UNIVERSIDADE DE SAO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

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# **Analysis of stress through salivary and blood cortisol: systematic review**

# **Análise do estresse por meio do cortisol salivar e sanguíneo: revisão sistemática**

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Dissertação constituída por artigo apresentada a Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do titulo de Mestre em Ciências no Programa de Ciências Odontológicas Aplicadas, na área de concentração Biologia oral, Estomatologia, Radiologia e Imagiologia.

Orientador: Prof. Dr. Flávio Augusto Cardoso de Faria

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**DEDICATORIA**

A Deus e minha família; as maiores
fontes da minha energia e forca,
Favio A. Noel;
Juana E. Polonia de Antonio;
Paola E. A. Polonia;
Fausto A. A. Polonia;
Pamela E. A. Polonia;
Letty, nossa cachorra.
Amo vocês.

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*Muito Obrigada!!*

*Lembre da minha ordem: “Seja forte e corajoso!*
*Não fique desanimado, nem tenha medo, porque eu,*
*o Senhor, seu Deus, estarei com você em*
*qualquer lugar para onde você for!”*

***- Josué 1:9 (NTLH)***

## RESUMO

O estresse na área de saúde, geralmente tem sido mensurado por meios objetivos e subjetivos. Respostas à questionários, escalas visuais e analógicas e preenchimento de formulários fornecem dados subjetivos, importantes para identificação do nível de estresse. Contudo, a dosagem do cortisol sanguíneo e salivar pode fornecer melhores indicadores para a avaliação objetiva do nível de estresse. Este trabalho de revisão sistemática teve como objetivo pesquisar estudos que avaliaram e compararam os níveis de estresse por meio de ambas as dosagens, de cortisol sanguíneo (padrão ouro) e salivar, identificando se existe ou não correlação entre os níveis desses biomarcadores durante situações de estresse. Amostras de saliva são obtidas mais facilmente do que amostras de sangue, sendo talvez mais indicadas para estudos experimentais que avaliem o nível de estresse. Métodos: foi realizada uma busca eletrônica meticulosa nas bases de dados PubMed; Scopus; Portal Regional da BVS; Embase e Web of Science, incluindo artigos publicados até janeiro 2023, usando os descritores e palavras chaves referentes a: “cortisol”, “saliva”, “blood”, “serum”, “plasma” e “stress”, combinados e correlacionados entre si, e também seus possíveis sinônimos em inglês, português e espanhol. Os estudos foram analisados por dois revisores independentes de modo a preencher os critérios de inclusão. Resultados: Na busca inicial foram encontrados, 1,054 estudos científicos, 216 artigos foram selecionados após leitura dos títulos e resumos que cumpriram os critérios de elegibilidade. Depois da leitura completa foram selecionados 68 artigos, mas apenas 18 artigos preencheram todos os critérios de inclusão e exclusão estabelecidos. Estes artigos foram divididos em seis tópicos: 1) Estresse psicológico [6 artigos encontrados]; 2) Fármacos que poderiam influenciar o nível de estresse [5 artigos encontrados]; 3) Estresse relacionado com a interrupção do hábito de fumar [3 artigos encontrados]; 4) Estresse em atividades esportivas e exercícios [2 artigos encontrados]; 5) Estresse relacionado com privação de sono [1 artigos encontrados]; e 6) Estresse em pacientes cirúrgicos [1 artigo encontrado]; Nossos resultados mostraram uma relação constante e consistente entre os níveis desses hormônios quando analisados na saliva e no plasma, tanto em situações basais quanto após situações de estresse agudo (em torno de 0,04). Conclusão: Diante do exposto, conclui-se que o cortisol coletado na saliva pode ser utilizado como um biomarcador confiável contra o padrão ouro (plasma) em pesquisas científicas.



