

Level of oxytocin prior to rugby and handball matches: An exploratory study among groups of Polish players

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ABSTRACT: The aim of the present exploratory study was to assess the changes in urinary oxytocin (OT) concentration during the period between five days before, and on the day of match, among rugby and handball players. Nine male rugby players with a mean age of 27.62 years (SD = 4.21) and 18 male handball players with a mean age of 17.03 years (SD = 0.57) participated. Urinary oxytocin level was measured by ELISA immunoassay as a ratio to the concentration of creatinine [mg/ml] measured through colorimetric detection. The relative level of OT to creatinine (OT/CRE) significantly differed between the type of player (rugby or handball) but not between times of measurements. Significant differences were only between OT/CRE level in a day of match in rugby players and in 5 days before match in handball players ($p < 0.05$). There was no change in oxytocin levels during the time periods between five days before and on the day of a match, in either of the two kinds of players. The change in oxytocin might be traceable during the match but not before a match and this perhaps depends on a more subtle context of competition, but not on the assumption of competition. Further studies are needed based on more homogenous group with higher number of matches.

KEY WORDS: oxytocin, sports, rugby, handball, competition.

Original article

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Introduction

Oxytocin (OT), the endogenous neuropeptide performing peripheral role as a hormone, is mostly known for facilitating uterine contractions during labour, facilitating maternal behaviour (Rilling et al. 2012), and also for initiating parenting behaviour (Gordon et al. 2010). However, OT has a broader role exerting modifying effects on human social cognition and behaviour (Bartz et al. 2011) that increase benefits of social interaction and promote social approach and affiliation (Heinrichs and Domes 2008; Gilbert and Basran 2019), empathy (Geng et al. 2018), build trust (Kosfeld et al. 2005), attachment (Donaldson and Young 2008) and group cohesion (De dreu et al. 2010; 2011) in humans and non-human animals (Rilling et al. 2012; Bartz et al. 2011; Chang et al. 2013; Neumann and Slattery 2016). The critical and intricate role of oxytocin has been implicated in the formation and maintenance of social groups by modifying several behaviours and human cooperative traits that are important in effectively functioning in a group (De Dreu 2012; Choi and Bowles 2007; Bowles 2009). However, there are recent reports that administration of oxytocin increased aggressive behaviour (Ne'eman et al. 2016) and that OT receptor gene polymorphism has association with aggression (Butovskaya et al. 2020) that might be important in combat with out-group enemies. According to a theoretical framework, OT modulates social behavior by means of increasing the salience of social stimuli and perhaps promotes a wide range of emotions and behaviors and not merely the positive and affiliative ones (Shamay-Tsoory and Abu-Akel 2016). Therefore, if indeed OT increases the salience of social agents, a plausible as-

sumption is that it will increase aggressive reactions in competitive situations involving aggressive provocations (Ne'eman et al. 2016). In a study of intergroup conflict in wild chimpanzees Samuni et al. (2016) reported that OT levels were elevated immediately before and during intergroup conflict compared with controls.

Human inter-group conflicts are part of our evolutionary history and success in such conflicts depends upon several adaptive mechanisms. Sports and games sometimes posit on humans a challenge situation that mimics inter-group conflict, as well as intra-group coordination to win over the opponent (Muñoz-Reyes et al. 2020). Sporting activities are very often chosen as a proxy to human combative situations. The sport competition model may involve several adaptive mechanisms, including aggression, and this model has been used in studies related to endocrine response or prenatal hormone exposure (Bogin et al. 2016; Longman et al. 2011). OT has been linked with human prosocial behaviour (Romney et al. 2019). On the other hand, there is evidence for prosocial behavior in enhancing team performance in sport (Moll et al. 2010). In a review, after considering several previous evidences, it was proposed that OT could be the bridging link between certain kinds of empathy as well as emotion transfer and enhanced group performance in team sports (for detailed review see: Pepping and Timmermans 2012).

