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SEÇÃO: REVISÃO

Relation between testosterone, cortisol and aggressive behavior in humans: a systematic review

Relação entre testosterona, cortisol e comportamento agressivo em humanos: uma revisão sistemática

Relación entre testosterona, cortisol y comportamiento agresivo en humanos: una revisión sistemática

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Abstract: Aggression is an evolutionary behavior as it has a role in survival, increasing one's access to food, shelter, status and reproduction. Testosterone and Cortisol are hormones often linked to aggressive behavior. We gathered and organized data from the last five years on the relation among Testosterone, Cortisol and aggression, while assessing the methods used by those scientific papers. A systematic review was made according to PRISMA guidelines. The search for indexed articles was performed in January 2019 using the keywords *aggress* AND Testosterone AND Cortisol* in three databases: Web of Science, SCOPUS and PsycInfo. The specific role of Testosterone and Cortisol in aggressive behavior is not unanimous. However, most articles found either an increase in Testosterone or a decrease in Cortisol associated with this behavior. There is the need for standardizing methods of triggering and assessing aggressive behavior, taking into account the assessment of social desirability and its impacts.
Keywords: PRISMA, hormones, aggression, HPA axis, HPG axis

Resumo: Agressividade é um comportamento evolutivo, tendo um papel na sobrevivência ao aumentar o acesso à comida, abrigo, *status* social e reprodução. A testosterona e o cortisol são hormônios frequentemente associados a comportamentos agressivos. Este estudo reúne e organiza dados dos últimos cinco anos sobre a relação entre testosterona, cortisol e agressividade, avaliando também os métodos utilizados pelos artigos. Uma revisão sistemática foi conduzida segundo as diretrizes do PRISMA. Uma pesquisa eletrônica de artigos foi realizada em janeiro de 2019, usando as palavras-chave agress* AND Testosterone AND Cortisol em três bancos de dados: Web of Science, SCOPUS e PsycInfo. O papel desses hormônios no comportamento agressivo não é unânime, porém a maioria dos artigos incluídos encontrou um aumento na testosterona ou uma diminuição no cortisol, associados a esse comportamento. Há a necessidade de padronizar os métodos de induzir e de avaliar agressividade, levando em conta a desejabilidade social e seus impactos.
Palavras-chave: PRISMA, hormônios, agressividade, eixo HPA, eixo HPG

Resumen: La agresividad es un comportamiento evolutivo, por desempeñar papel en la supervivencia, aumentando el acceso a comida, refugio, estatus social y reproducción. La testosterona y el cortisol son hormonas frecuentemente relacionadas con el comportamiento agresivo. Este estudio reúne y organiza datos de los últimos cinco años sobre la relación entre testosterona, cortisol y agresividad, junto a los métodos de evaluación utilizados por los artículos científicos. Se realizó una revisión sistemática de acuerdo con las directrices PRISMA. La búsqueda electrónica de artículos indexados fue realizada enero de 2019, utilizándose de las palabras clave aggress * AND Testosterone AND Cortisol en tres bases de datos: Web of Science, SCOPUS y PsycInfo. El papel de

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esas hormonas en el comportamiento agresivo no es unánime, aunque la mayoría de los artículos incluídos hayan hallado aumento de testosterona o disminución en cortisol, asociados a ese comportamiento. Se necesita la estandarización de los métodos de inducción y evaluación de la agresividad, teniendo en cuenta la deseabilidad social y sus impactos.

Palabras clave: PRISMA, hormonas, agresividad, eje HPA, eje HPG

Aggression is an evolutionarily-conserved behavior present in most species as it plays a role in survival, increasing an individual's access to food, shelter, status and mating opportunities (Carré & Olmstead, 2015; Waltes, et al., 2016). In the human species it can take a wide range of forms, varying from verbal aggression to physical fighting and, unfortunately, killing (Allen & Anderson, 2017). Still, all aggressive behavior has a common core, they are behaviors that are meant to be hurtful towards another being who is motivated to avoid such injury (Allen & Anderson, 2017). Even though aggression is a primitive behavior and deeply studied, its complex underlying mechanisms have not been completely elucidated.