**Palavras-chaves:** cortisol salivar, cortisol sanguíneo, estresse.

## ABSTRACT

**Analysis of stress through salivary and blood cortisol. Systematic review**

Stress in health area, generally has been measured by objective and subjective means. Subjective data through answering questionnaires, forms, visual and analog scales, are important to identify stress level. However, the dosage of blood and saliva cortisol can provide better indicators for the objective assessment of stress level. The aim of this systematic review was to investigate studies that evaluated and compared stress levels through blood (gold standard) and salivary cortisol levels, identify whether or not correlation between levels of these biomarkers during stressful situations. Saliva samples are easier to obtain than blood samples and may be more suitable for experimental studies evaluating stress level. Methods: a meticulous electronic literature search was performed in PubMed; Scopus; VHL Regional Portal; Embase and Web of Science, searches were carried out up to January 2023, using descriptors and keywords concerning to: “cortisol”, “saliva”, “blood”, “serum”, “plasma” and “stress”, combined and co-related with each other, and also their possible synonyms in English, Portuguese and Spanish. The studies were analyzed by two independent reviewers in order to meet the inclusion criteria. Results: initial search found, 1,054 scientific studies, 216 selected articles after reading the titles and abstracts that meet the eligibility criteria. With the complete reading of the articles were selected 68, but finally 34 articles met all the inclusion and exclusion criteria established in this systematic review. The scientific studies that entered in our systematic review could be divided into six lines: 1) Psycological stress [6 articles found]; 2) Drugs that could influence the stress level [5 articles found]; 3) Stress related to stopping smoking [3 articles found]; 4) Stress in sports activities and exercises [2 articles found]; 5) Stress related with sleep deprivation [1 articles found]; and 6) Stress in surgical patients [1 article found]; Our results showed a constant and consistent relationship between the levels of these hormones when analyzed in saliva and plasma, both in baseline situations and after acute stress situations (around 0.04). Given the above results, we concluded that cortisol collected in saliva can be used as a reliable biomarker against the gold standard (plasma) in scientific research. Conclusion: Given the above, we concluded that cortisol collected in saliva can be used as a reliable biomarker against the gold standard (plasma) in scientific research.

**Keywords:** salivary cortisol, blood cortisol, stress.

# LISTA DE FIGURAS

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# LISTA DE ABREVIATURA E SIGLAS

| | |
|---|---|
| BVS | Biblioteca Virtual em Saúde |
| VHL Regional Portal | Virtual Health Library Regional Portal (BVS) |
| PROSPERO | International Register of Systematic Reviews |
| WOS | Web of Science |
| HPA | Hypothalamic-Pituitary-Adrenal |
| HHA | Hipotàlamo-Hipòfese-Adrenal |

# SUMÁRIO

# 1 INTRODUÇÃO E REVISÃO DE LITERATURA

# 1 INTRODUÇÃO E REVISÃO DE LITERATURA

***Estresse e cortisol***

O estresse ativa o sistema nervoso simpático causando a liberação de catecolaminas, dando lugar a uma resposta bioquímica que aumenta os níveis plasmáticos de vasopressina, oxitocina e o fator liberador de corticotropina, hormônio que induz a síntese e liberação do cortisol (Briegel *et al.*, 2020; Young Kuchenbecker *et al.*, 2021). A corticotropina aumenta a quantidade disponível do hormônio adrenocorticotrófico (ACTH), responsável pelo controle da atividade secretória do córtex da glândula supra-renal, induzindo a secreção de hormônios mineralocorticóides, glicocorticóides (como o cortisol) e hormônios sexuais (androgênios e estrogênios). A regulação da atividade do eixo Hipotálamo-Hipófise-Adrenal (HHA) é realizada especialmente pelo cortisol e pode ser indicada pelo nível plasmático desse hormônio e de outros biomarcadores inflamatórios (Briegel *et al.*, 2020; Sherin e Nemeroff, 2011; Young Kuchenbecker *et al.*, 2021).

O estresse afeta profundamente a saúde e o desenvolvimento humano. O atendimento odontológico está entre as cinco situações mais temidas e, por isso, inúmeras investigações têm estudado tratamentos farmacológicos e paliativos em busca de amenizar o estresse associado a tratamentos dentários ou à consulta odontológica (Aravena, Almonacid e Mancilla, 2020; Young Kuchenbecker *et al.*, 2021). De acordo com a Associação Americana de Psicologia (American Psychological Association), o estresse é uma reação normal às pressões diárias, mas níveis constantes muito altos não são saudáveis, podendo reduzir a qualidade de vida. A produção de cortisol é induzida especialmente pela atividade do sistema simpático, devido à ativação do Eixo Hipotálamo-Hipófise-Adrenal (HHA), notadamente responsável por condições estressantes e de medo que, embora sejam classificados como processos distintos, estão intimamente relacionados pela interação neural e neuroendócrina (Ballhausen, Kliegel e Rimmele, 2019; Davies *et al.*, 2022; Field *et al.*, 2005).

