovery yield is similar for OT in both spiked, saliva, and sweat samples, amounting to 87.9%, 82.5% and 86.6% respectively. Therefore, we carefully concluded that for determining OT by EIA, extraction is not an essential step. To illustrate when extraction is a "must" step before analysis, we present data from previous blood analysis conducted in our lab on Table 2 D and 2 E. We show here that for blood samples extraction is essential when OT is measured by the new ENZO kit (Table 2 D). In this case, OT without extraction was abnormally high, in the range of the thousands, and the recovery yield was very low, standing at 19.0%, which indicates sample interference. In contrast, for the previously available ENZO kit 901–153, extraction was not required (Table 2 E), as can be seen from the high recovery yield of 92.5%. Therefore, in the current research, we assayed OT by EIA without extraction.

3.3. OT is detected in sweat collected from various body regions

3.3.1. Experiment 1

The next experiment aimed to pinpoint the body surface region that provides samples with the highest OT concentrations. To this end, sweat was collected by 14 trainees, who cycled indoors, from various body regions (the underarm, forehead, upper chest and forearm) throughout their workout. Table 3 presents the OT values for these regions. Of note is that axillary OT is significantly higher compared to other tested regions and that no correlation was found between axillary OT and OT in other sweat regions (Table 3). We wish to emphasize that there were cases in which we could not detect OT in non-axillary sweat regions. We cannot exclude the possibility of overcollection from these areas. It

might be that more is less, as is the case when collecting saliva, for which there is an optimal amount (0.5 up to 2 ml) for the detection of the highest OT levels. In our experience sample volumes beyond 2 ml yield lower levels of OT in serial collection (data not shown). It might be the same case for sweat, that initial drops of sweat contain higher concentrations of OT, meaning that collecting excessive sweat from the same region might result in the detection of biased low OT.

3.3.2. Experiment 2: OT administration study

To further reveal the optimal region from which the highest concentration of OT can be sampled, we administered OT intranasally and measured OT and TS in post-exercise sweat from the forehead, upper-chest, arms, and axillary sweat. We also measured OT and TS in saliva at baseline (before OT administration), 15 min post-administration, and after exercise. As expected, OT administration markedly increased peripheral OT in both sweat and saliva. Salivary OT increased pre-activity and remained high post-activity (Table 3). Post-activity sweat samples (collected 45–60 min after initiation of workout) showed sweat in the facial forehead region, the upper part of the chest, arm, and underarm. Of these areas, axillary sweat contained significantly higher levels of OT (Table 3). Only half of those who produced axillary sweat managed to provide sweat from the forearm and therefore these data are not presented in Table 3. TS levels, in pg/ml analogously to OT, were similarly highest in axillary sweat (Table 3).

In summary, for the purpose of the current research, we collected sweat samples from the axillary and instructed subjects to provide sweat with a specific volume range.

3.4. Experiment 3 Naturalistic study; OT and TS in saliva and sweat

Eighty-four individuals participated twice in an aerobic physical activity. At each time point (T1 and T2) they provided two saliva samples, one at baseline and another post exercise, as well as a sweat sample post activity.

OT: Physical activity increased salivary OT at both T1 and T2. Within each time-point, there was a high correlation between salivary OT levels at baseline and post exercise (Table 3), suggesting high individual stability. There were no correlations between the sweat and post-exercise saliva samples. The average baseline and post-exercise OT levels for both times were M (162) = 61.25, SE = 5.11 (baseline) and M (163) = 97.01, SE = 8.8 (post-exercise). There were no observed mean-level differences between T1 and T2 times for saliva OT baseline and post exercise or in sweat OT (Table 3). This further supports the individual stability of the OT measurements.

Testosterone: TS was measured only at T2 of the study. Results (Fig. 1 and Table 3) show that physical activity significantly increases salivary TS for women and men. Additionally, TS concentrations in sweat were significantly higher than those found in post-activity saliva for both women and men (Table 3). Correlations between OT and TS were not significant for salivary assessment at baseline and post-exercise of for sweat.

———Insert Table 3 here———

3.5. OT and TS levels as a function of solitary versus social training

Interestingly, solitary training induced significantly higher levels of sweat OT than social training.