Several environmental and biological factors have been linked to aggressive behavior. From an environmental perspective, we can cite maladaptive families or parenting, difficult life conditions, chronic exposure to violent media, among others, as factors that could increase one's likelihood of being more aggressive (Elsaesser et al., 2013; Anderson & Bushman, 2018). On the other hand, from a biological perspective, genetics and various imbalances are involved in the increase of aggression, such as in the amygdala, basal ganglia, periaqueductal grey, hypothalamus and ventromedial prefrontal cortex, but also neurotransmitters such as dopamine, serotonin and GABA (Miczek et al., 2007; Willner, 2015).

The hypothalamus is an important diencephalic structure, involved in human behavior, emotions and stress. This structure, along with the pituitary gland, conducts a hormonal orchestra that keeps the body in homeostasis, acting as a bridge between telencephalic control and the endocrine system. Among these functions, we can highlight two hormonal axes in which behavioral effects have been studied: the hypothalamus-pituitary-adrenal (HPA) axis and the hypothalamus-pituitary-gonadal (HPG) axis. In the HPA axis, corticotropin-releasing hormone (CRH) is produced by the paraventricular nucleus of the hypothalamus, which promotes release of adrenocorticotropic hormone (ACTH) by the pituitary, leading to Cortisol production by the adrenal glands. Cortisol (CRT) is critical to body functioning as it regulates several processes, such as lipid and glucose metabolism (Karacabey, 2009); blood pressure (Turner et al., 2020) and immune and inflammatory responses (Staufenbiel, et al., 2013).

In the HPG axis, the hypothalamus produces gonadotropin-releasing hormone (GnRH), which stimulates the secretion of two hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH); both of which activate Testosterone (T) release by the gonads (Montoya et al., 2012). T is primarily produced by Leydig cells in the testicles, ovaries and placenta, and by the adrenal cortex, and is responsible for the increase in bone and muscle mass and the secondary male characteristics (Eisenegger et al., 2011).

Although the biological functions of CRT and T have been widely studied, their behavioral role has been less elucidated. Studies show that variations in CRT level might be linked to behaviors such as food intake (Newman, O'Connor, & Conner 2007), suicide (van Heeringen et al., 2000), depression (Susman, Dorn, Inoff-Germain, Nottelmann, & Chrousos, 1997) or even psychopathic traits (Cima, Smeets, & Jelicic, 2008). With regard to T, studies have demonstrated that a rise in salivary T is related to aggressive behavior, competitive behavior or interaction with attractive members of the opposite sex (Carré, et al., 2009; Turanovic, et al., 2017). CRT and T have an inhibitory relation, so in order to maintain balance between avoidance behavior and approaching behavior to a stimulus, the HPA and HPG axes work together increasing fitness (to attract a mate) or destroying something harmful (Glenn et al., 2011). Other chronobiological, such as puberty (Fragkaki, et al., 2018) and menstrual cycle (Montero-López et al., 2018), and pathological matters, like anxiety disorders

(Giltay et al., 2012; Staufenbiel et al, 2013), can also cause alterations in hormonal levels, causing physical and further psychological changes when influenced by stress. Although these and other recent studies (Grebe et al., 2019; Cheng & Kornienko, 2020) have sought to understand this interaction between neurobiology and human behavior, more studies are needed to better understand this complex issue.

On the basis of the current evidence linking aggression to T and CRT, this review aimed to collect and organize recent data from studies in the past five years detailing a) how aggression and those hormones have been assessed in behavioral studies and b). how T, CRT and aggression relate to each other.