Clinicamente, o principal uso da avaliação do cortisol é sua superprodução na síndrome de Cushing ou hipercortisolismo, na doença de Addison, bem como em casos de estresse, obesidade, diabetes, doenças psiquiátricas, gravidez e alcoolismo (Turpeinen e Hämäläinen, 2013). Em raros defeitos de enzimas adrenais e após estresse prolongado podem ser observados baixos níveis de cortisol; para fins de diagnóstico, são utilizados cortisol sérico total e livre, excreção noturna ou urinária de cortisol de 24 horas, soro da meia-noite ou cortisol salivar (Turpeinen e Hämäläinen, 2013).

Um grande corpo de evidências científicas indica que estressores agudos e crônicos (por exemplo, estresse no trabalho, isolamento social) e estados emocionais negativos (por exemplo, depressão, ansiedade) influenciam o desenvolvimento de doença cardiovascular (DCV), independentemente dos fatores de risco pré-existentes (Iob e Steptoe, 2019).

Muitas vezes, considera-se que o tratamento odontológico produz ansiedade e estresse, relacionando-o com respostas de luta ou fuga em crianças (Akyuz, Pince e Hekim, 1996; Patil *et al.*, 2015). Antes da administração de anestesia local para extração dentária em homens saudáveis, foi identificado um aumento sérico de 17-hidroxicorticosteróides (17-OHCS), indicando uma estimulação importante no córtex adrenal antes do tratamento dentário (Akyuz, Pince e Hekim, 1996; Patil *et al.*, 2015). Tal grupo de esteróides detectados em amostras de sangue, incluem todos os corticóides que possuem grupamento hidroxila, incluindo a aldosterona, corticosterona, hidroxicortisona, cortisona, e hidrocortisona.

Pessoas com medo ao tratamento odontológico podem ser difíceis de cuidar, requerem mais tempo de atendimento clínico e seus problemas de comportamento na cadeira odontológica podem resultar em uma experiência estressante e desagradável, tanto para o paciente quanto para o profissional (Armfield e Heaton, 2013). Prete et al (2020), em estudo realizado em pacientes com insuficiência adrenal, evidenciou aumento significativo do cortisol sérico em condições estressantes, principalmente em cirurgias e sepse.

Em estudo randomizado controlado que utilizou musicoterapia antes do tratamento endodôntico, com intenção de reduzir o estresse dos pacientes, foram registrados níveis mais baixos de cortisol plasmático no grupo teste (musicoterapia), embora não tenham sido encontradas diferenças estatisticamente significativas quando comparadas ao grupo controle (Wazzan *et al.*, 2022).

### *Ritmo circadiano e estresse*

O sono é essencial para uma boa saúde humana em geral (Vasey, Mcbride e Penta, 2021). O sono pode ser considerado como resultado da inibição motora e, em qualidade e quantidade adequadas, mantém a saúde cerebral, cardíaca e imunológica; graças ao controle circadiano, os seres humanos experimentam o sono ciclicamente uma vez por dia (Vasey, Mcbride e Penta, 2021). A interrupção do sono e a perda do ritmo circadiano afetam os sistemas reguladores homeostáticos nas vias periféricas e centrais, de forma semelhante ao estresse agudo ou crônico; desempenham um papel importante no desenvolvimento de distúrbios relacionados ao estresse (Agorastos e Olff, 2021; Wright *et al.*, 2015).

O sistema de estresse e o ritmo circadiano humano estão intimamente ligados; durante as 24 horas um regula a atividade do outro e vice-versa; estresse afeta o ritmo circadiano em grande medida, pode levar a uma desregulação circadiana aguda/reversível ou sustentada (Agorastos e Olff, 2021). Cortisol, um hormônio endócrino, é um biomarcador útil na doença do estresse; seu padrão diário é conduzido pelo relógio circadiano localizado no núcleo do Sistema Nervoso Central (SNC) do hipotálamo (Wright *et al.*, 2015).