Success in sports has been often linked with motivation and positive emotions (McCarthy 2011). Prosocial behaviours, such as the high-five, the fist-pump, and the group hug, during a game, remain staple elements of success in sporting life (Kraus et al. 2010). These behaviours may enhance OT (Uvnäs-Moberg et al. 2015). But it is not known if such increase also oc-

curs as a preparatory mechanism for a motivational adaptation among elite sports persons before a match. Pre-match elevation of OT might be important for soothing of stress and enhancing empathy and greater receptivity for collective emotions for teammates or rivalry against opponents.

With such background information, the present exploratory study was conducted with the aim to observe if some change occurred in urinary OT concentration between two occasions, 5 days before, and on the match-day, among the selected groups of rugby and handball players in Wroclaw, Poland. The primary objective was to test if OT level would increase before the match or not, as the increase would be indicative of some change in oxytocinergic system. For this primary exploration, the present study chose rugby and handball matches as these are known to regularly generate high level of competitive excitement both among the players and the spectators. Rugby involves high level of contact, aggressive interactions, and hence high prevalence of injuries; handball on the other hand requires specialized team coordination (Uvnäs-Moberg et al. 2015). In this study, these two kinds of sports were chosen to proxy high aggressive competition and sociability, respectively. Although the study was a preliminary exploration, the findings were expected to set a direction towards further research on the adaptive response to a forthcoming challenge situation and building a capacity for enhanced team cooperation through a biological pathway of oxytocinergic response.

Materials and Methods

This preliminary exploratory study followed a repeated measures design of the outcome variable (OT) at two subsequent

points of time. Nine male rugby league players with a mean age of 27.62 years ($SD = 4.21$), participating in games of regional rugby league (Lower Silesia), and 18 male handball players with a mean age of 17.03 years ($SD = 0.57$), taking part in games of academic league, were included in the study. All participants provided informed consent before participation. The rugby players were included in the study in a competition season during the matches of Second Polish League of Rugby. At this time, the player-participants were undertaking two trainings per week. The handball player-participants were attending the Junior Sport Championship School in Wroclaw, undertaking five training sessions a week, and at least, 10 hours per week of physical education lessons in school. Their handball teams were ranked 3rd–4th among 10 teams in the Third Senior League in Poland. Thus it was assumed that the participants were all potentially dedicated and whole time players.

The urine samples for study were collected by the participants on their own at their respective homes. They collected the morning samples of urine in two occasions, 5 days before and on the day of match. For this purpose, sterile plastic containers (100 ml) were distributed to each participant before the day of collecting sample. They were asked to collect the samples of the first urine in one sterile container after waking up in the morning. After collection of these containers from the participants on the same day, each sample was stored in two sterile plastic tubes (30 ml) at minus 20° Celsius temperature till the laboratory analyses were conducted. The rugby players were followed for 3 matches (during April and May 2017), whereas handball players for 2 matches (during October 2017). Together, the 112 urine samples were

collected, 51 from rugby and 61 from handball players.

After thawing, the samples were centrifuged at 1500 x g for 10 minutes at 4° Celsius temperature and supernatants were collected. OT concentration was estimated by ELISA immunoassay (Catalog #: ADI-901-153A; Enzo Life Science, USA). Creatinine (Cr) concentration was measured with the Creatinine Colorimetric Detection Kit (Catalog #: ADI-907-030A; Enzo Life Science, USA). Both analyses were conducted according to the assay procedures described in provided manuals. All standards and samples in both assays were run in duplicate. We calculated intra-class correlations (ICCs) of methods of estimating oxytocin ($r=0.92$, $r^2=0.84$) and creatinine ($r=0.997$, $r^2=0.995$). Relatively high values of ICCs showed confirmed the high precision of estimation. OT concentration in urine samples was expressed as a ratio of OT to creatinine: OT pg/ creatinine mg (OT/CRE) and used in further calculations. Analyses were conducted in a laboratory of Institute of Immunology and Experimental Therapy, Polish Academy of Sciences by two trained members of the institute (AS, DL).