Methods

To achieve our objective, we performed a systematic review of the literature following the PRISMA guidelines (Galvão et al., 2015). The electronic search for articles was done on three databases: Web of Science, SCOPUS and PsycInfo, in January 2019, using the search terms: *aggress* AND Testosterone AND Cortisol*. Articles were included in this review if they were/had: (1) published between 2014 and 2019; (2) humans adults as the object of study, as children and teenagers still have developing neuroendocrinologic features; (3) participants with no psychopathologies, as they might cause changes in their hormonal profiles; and (4) had the full text available in English on online databases. Articles were excluded if they were/had: review articles, book chapters, thesis and/or dissertations; no measures of T and CRT; no induction or measures of aggressive behavior or anger; and no examination of correlations between the hormones and aggression. As a result, 327 articles were obtained from the databases. After the removal of duplicates, the abstracts of 240 unique remaining papers were screened by two independent reviewers (MP and AR). Divergences between those reviewers were assessed by a third judge (RMMA). A total of 106 articles which met the inclusion criteria were assessed for a second time, when the full text was analyzed according to the inclusion and exclusion criteria. Again, this process was performed by two independent reviewers (MP and AR), and when in disagreement, a third reviewer was asked to assist (RMMA).

Full text analysis led to 10 articles being included in this review. Ten studies investigated the role of Testosterone and CRT on aggressive behavior and fulfilled our criteria. An overview of the selection process is shown in Figure 1. The data extracted for the review consisted of: (b) objective of the study; (c) sample size and gender; (d) grouping criteria; (e) age of participants; (f) type of aggression induction; (g) hormonal assessment method; (h) psychological assessment method; (i) main results. Main sources of bias in the studies were also assessed by reviewing sample randomization processes, presence of drug screening, presence of psychological assessments, temperature for hormonal storage, and hormonal assessment method (Table 1).

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Table 1 – Main sources of bias in the studies where the column (a) represents the author(s) and year of publication; (b) Presence of sample randomization; (c) Presence of a drug screening; (d) Sample collection time; (e) Presence of a psychological assessment; (f) Hormone storage temperature; and (g) Hormone assessment kit used

Author, year	Sample rando-mization	Drug scree-ning	Sample collection time	Psychologi-cal screening	Hormone storage temperature	Hormone assessment kit
Pesce et al., 2015	No	Yes	Between 3:30pm and 4:00pm	Yes	–80°C	Enzyme immunoassay
van der Meij et al., 2015	Yes	Yes	Not disclosed	Yes	–20°C	Enzyme immunoassay for Testoste-rone Luminescence immunoassay for Cortisol
Buades-Rotger et al., 2016	No	No	Between 6am and 8am; 30 min later; 1 hour later; and between 6pm and 8pm	Yes	–20°C for saliva; –80°C for blood	Liquid chromatography–tandem mass spectrometry
Perna et al., 2016	No	Yes	Not disclosed	Yes	–20°C	Chemiluminescence immunoassay
Ribeiro Jr et al., 2016	No	No	Between 9am and 6pm	No	–80°C	Enzyme immunoassay
Romero-Martínez & Moya--Albiol, 2016	No	No	Between 4pm and 7pm	No	–20°C	Chemiluminescence immunoassay
Oxford et al., 2017	Yes	No	After 1:30 pm	No	Not disclosed	Radioimmunoassay
Walther et al., 2017	No	Studies 1 and 2: Not disclosed Study 3: Yes	Studies 1 and 2: Not disclo-sed Study 3: Around 6:30am	Yes	–20°C for saliva (for hair, it was not specified)	Enzyme immunoassay for saliva Liquid chromatography –mass spec-tometry for hair
Cabral & de Almeida, 2018	Yes	No	Not disclosed	No	–20°C	Enzyme immunoassay
Probst et al., 2018	No	Yes	Not disclosed	No	–28°C	Radioimmunoassay

Results

Group size varied between 19 and 56 participants. The average age of the participants varied between 20.84 and 57.1 years old. Most articles (8/10) studied subjects aged between 20 and 30 years. With regard to the gender of participants, half of them (5/10) included only men, while three (3/10) included men and women, only two (2/10) included only women.