### *Cortisol salivar*

Entre os métodos para avaliar o medo odontológico em crianças está o cortisol salivar, uma opção simples e fácil que reflete de perto os níveis séricos de cortisol livre, independentemente da taxa de fluxo salivar (Armfield e Heaton, 2013). A saliva é o espelho do sangue; especialmente em crianças, a amostragem de saliva é fácil, não invasiva (Patil *et al.*, 2015)

O cortisol salivar é um ultrafiltrado do cortisol plasmático que reflete os níveis de cortisol sérico biologicamente ativos, não ligados a proteínas; sua variação circadiana segue a do cortisol sérico, com níveis mais altos pela manhã e mais baixos à meia-noite. Por este motivo, o cortisol salivar é comumente usado para o diagnóstico de hipercorticismo, como na Síndrome de Cushing (SC), ou para diagnóstico da hipofunção do córtex da adrenal, como na Doença de Addison. Métodos de imunofluorescência e outros tipos de imunoensaios são frequentemente utilizados para este fim; mais recentemente, métodos mais sensíveis e mais específicos, como a espectrometria de massas LC-MS/MS, têm sido utilizados (Turpeinen e Hämäläinen, 2013).

***Cortisol no sangue, soro ou plasma***

Em estudo sobre a relação entre obesidade e cortisol, a concentração plasmática é parece ser mais crítica e precisa para pacientes com hipercortisolismo (Salehi, Ferenczi e Zumoff, 2005). Devido à variação circadiana da liberação de cortisol, uma amostragem incorreta pode influenciar significativamente os resultados (Turpeinen e Hämäläinen, 2013). A alta especificidade de LC-MS/MS (Cromatografia Líquida com Espectrometria de Massa) facilita a medição confiável de cortisol em amostras de plasma, urina e saliva(Turpeinen e Hämäläinen, 2013). No entanto, a interpretação dos valores de cortisol plasmático ou em outros fluidos é complicada, dada a segregação episódica do hormônio (um valor de ponto único não é representativo); tal problema pode ser resolvido medindo as concentrações plasmáticas de cortisol por 24 horas (Salehi, Ferenczi e Zumoff, 2005).

O cortisol, sem dúvida, é o hormônio do estresse, um importante biomarcador que regula processos importantes relacionados ao equilíbrio e desenvolvimento neuroemocional do ser humano, incluindo nutrição, sono, controle emocional, dentre outros. O objetivo desta revisão sistemática foi comparar os níveis de cortisol quando este hormônio foi dosado simultaneamente em dois fluidos distintos, sangue e saliva. Pretende-se, assim, verificar a correlação existente entre estas dosagens, de maneira a poder utilizar formas mais simples e menos invasivas de coleta de material biológico em futuras pesquisas científicas.

# 2 ARTIGO

# 2 ARTIGO

The article presented in this Dissertation was written according to the **Brazilian Journal of Oral Sciences** instructions and guidelines for article submission.

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**Analysis of stress through salivary and blood cortisol: systematic review**

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**ABSTRACT**

In the health area, stress has generally been measured by objective and subjective means. Subjective data, collected through questionnaires, forms, visual and analog scales, are important to identify stress level. However, the dosage of blood and saliva cortisol can provide better indicators for the objective assessment of stress level. The aim of this systematic review was to investigate studies that evaluated and compared simultaneously blood and salivary cortisol levels, and identify whether there is a correlation between these biomarkers during basal and stressful situations. Saliva samples are easier to obtain than blood samples, and it may be more suitable for experimental studies that evaluate the stress level. Methods: an electronic search was performed in PubMed; Scopus; VHL Regional Portal; Embase; and Web of Science databases. The searches were carried out up to January 2023, using descriptors and keywords as "cortisol", "saliva", "blood", "serum", "plasma" and "stress", which were combined and co-related with each other, and also with their possible synonyms in English, Portuguese and Spanish. The studies retrieved were analyzed by two independent reviewers to meet the inclusion criteria. Results: 1054 studies were retrieved from the databases. After reading the titles and abstracts, 216 articles, that could meet the eligibility criteria, were selected for complete reading. After the complete reading of the articles, 18 met all the inclusion and exclusion criteria established for this systematic review and were eligible for this study. The studies eligible for our systematic review can be classified into six topics: 1) Psychological stress [6 articles found]; 2) Drugs that could influence the stress level [5 articles found]; 3) Stress related to smoking abstinence [3 articles found]; 4) Stress in sports activities and exercises [2 articles found]; 5) Stress related with sleep deprivation [1 articles found]; and 6) Stress in surgical patients [1 article found]. Our results showed a constant and consistent relationship between the levels of cortisol, when analyzed in saliva and plasma, both in baseline situations and after acute stress situations (around 0.04). Thus, we concluded that cortisol collected in saliva can be used as a reliable biomarker against the gold standard (plasma) in scientific research.