Statistics of mean, standard deviation (SD), median, maximum-, and minimum values were used to describe the measures of anthropometry as well as OT/CRE level. There was not any significant correlation between age and

OT level, neither in rugby players not in handball players. Differences in ratios of OT, between 5 days before and a day of match, were assessed by ANOVA with repeated measurements, where appropriate team and repeated estimation of OT were factors. Post hoc comparison was done of the means of Tukey's Test for unequal sample size. All calculations were done using STATISTICA 13.1.

The study protocol has been approved by the Senate Ethical Committee Scientific Research of the University School of Physical Education in Wroclaw. Although the study used non-invasive measures, the ethical guidelines as laid down by the Helsinki Declaration were sincerely adhered to (Goodyear et al. 2007).

Results

Table 1 shows the mean age, height, weight and BMI in Rugby and Handball players and the difference in these measures between these two groups of players as assessed by student t test. There were significant differences in age ($p<0.001$) and BMI ($p<0.05$), but not in height and weight. The rugby players were older and had higher BMI than the handball players.

Table 2 presents the descriptive statistics of the ratio of oxytocin-to-creatinine levels [OT/CRE, pg/mg] for each subject. For each rugby player six estimations were done, whereas for handball players only four.

Table 1. Descriptive statistics of age and body dimensions of the players

	Age		Height		Weight		BMI		
	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rugby	9	27.6	4.2	181.3	6.4	90.0	7.6	27.3	1.2
Handball	18	17.8	0.60	183.3	6.1	83.4	12.8	24.7	3.0
t-test		t=10.11; $p<0.001$		t=0.83; $p>0.05$		t=1.49; $p>0.05$		t=2.63; $p<0.05$	

Table 2. Descriptive statistics of the ratio of oxytocin-to-creatinine [pg/mg] of urine samples of all individual players collected at all occasions

Players' ID	N-urine samples	Mean	SD
Rugby (N = 52 samples)			
2	6	437.6	188.9
3	6	608.6	162.6
5	6	684.9	176.0
7	6	648.0	262.5
8	6	665.7	108.0
9	6	663.6	58.7
10	6	864.0	143.6
11	6	724.8	172.7
12	4	514.0	78.1
All rugby players	52	650.7	189.4
Handball (N = 61 samples)			
1	2	902.2	14.9
2	3	760.7	28.3
3	3	629.5	125.9
4	3	839.5	96.7
6	4	597.8	106.2
7	4	703.7	62.1
8	4	614.9	112.3
9	4	777.9	171.3
10	4	565.7	130.9
11	3	637.0	121.4
12	4	737.0	113.0
13	2	687.3	3.2
14	2	918.4	271.0
15	4	857.6	313.1
16	4	941.5	204.6
17	4	891.0	120.3
18	3	773.4	93.5
21	4	747.5	167.5
All handball players	61	748.8	172.5

Results of ANOVA revealed that only the type of player (rugby or handball) significantly differentiated OT/CRE levels, but no effect of repeated measurements were found (Table 3).

Tukey's post-hoc tests showed only differences between OT/CRE level in a day of match in rugby players and OT/CRE level in 5 days before match in handball players ($p < 0.05$).

Table 3. Results of ANOVA with repeated measurements of the oxytocin-to-creatinine ratio [pg OT/mg CRE] in 5 days and in a day of match among rugby and handball players (Size effect was expressed by partial eta square)

	5 days before		In a day of match	
	Mean	SD	Mean	SD
Rugby	672.21	196.52	625.78	171.13
Handball	754.74	194.16	743.21	167.73
	F	<i>p</i>	Partial η^2	
Players	6.63	<0.05	0.11	
Repeated measurements	1.17	=0.285	0.02	
Interaction	0.26	=0.610	0.005	

Figure 1 demonstrates the changes in the OT/CRE levels in each player between the two occasions, five days before match and on the day of match. In this

graph no definite pattern or regularity was observed indicating any difference in ratios 5 days before and on a day of match.