Even though most articles (8/10) inducted aggression or anger experimentally, several methods were used: presentations videos as only inductors; both videos and online arguments; Trier Social Stress Test (TSST); Point-Subtraction Aggression Paradigm (PSAP); Social Threat Aggression Paradigm (STAP); or the Ultimatum game. The assessment of the anger or aggression of participants was also diverse. The most common way (4/10 studies) to assess anger was by measuring how subjects punished their opponents, other ways were also exploited such as: the State-Trait Anger Expression Inventory 2 (STAXI-2) alone or alongside other subscales; Buss-Perry Aggression Questionnaire (BPAQ) alone or combined with the Hot Sauce Allocation task or the Brief Symptom Inventory-Aggression; 100-mm Visual Analogue Scale (100VAS) with the Single Category Implicit Association Test (SC-IAT); or through the performance in the Ultimatum game. Regarding psychological assessment, only five (5/10) were assessed.

Most studies (8/10) used saliva samples to measure the CRT and T levels of the participants, while the others used blood, blood and saliva or hair and saliva. Regarding the storage temperature of collected samples (if blood or saliva), the most used temperature was -0°C, but varied from -20 °C to -80 °C, sometimes varying temperatures for blood (-80 °C) and saliva (-20°C). To analyze the samples the majority of the studies used the same techniques to analyze both hormones. The most common technique was enzymatic immunoassays, followed by chemiluminescence immunoassays, radioimmunoassays and mass spectrometry, utilized in only one study. Different techniques of analysis were performed for each hormone in two situations: 1) Cortisol by luminescence immunoassay and Testosterone by enzymatic immunoassay; and 2) hormones in saliva samples were measured by enzymatic immunoassay and in hair samples by mass spectrometry.

Only three studies (3/10) were randomized, and drug consumption that could possibly interfere with the results was assessed in only four studies (4/10). Also, the time of the sample collection was not unanimous considering some studies executed the study in the afternoon, while others collected samples either in both in the morning and afternoon, many samples throughout the day, or in the morning. Still, four (4/10) did not indicate at what time the collection of samples was done.

Articles included in this review were not unanimous about how T and CRT levels play a role in the aggressive response. According to Terburg et al. (2009; Popma et al. 2007; Denson et al., 2012), the ratio between T and CRT can be involved with more aggression. Among our selected studies, only two studies (2/10) exhibited the hormonal profile described by Terburg et al. (2009). Most articles (6/10) found either an increase in T or a decrease in CRT, partially agreeing with Terburg et al.'s hypothesis and corroborating with other studies. Only Buades-Rotger et al. (2016) (1/10) presented findings contrary to Terburg et al.'s hypothesis, as the saliva T level decreased. Two studies (2/10) did not find any hormonal changes in their subjects. However, the results of Perna et al. (2016) might have been impaired due to the wrong storage conditions, and Cabral and de Almeida (2018) did not disclose their sample collection time, which could have interfered with their results.

Most results (8/10) indicated a relation between aggression and the assessed hormones. Four studies presented a positive correlation with aggression when T was analyzed and negative when the same was done to CRT; two studies (2/10) found the opposite. A T/CRT ratio was also correlated with aggression in two articles (2/10). An overview of the characteristics of the studies that were included is shown in Table 2.

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Table 2 – Informative table of characteristics of each selected study where column (a) presents the Author and year of publication; (b) Objectives; (c) Subject count and gender; (d) Grouping; (e) Average of subjects; (f) Method of aggression induction; (g) Method of hormonal assessment; (h) Method of psychological assessment; and (i) Main results found