F (1,76) = 5.073, p = .027. Axillary OT in solitary workout was M (43) = 234.2, SD = 73 and in social work out M (39) = 97.0, SD = 40.9 (Fig. 2). We then examined the joint association of personality traits and training context with OT in axillary sweat using univariate ANOVA. No personality-related findings emerged for neuroticism. With regards to extroversion, results (Fig. 3) showed a main effect for solitary/social training, F (1,76) = 7.07, p = .01; no main effect for high/low extroversion, F(1,76) = 2.03, p = .158; and a significant interaction effect between the two, F (1,76) = 4.21, p = .04. Examination of the means

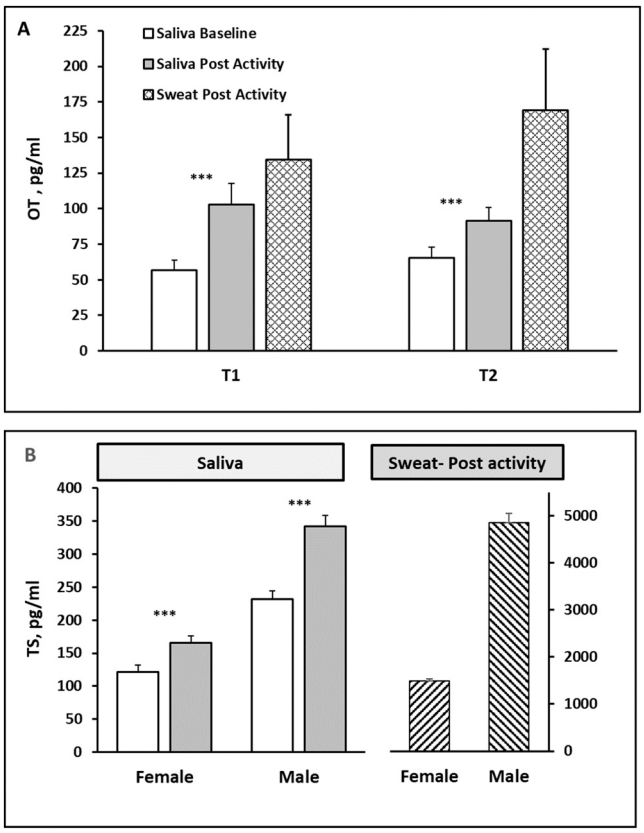

Fig. 1. OT and TS in saliva and sweat in response to physical activity. The Salivary baseline and saliva post activity and sweat levels of OT (A) and TS (B), of experiment 2. The OT levels of all the participants in T1 (left panel) and T2 (right panel). While the TS of male and female saliva (left panel) and the sweat (right panel) of experiment 2 T2 were measured. Saliva pre-activity, post activity, and sweat post activity represented in white gray and cross lines respectively. Results presents in average and the error bar as SEM, significancy * ** $p < 0.001$.

show that the highest level of axillary sweat during aerobic training was found among individuals high in extroversion who are working out alone. We want to emphasize that these results are preliminary and require further research. We did not have directional a-priori hypotheses and the findings do not stand correction for multiple comparisons. Since this is the first study on OT in sweat, we bring these results as a highly tentative ground for future research on the associations between sweat OT and personality.

Similar analyses were conducted for TS for women and men separately. We found no main or interaction effects for solitary/social training, extroversion, neuroticism, or their interaction for either gender.

4. Discussion

To the best of our knowledge, this is the first study to show that OT is present and can be measured in human sweat. By detecting OT in sweat, we have extended the sources of OT measurement in humans from exclusively internal sources to include a source that is peripheral to the body. Our finding may therefore lead to expanding the role for OT in human social life beyond those which have been previously conceptualized. It is possible that OT concentrations in the periphery, in fractions such as sweat, provide a template for multiple processes that underpin social communication, interpersonal transmission, and group coordination. As such, our findings open a wide field for future research on the role of human sweat OT in a variety of social and affiliative processes between partners and among social groups ranging from small families