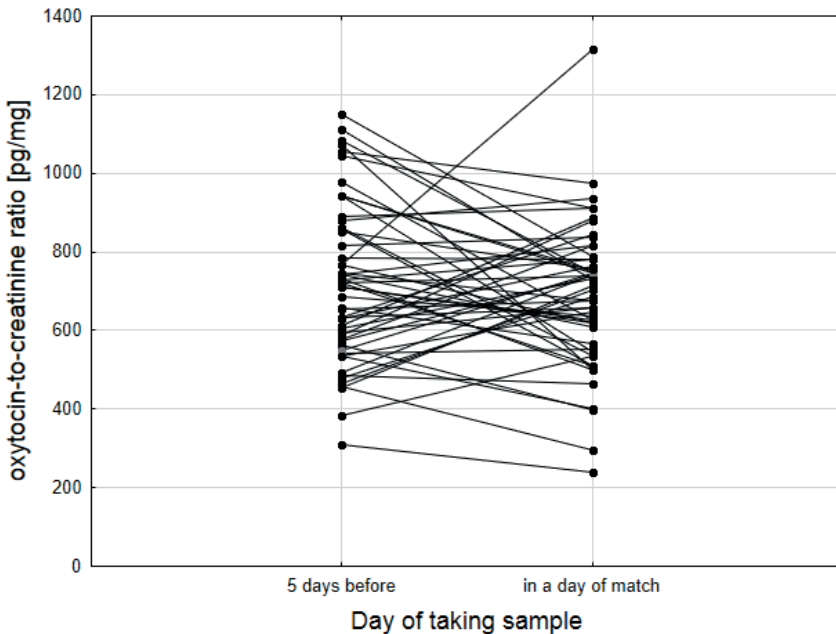


Fig.1. Changes of individual level of oxytocin-to-creatinine ratio [pg/mg] in all players estimated 5 days before and in a day of the match

Discussion

The effects of OT on adaptive social behaviour have been the topic of increasing scientific interest in recent years, both in animal and human studies. In the present study, we investigated whether urinary OT level among the players would rise up on the day, as compared to five days, before highly competitive rugby and handball matches. Results of the present study showed that the OT level differed between rugby and handball players. However, it did not differ between two time points, in either group or when participants from both sports were clumped together. There was no indication of any difference in OT between five days before and on a day of match.

Most previous research evidence was obtained in laboratory settings using intergroup social-dilemma games and focused on human male participants. Only a handful of studies have studied intergroup contexts in captive or wild non-human animals. In a study among wild chimpanzees, during intergroup conflicts in natural habitat, a significantly higher urinary OT level was recorded in both sexes immediately before and during the intergroup conflicts (compared to controls). Both anticipatory response to, and participation in the intergroup conflicts involved high urinary OT levels compared to control conditions (Samuni et al. 2016, 2018). The present exploratory research, however, could not measure OT concentration just before or after a match. But it at least, does provide a preliminary database up to a certain stage before competitive situation.

During intergroup conflict, elevated levels of both cortisol and OT are expected to have adaptive importance. Whereas the former facilitates rapid production

of energy required for combat or flight (McEwen 2007; Samuni et al. 2016), the latter may promote the essential intergroup cohesion leading to cooperative responses (De Dreu et al. 2010, Samuni et al. 2016). Studies on both humans and chimpanzees indicate an oxytocinergic system involvement during intergroup conflict (De Dreu et al. 2010, Samuni et al. 2016). It is widely believed that sports and games could closely imitate a real-life inter-group conflict and intra-group cohesion among humans (Muñoz-Reyes et al. 2020). It was therefore hypothesized that similar effect could also be visible shortly before participation in highly competitive games. In Poland, rugby is played by a very few teams and the handball players selected for this study were relatively top ranking. Since the participants were dedicated players, it was possible that they had developed ability to perform at high level of sports by several years of practice and adjustment to the specific requirements of the game. Based on the theoretical framework, we tried to explore whether they might show elevated OT a few days before a game, as a physiological response, that might support them in performance. However, the results of the present study did not provide supportive evidence. It showed no definite pattern or regularity of changes in OT level from five days before and on a day of match. In both types of games there were no significant differences between those two time points. The result was identical even when data for all players in two games were analyzed together.