(a) Authors, year	(b) Objective	(c) Subjects (gender)	(d) Grouping (*n*)	(e) Age (years)	(f) Aggression induction	(g) Hormonal assessment	(h) Psychological assessment	(i) Main results
Pesce et al., 2015	The aim was to investigate the individual hormonal (Testosterone and Cortisol) and IL-1 variation, and also the expression of anger and anxiety in kickboxing athletes	25 Kickboxing athletes (male)	No grouping	M=28.68 SD=5.34	No experimental induction	Saliva	STAXI-2	Close to an official competition, the anger score, Testosterone, Testosterone/Cortisol ratio and IL-1b salivary concentrations were significantly higher, and then decreased during competition.
Van der Meij et al., 2015	To investigate if media exposure had the ability to modulate aggression	74 Football fans (male)	Positive rival fan video condition (26) Negative rival fan video condition (24) Neutral rival fan video condition (24)	M=20.84 SD=3.17	Emotion-eliciting films	Saliva	Buss-Perry Aggression Questionnaire Hot Sauce Allocation task	Aggression was higher in fans with lower basal Cortisol levels. No correlation with Testosterone
Buades-Rotger et al., 2016	The study aimed to establish whether reactivity to angry faces and aggression in direct social interaction is modulated by basal and/or acute levels of endogenous Testosterone and Cortisol	39 Young college students (female)	No grouping	M=23.22 SD=3.2	Social Threat Aggression Paradigm (STAP)	Saliva and blood	Opponent punishment Aggression questionnaire	Salivary Testosterone at scan-time was negatively related to aggression whereas Cortisol had no effect

Perna et al., 2016	To investigate the acute effects of alcohol and cannabis on subjective aggression in alcohol and cannabis users following aggression exposure	61 Participants (35 male)	Heavy alcohol users (20) Regular cannabis users (21) Control group (20)	M=22.5 SD=2.3	Point-Subtraction Aggression Paradigm (PSAP)	Blood	100-mmVisual Analogue Scale (VAS) Single Category Implicit Association Test (SC-IAT)	Changes in aggressive feeling or response were not correlated to Cortisol or Testosterone
Ribeiro Jr., 2016	Investigate whether 2D:4D strength correlation becomes stronger in challenge conditions when steroid hormones change	89 Participants (male)	No grouping	M=23.51 SD=6.27	An aggressive video containing rugby tackles	Saliva	Buss-Perry Aggression Questionnaire	Challenge group had higher Testosterone and anger scores
Romero-Martínez & Moya-Albiol, 2016	The study aimed to establish whether the salivary Testosterone/Cortisol ratio response to acute stress could be employed as a marker of proneness to anger	70 Parents (26 male)	Parents of offspring with Autism Spectrum Disorders (ASD) (35) Control group (35)	ASD fathers: M=45.46 SD=1.22 ASD mothers: M=45.27 SD=1.71 Control fathers: M=39.92 SD=1.38 Control mothers: M=45.00 SD=0.90	Trier Social Stress Task (TSST)	Saliva	STAXI-2 Three anger subscales	Testosterone/Cortisol ratio response to stress was significantly associated with high anger feeling increases, trait and expression in caregivers
Oxford et al., 2017	The study aimed to examine hormonal responses to competition in relation to gender and implicit motives	326 Participants (165 male)	Men, oral contraceptive women (OC) and non-using oral contraceptive women (NC): one-on-one and team conditions (not disclosed)	M =20.84 SD=3.17	Trier Social Stress Test (TSST)	Saliva	Opponent punishment	Cortisol had a negative correlation with aggression, except in women in one-on-one competitions

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Walther et al., 2017	The study examined age-related diferences in emotional experience and the moderation of steroid hormones	Study 1: 271 healthy participants (male) Study 2: 121 vitally exhausted participants (male) Study 3: 384 participants (male)	Study 1: No grouping Study 2: Mildly vitally exhausted (48); substantially vitally exhausted (56); severely vitally exhausted (20) Study 3: No grouping	Sudy 1: M=57.1 SD=10.7 Study 2: M=52.7 SD=8.4 Study 3: M=43.75 SD=10.72	No experimental induction	Study 1: Saliva and hair Study 2: Saliva and hair Study 3: Saliva	Studies 1 and 2: Buss-Perry Aggression Questionnaire Study 3: Brief Symptom Inventory-Aggression	Higher Cortisol intensifies aggression; age-related decrease
Cabral & de Almeida, 2018	The study aimed to investigate whether anger causes agonistic, dominance-seeking tendencies in men	83 Healthy undergraduates (male)	Control group (42) and Anger group (40)	M=21.20 SD=2.21	Online debate and emotion-eliciting film clips	Saliva	Opponent punishment	There were no differences between groups for hormonal responses, either for Testosterone or for Cortisol
Probst et al., 2018	The study aimed to reassess the relationship between Testosterone and reactive aggression in naturally cycling women	40 Healthy participants (female)	Late follicular and late luteal (not disclosed)	M=25.91 SD=5	Ultimatum game	Saliva	Aggression was assessed by their performance in the Ultimatum game	They found that women with generally high Testosterone levels showed higher reactive aggression behavior in response to extremely unfair offers than women with low Testosterone levels