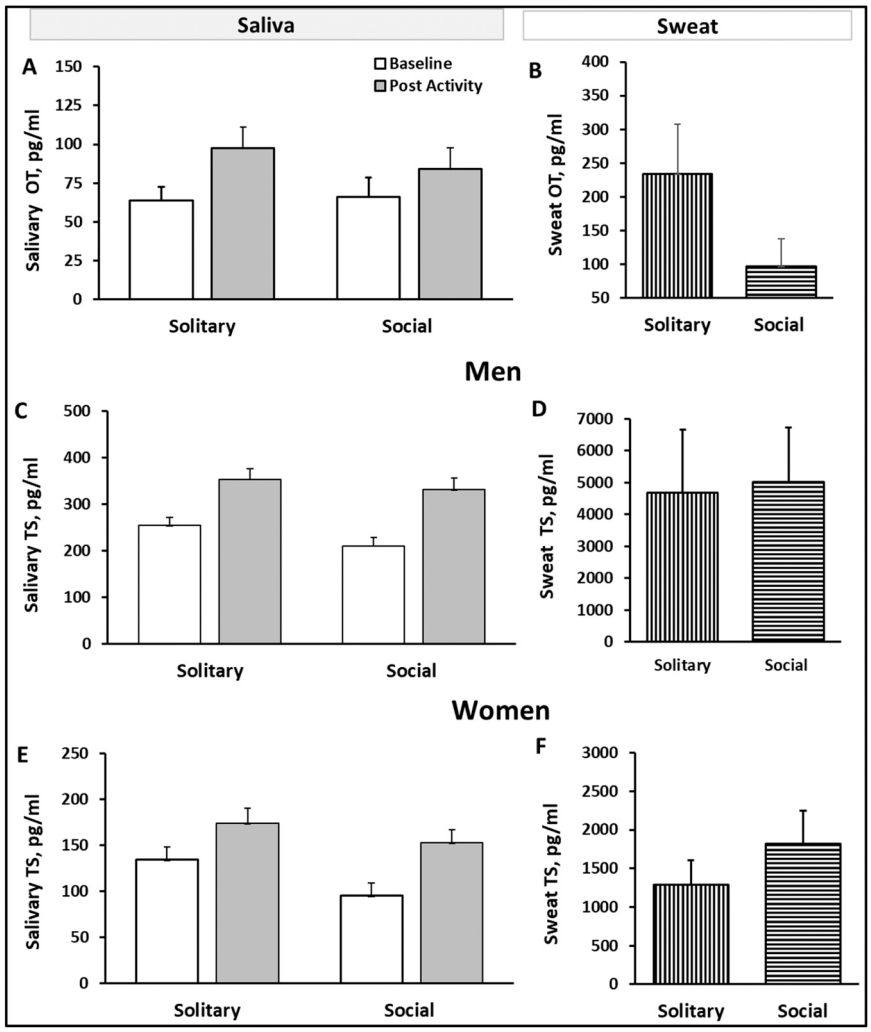

Fig. 2. OT and TS in response to physical activity in solitary or social training. The salivary baseline and post training and sweat post training of OT and TS levels of according the social condition; solitary or social. A) salivary OT, B) Sweat OT, C) Salivary Male TS, D) Sweat male TS, E) Salivary female TS, F) sweat female TS. Saliva baseline (white), post activity (gray), and sweat post activity (slanted lines) are represented. Results presents in average and the error bar as SEM.

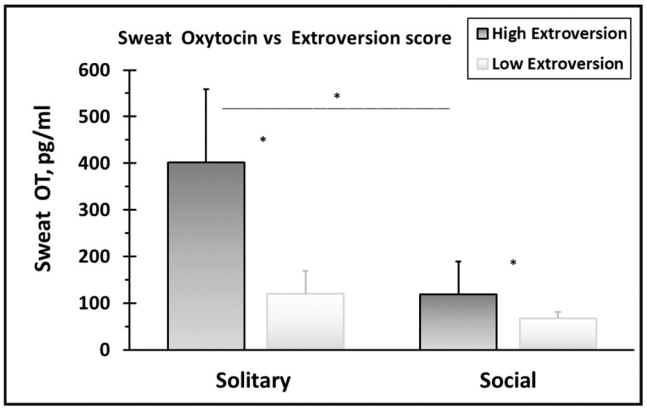

Fig. 3. Sweat OT in solitary or social training, of high and low extrovert trainees. Sweat OT levels according to the social conditions and extrovert score, highest half (dark grey) or lowest half (light gray). Results presents in average and the error bar as SEM. * $p < 0.05$.

to communities to inhabitants of entire ecologies.