There are several probable scientific reasons for the 'negative' result. With growing number of investigations on the effects of OT on behavior, cognition, and neuropsychiatry, it is becoming clear that its functions are far more complex than

initially was presumed (Bartz et al. 2011; Chang et al. 2013). OT release during intergroup conflict as observed by Samuni et al. (Samuni et al. 2016) was postulated to be triggered by the social context that was linked with the stressor rather than the stressor itself (Samuni et al. 2019). Accumulated evidence from several studies indicates that social contexts and individual factors, such as sex, early experience, or health, influence oxytocinergic effects (Bartz et al. 2011; Rilling et al. 2012). Perhaps, the context before actual commencement of the matches, or during the matches, such as, physical-aggressive contact during the match, would raise OT levels. Unfortunately, we could not measure OT/CRE ratios during or after the game.

Several studies, e.g., Striepens et al. (2011), suggested that OT mediates behaviors which are mainly prosocial. Nonetheless, other studies from animal research contradicted this by showing that OT can also enhance anxiety (Guzmán et al. 2013). Similarly, among humans, recent studies demonstrated its effects that might promote conflict rather than cohesion. For example, OT was found to increase envy and gloating (Shamay-Tsoory 2009), decrease trust and the inclination to cooperate in individuals with borderline personality disorder (Bartz et al. 2011), and facilitate out-group derogation (De Dreu et al. 2011). Thus, although OT is widely viewed as a prosocial compound, it may also promote antisocial responses, thus suggesting a context-dependent effect (Goodson and Thompson 2010). Thus, it seemed that OT level is not raised, at least, at this stage (before a few hours of match), but maybe just before game or during game. Instead, prosocial behaviors, such as mutual touching and hugging among teammates during the game (context) might increase the OT level (Kraus et

al. 2010; Uvnäs-Moberg 2015). Although sports and games could be suitable proxies for real-life competitions (Longman et al. 2011), there are certain differences between sport, game, and real-life challenges. It would be ideal to measure OT in soldiers at war, as was done for fighting chimpanzees. The present study, however, showed that although some oxytocinergic response during preparatory period of a competitive sport was assumed, it might not have been activated even a few hours before the match. The social context immediately before a conflict to protect territory among the chimpanzee is expected to be very different from that of a human sports person before playing in a match. The game was only a part of their life but for chimpanzees the fights are to protect their entire world. Studies on animal models have also demonstrated that social context might modify the regulating effect of OT on social play (Bredewold et al. 2014).

Several limitations of the present study should also be worth discussing. First, this study was an exploratory attempt with limited resources. The sample sizes were small. Secondly, we compared players from different sports disciplines that might have different effects. The two teams also had different popularity and ranking scores, and perhaps, not exactly comparable. Third, we could not collect reliable data on the extent of training, how often the players worked in group, the immediate social conditions, the exact activities during a certain period before sample collection, etc. These factors including individual characteristics could have confounding effects (Bartz et al. 2011). Fourth, the players of handball and rugby were followed in different seasons of the year. Thus, potential seasonal variation in OT also could be responsible

for lack of relationship and differences between sport groups. Fifth, the differences in OT between two groups also could be age related since there was also significant age difference. Despite these inherent limitations, this study, with its purely exploratory objectives, provided the first preliminary information on the topic. Future research might best be conducted on several teams of a single discipline, assess OT before, during and after several games, precisely control for confounders, such as, age, nature of training, seasonal effect, and also include more aggressive games, such as, martial arts.

Conclusion

The present exploratory study showed that the OT level in professional sportspersons did not increase with the nearing of a match. Perhaps the increase might be noted in a more critical context, such as, during the game. Further studies are needed based on more homogenous group with higher number of matches.

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Conflict of interests

The authors do not have any conflict of interests.

Authors' contributions

MK – collected the urine samples, prepared samples for analysis, build the database, ZI, AR, IC, AD, MŚ, KK, AS

– recruited and instructed participants, conducted measurements and prepare database, prepare first draft; AS, DL – carried out biochemical analysis, prepared part of the draft, checked final draft; RC, BB – prepared and edited the final draft, collected literature, corrected statistical analysis; SK – designed the study, made the analysis and prepared the draft and checked the final version.

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