Discussion

We hypothesize that there is no consensus in the literature due to the lack of methodological conventions, therefore, this review aimed to understand the underlying differences in the methodology in studies regarding aggressive behavior, and cortisol and testosterone, while looking for evidence of the relation among those variables. Ten articles published from 2014 onwards were found through the PsychInfo, SCOPUS and Web of Science databases that met the analysis criteria. Even though there are evident similarities between human and animal models (Haller & Kruk 2003), our search was restricted to studies on humans. Furthermore, samples were composed of adults, since puberty causes significant changes in the neuroendocrinological profile (Fragkaki et al., 2018). Findings suggest that psychopathologies can be related to changes in CRT and T levels. According to Giltay et al. (2012), lower saliva T levels were found in women diagnosed with mood and anxiety disorders. Also, Staufenbiel et al. (2013) found reduced hair CRT levels in patients with anxiety disorders.

Studies indicating differences between men and women in the capability for emotional regulation also suggest that women tend to be less impulsive and aggressive during the fertile stages of their menstrual cycle (Kaighobadi & Stevens, 2013), such as menstrual cycle tended to influence cortisol response to stress with more reactivity observed in the luteal phase (Montero-López et al., 2018). In this sense, the results of those studies indicate that differences between men and women fluctuate according to the menstrual cycle from the perspective of behavioral self-control strategies. As Perna et al. (2016) did not verify the use of contraceptive methods, this might explain their inconclusive results. Oxford et al. (2017) did not group women according to their cycle phase, which might explain their results.

In relation to hormonal measurement of the participants, the literature indicates that the saliva hormonal profile is sensitive to changes in HPA activity. In sport, saliva T and CRT are widely used biomarkers for assessing competition and aggression effects because they reflect circulating blood concentrations (Crewther et al., 2010; Pappacosta et al., 2016). Moreover, saliva collection can be seen as a useful and efficient method for allowing repeated sampling of hormones in a short time, considering it is not invasive, generating a much lower stress response in comparison to venous puncture, and it is a cheaper option (Lewis, 2006; Kudielka et al., 2012; Hayes, 2014).

In relation to the induction of aggression, the literature suggests that videos are widely used to elicit emotions in research (Kreibig 2010). Such videos allow the dynamic situation to be re-created by bringing together visual and auditory stimuli, thus increasing the ecological validity of the procedure (Rottenberg & Gross 2007; Schaefer et al., 2010). Furthermore, they have the advantage of standardization, which enables data replicability (Fernández et al. 2012). In the study of Van der Meij et al. (2015), participants in a negative humour condition – in which they watched an adversary team-fan making negative comments about their team – watched a summary of a football match in which their team lost to their opponents. The results showed that exposure to the videos did not affect aggression directly. However, participants exhibited high levels of aggression and anger after watching the match. Also, aggression was higher in team supporters whose basal CRT levels were lower, which suggests that part of that aggression was proactive and related to emotions induced by the videos. In the study of Ribeiro et al. (2016), the results showed that the mean T level in the participants after induction of aggression by videos was significantly higher than in the control group, even though the CRT levels did not differ between groups.