Due to the novelty of the current research, we began our project with a pilot study to determine what types of physical activity would produce sufficient volume of sweat, which part/parts of the body could provide an effective source of sweat, and by what means. From the first part of the pilot, we learned that there are adequate amounts of underarm sweat for measuring OT by EIA that can be collected in test tubes following at least 25 min of aerobic activity or intense cardiovascular activity. We chose to collect sweat resulting from physical activity and not from wearing heavy clothes because the latter induces thermoregulatory perspiration from both eccrine and apocrine glands which may exhibit different hormonal patterns. We succeeded in detecting OT in all axillary sweat samples without exception. In addition, we found that levels of axillary OT post-activity were higher than those detected in saliva.

There is an ongoing debate in the field of OT measurement whether extraction is essential for determining OT in biological samples (MacLean et al., 2019; Gnanadesikan et al., 2021; Gan et al., 2023). Our laboratory has measured OT for two decades and we are aware of the necessity to extract blood and urine samples for measuring OT. The first kit we ever used by Assay Design (Feldman et al., 2007) did not require extraction for blood, but only for urine, samples. However, we learned that with the ENZO kit 900–153 A that was available since 2013 extraction is needed for measuring blood samples and a similar practice has been suggested by others for that kit (Gan et al., 2023; Gnanadesikan et al., 2021). In contrast, saliva sample did not required extraction at any point. The necessity of extraction for saliva and sweat samples was tested here by comparing the OT concentration of extracted and

unextracted samples.

Based upon these comparisons (Table 2) across fractions, we concluded that sweat and saliva samples can be measured directly with the appropriate steps of preparation (see Methods). We should note that the yield is in the range of spike samples, which confirms the reliability of the method. Another indication for the reliability of our measurement of OT in sweat without the need for extraction is that Cayman and ENZO kits yielded highly correlated values that were within the same range, providing further validation for the detection of OT in sweat. Gan and colleagues (2023) showed that there is no need for extraction in plasma when using the Cayman EIA OT kit.

The next question we addressed in our effort to measure OT in sweat is what body area was the best source from which sweat should be collected. To address this question, we compared sweat from underarm, forehead, upper chest, forearm. We conducted two tests; in the first we asked trainees to provide sweat while cycling indoor, in the second, we used an OT administration design. Intranasal OT administration is known to increase levels of OT in saliva (van IJzendoorn et al., 2012; Weisman et al., 2012; Shimon-Raz et al., 2021) and we show here, for the first time, that it also enhances OT levels in human sweat. We therefore used this enhancement to pinpoint those body parts for which OT elevation is most notable, as noted in both indoor cycling and following administration. Such findings can provide some indication of central-peripheral associations for our results on OT in sweat. Sweat was collected 45 min after OT administration (24IU of syntocinon) and 30 min from the beginning of exercise. We found that for individuals who provided sufficient sweat from the axillary- most of them provided sweat from forehead and upper chest regions, while a third provided from the forearm as well. These results indicate that axillary sweat yielded the highest OT values as compared to both forehead and upper chest sweat, suggesting that OT might be produced in apocrine sweat glands. Similarly, axillary sweat contained the highest TS levels as compared to forehead, upper-chest, and forearm sweat. This is in line with prior studies reporting that axillary perspiration of young men often contained extraordinary levels of TS and estradiol (E2) steroids, with average concentrations exceeding those in facial perspiration for both men and women (Elliott et al., 2017). The presence of apocrine sweat glands in the underarm may explain the high levels of hormones in axillary perspiration. Apocrine glands are located primarily in the underarm but are present in breasts, face, scalp, and the perineum as well. Particularly relevant to the current study is the fact that apocrine glands produce viscous, lipid-rich sweat, which is comprised of proteins, sugars, ammonia, as well as steroids (Baker, 2019).