Literature also suggests STAXI-2 as an aggression-measuring tool. This is the most common instrument for assessing anger and is also regarded as trustworthy and valid for measuring anger experience and control (Lievaart et al., 2016; (Dalton et al., 1998). Nevertheless, STAXI-2 does present some bias from social desirability because its objectives are known to the respondents. Social desirability response bias has been shown to be present even when the individual makes no conscious efforts at a deliberate overly positive self-reporting on psychometric measures, but has a characterological tendency towards selfenhancement (McEwan et al., 2009). The study of McEwan et al. (2009), indicates that social desirability response bias is associated with lower reported anger expression and higher reported anger control, as measured using common psychometric instruments. Among the articles that used STAXI-2, none made any mention about the possibility of bias but, even so, a positive correlation was found between T or T/CRT ratio and the STAXI-2 scores. The BPAQ is another self-report scale, published in 1992 that quickly became the gold standard for measuring aggression (Gerevich et al., 2007), consisting of 29 items that measure different dimensions of aggression through four subscales that assess anger, hostility, verbal aggression and physical aggression (García-León et al. 2002). Although the BPAQ tried to create mechanisms to mitigate social desirability bias, it still could not overcome it (Becker et al., 2007). Bias risk was not mentioned among the three studies that used the BPAQ: in one, the BPAQ scores coincided with a decrease in CRT levels; another had higher CRT levels with an increase in BPAQ scores; and the other had an increase in T levels as the BPAQ scores increased. In three other studies opponent punishment was used as a measure of aggression of the participants. Although the Hot Sauce Allocation task might have different dynamics, its fundamentals are very similar to other punishment tasks. The purpose of this kind of measure of aggression is based on the definition of aggression, as aggression increases the damage one wants to inflict on others. The Hot Sauce Allocation task did not find any hormonal correlations. In the other tasks, one demonstrated a negative correlation with T, one showed a negative correlation with CRT and one did not present any correlations.

The sample collection time is very important due to CRT and T circadian characteristics. As one awakes CRT is at its peak concentration, which varies according to the individual's chronotype, decreasing during the day until it reaches its minimum at night (Gagnon et al., 2018). T also varies over the day, peaking mainly at night and decreasing during the day (Wittert 2014). The minority of the articles collected the samples in the afternoon, which is considered the ideal time. This combination of factors makes the data incomparable with other articles and might influence results if collection is made both in the morning and in the afternoon.

The majority of the articles are in agreement with the procedures for storing samples. It is recommended that saliva samples be stored at or below -20°C, as higher temperatures might alter values in the medium and long term (Toone et al. 2013). For blood samples the temperature should be even lower, at -70°C, to avoid unbinding of hormones and serum globulins (Tworoger & Hankinson, 2006). For hair samples, storage at room temperature is sufficient (Sauvé et al., 2007).

Final remarks

Although there are other studies that demonstrate this interaction between cortisol and testosterone in human behavior, this review is justified by the fact that these results are not consistent, as there is no consensus in the literature. In addition, evaluating the methods used in previous studies is of fundamental importance so that we can think about more robust future studies and so that we can finally get closer to understanding this relationship. So, this review served to enlighten the issues that might affect the results of studies involving aggressive behavior and steroid hormones in healthy young adults. We were able to establish a trend of an association between increased testosterone/cortisol ratio and aggression. However, our sample consisted of a small number of studies, possibly due to the strict methodology applied, added to the inclusion and exclusion criteria.

Studies involving hormones in women and in both genders are still not as prevalent as in men, possibly because of the difficulties brought about by having to control the menstrual cycle in women. But to understand aggression as a whole, more studies must include both men and women. Studies also did not have consensus in their methods of assessment and induction of anger, for which there are many forms. In this sense, videos and films have been widely used to elicit emotions in the laboratory. We believe that measuring opponent punishment might be a less biased method for assessing aggression, although this still needs a standard protocol. Also, we suggest that future studies should use more than one method of anger induction in order to potentiate effects. The predominance of self-report measures may be associated with a lack of consensus among studies on the specific role of Testosterone and Cortisol in aggressive behavior. We also encourage studies to discuss important issues about social desirability when using instruments that assess aggressive behavior. Thus, more studies are necessary to strengthen empirically the relation between aggressive behavior and T and CRT levels. Furthermore, future studies need more standardized methodologies and must extend their samples in order to obtain more significant results.

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