OT and TS are considered to be "social hormones" and participate in multiple processes of sociality and affiliation, for instance, in parenting and parent-child attachment (Feldman, 2019; Weisman et al., 2014; Schneiderman et al., 2014; Gordon et al., 2017), early stages of romantic love (Schneiderman et al., 2014), long-term couple relationship, and close friendship (Djalovski et al., 2021). OT and TS also play important roles in general social functions, such as empathy, collaboration, and social group functions (Crespi, 2016). While OT and TS are often not directly correlated, they impact upon each other in multiple indirect ways that are mediated by contextual factors and personality traits. The abundance of OT and TS in underarm sweat may point to this channel as a potential pathway by which these hormones exert their social effects, and this hypothesis should receive much further research.

Interestingly, after intranasal OT administration, participants reported difficulty in performing their training with the typical intensity. Moreover, they felt a reduction in sweat secretion, and it took longer (up to an hour) to provide samples after exercising. This may be related to the anxiolytic effect of OT, which has been reported to reduce heartbeat and blood pressure (Buemann and Uvnäs-Moberg, 2020; Carter et al., 2020). OT at post administration and training showed a significant increase in salivary OT from baseline, consistent with our previous study (Weisman et al., 2012). In that study, we showed that salivary OT levels remained significantly high even after four hours in most participants, while several participants showed a decline already within the first hour. Similar variability among participants was detected in the current study and further research is required to examine the factors associated with such a rapid decline, whether they relate to personality traits, psychopathological conditions, or physiological factors.

The naturalistic experiment of sweat collection "in the wild" was conducted twice. In T1 all volunteers (N = 435) were asked to provide saliva at baseline and at post training as well as sweat at post training. Although all volunteers were given specific instructions to perform intense activity for at least 25 min, only 21.6% (94 of 435 volunteers) managed to provide adequate amounts of sweat. The findings that as many as 80% of the volunteers did not provide adequate amounts of sweat was somewhat surprising. It is important to note that, in this naturalistic experiment, participants performed their regular training in their natural environment. Therefore, we cannot exclude the impact of self-perception on the quality and intensity of their training and individual fitness. Perspiration is known to be influenced by various factors amongst them are the number and properties of various types of sweat glands (Baker, 2019). Men sweat more than women (during menses sweating is lower), adults sweat more than children, and athletes sweat more than non-active individuals (Baker, 2019).

At the second time-point (T2) of the naturalistic experiment, we recruited the 94 volunteers who successfully provided sweat at T1. These subjects were asked to perform the same activities as in T1 and, in addition, completed self-report measures. Of these subjects, only 84 were able to provide adequate amounts of sweat at T2. Of note, we found that OT in sweat was individually stable from T1 to T2, as was OT in saliva. Studies have shown individual stability in OT across measurement times that span weeks, months, and even years as seen in both plasma (Feldman et al., 2007, 2013) and saliva (Priel et al., 2019). In urine, individual stability was similarly found across several measurement points (Pratt et al., 2015). The individual stability in OT in sweat found here in assessments that took place several months apart indicate that OT in sweat yields similar stability. Overall, the individual stability of OT across fractions lends further support to the notion that OT is a "trait like" characteristic of the individual and can serve as a bio-index of sociality, affiliation, and personality (Feldman, 2012, 2016).

We found no significant correlation between axillary perspiration and saliva OT or TS. Our findings are consistent with previous research showing that, whether or not and to what degree these hormones are correlated, may differ according to the bodily fluids being tested. To illustrate, previous research did not find any correlation between axillary and urinary TS (Elliott et al., 2017), urinary and salivary OT (Feldman et al., 2011) or urinary and blood OT (Feldman et al., 2011). Alternatively, studies found correlations between blood and salivary OT (Feldman et al., 2011), CSF and saliva OT (Martin et al., 2018); and CSF and blood OT (Martin et al., 2018). The discrepancies between levels of hormones, such as OT and TS, may be due to variations in their production sites as well as in their distribution across different body fluids. The local production of OT on skin cells (Deing et al., 2013) might contribute to this phenomenon.

Our findings highlight the influence of the social context on OT and TS production. Salivary OT and TS at post-raining increased both for the solitary and the social conditions. As opposed to salivary OT, axillary OT was significantly higher in the solitary compared to the social training. We anticipated sweat TS to be influenced by the social condition. This was based on results from studies showing the impact of the social environment on steroid levels. For example, men who interacted with women exhibited significant elevations of salivary TS relative to both their own baseline concentrations and to the scores difference among the men who interacted with other men (Roney et al., 2007). In addition, experimenter gender affected axillary TS and E2 levels in males. The literature underscores the need to consider situational variables and to standardize methods of perspiration sample collection for steroid analysis (Elliott et al., 2017). The notion that axillary TS may be influenced by group versus solitary activity was not upheld, which might have been

due to insufficient sample size. Zouboulis and colleagues (2007) reported that the skin locally synthesizes significant amounts of sexual hormones with intracrine or paracrine actions. The local level of each sexual steroid depends upon the expression of each of the androgen and estrogen synthesizing enzymes in each cell type, with sebaceous glands and sweat glands being the major contributors. However, DHEA, androstenedione, and possibly DHEA-S as well, can be converted by sebocytes, sweat glands, and probably dermal papilla cells as well, into more potent androgens such as testosterone and DHT. All the above indicates that the lack of correlation between salivary and sweat OT and TS might be derived from local production of these hormones (Zouboulis et al., 2007).

Our finding on the presence of OT in human sweat may support the proposition of local OT production at the skin level (Grinevich and Charlet, 2017). Skin is an intricate self-renewing organ that serves as the primary defense barrier against a hostile environment. It protects against harmful antigens and chemicals, dehydration and overhydration, and ultraviolet radiation. It provides structural integrity and resilience, allows selective absorption and antioxidant storage, controls thermoregulation through fluctuations in cutaneous blood supply and perspiration, and stimulates epidermal regeneration when injured (Abdo et al., 2020). Our findings may open a new way to explore how OT may have a non-centrally modulated role in social interactions both proximally (i.e. though touch), and, perhaps even more importantly, non-proximally.

Our post-hoc analysis showed that individuals with high trait extroversion and who exercised alone exhibited axillary OT levels four times greater than who scored low extrovert subjects (Fig. 3). It is important to note that these finding should be treated with caution, as we did not have a priori hypothesis and the findings may not stand multiple correction, and thus much further validation is needed. These tentative findings may further indicate that biology and personality are intertwined so that a person extrovert in character secretes more substances in his sweat. Further research is needed to determine whether OT in sweat is associated with personality traits and the social setting, as it is known to be the case for steroids hormones (Elliott et al., 2017).

Several limitations of this study should be noted. First, there is a minimal amount of sweat that the body needs to produce for the assay of OT. Only 20% of the participants were able to collect the quantity of sweat required for analysis. Another major limitation is that the study was conducted in a naturalistic setup where each participant performed his/her regular activity, and we did not have control over the degree of intensity nor duration of training. In future, we aim to establish a uniform protocol to enable control over the setting, type of activity, and social context. Additionally, due to the complexity of the interactions being examined, the number of conditions and the sample size may not have been enough to reflect the hormonal behavior. For example, we expect different patterns for men and women according to gender and the gender they exercise with. Our study grouped the participants according to gender and social condition during exercise (solitary versus social). With a total sample size of 82 participants in the main experiment, there were only 15–27 individuals in each group. Further division into more homogenous groups may have been illuminating and we hope to look at those in future research.

In sum, the current study is the first to clearly demonstrate the presence of OT in human sweat. OT levels in saliva increased under all conditions of physical activity, both solitary and social, whereas OT levels in sweat were higher while training in solitary conditions as compared to social conditions. Overall, our results suggest that OT in sweat is sensitive to factors such as social contexts and personality traits. Much further research is needed to elucidate the associations between OT in human sweat in relation to a variety of naturalistic ecologies, social contexts, personality factors, and psychopathological conditions.

Declaration of Competing Interest

Drs. Zagoory-Sharon, Levine, and Feldman declare no conflict of interest.

Acknowledgement

The study was supported by the Simms/Mann Foundation.

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