Keywords: salivary cortisol, blood cortisol, stress.

## Introduction

In the health area, stress has generally been measured by objective and subjective means. Subjective data, collected through questionnaires, forms, visual and analog scales, are important to identify stress level. However, the dosage of blood and saliva cortisol can provide better indicators for the objective assessment of stress level.

Cortisol has been used for many years as a specific stress biomarker, and many researchers have studied this hormone in different health areas. A systematic review published in the "Oral Biology Files" journal states that some studies included in that research claim that cortisol in saliva is correlated with blood cortisol levels in patients with periodontitis, and that oxidative stress can greatly contribute to the development of periodontal disease, but more studies are needed to understand this issue (Botelho *et al.*, 2018).

In Sweden, Ahs et al. 2006, revealed a positive correlation between Hypothalamic Regional Cerebral Blood Flow (Hypothalamic Regional Cerebral Blood Flow - rCBR) and salivary cortisol levels in patients with Social Anxiety Disorder, but did not measure cortisol in blood (Ahs *et al.*, 2006). Kirschbaum and Hellhammer (1994), in a review study, states that psychological stress has been accepted as a determining factor in increasing the activity of the Hypothalamic-Pituitary-Adrenal (HPA) axis.

The stress status can be measured by the level of cortisol in saliva in newborns and adults. About the most obvious advantages of measuring cortisol in saliva is the fact that it is ease to obtain and, as it is noninvasive, it avoids puncture stress. Because it is measured at almost unlimited frequency, in a wide variety of clinical and field settings, regardless of sampling, salivary cortisol is therefore an easy method to measure the free unbound hormone fraction (Kirschbaum and Hellhammer, 1994).

Although studies refer not only to the similarity between these biomarkers, but also highlight the preference for measurements in saliva as an outpatient, painless, minimally invasive method with a reliable reflection of blood values (Bhake *et al.*, 2020; Bhattarai, Kim e Chae, 2018; Duinen, van *et al.*, 2005; Ho *et al.*, 2020; Šimůnková *et al.*, 2007), in the 1990s, studies expressed that there was considerable doubt whether salivary cortisol really provided a valid and reliable correlate of serum or plasma cortisol concentration (Kirschbaum e Hellhammer, 1994).

Van Duinen et al. 2005, found a significant increase in cortisol levels both in saliva and blood when CO2 at 35% is inhaled, however the authors state that the increase in the free fraction of serum cortisol is more pronounced than salivary cortisol, justifying these discrepancies with the advantages of salivary cortisol of not performing venous puncture, which can cause stress and therefore a release of cortisol (Duinen, van *et al.*, 2005). Another study suggests that salivary cortisol measurements are less reproducible than blood measurements for low-dose Dexamethasone Suppression Tests (DST), which are important for diseases such as depression, metabolic syndrome, coronary heart disease and arterial disease (Reynolds *et al.*, 1998). For saliva, the repeated measurement was as much as five times as small or five times as large as the first for 95% of the time, while for plasma a repeated measurement was only half as small or twice as large as the first (Reynolds *et al.*, 1998).

In another aspect, circadian and circannual rhythms also influence unstimulated salivary flow rates (Bhattarai, Kim e Chae, 2018). The possibility of sampling error is highest during saliva collection and processing. Thus, incorrect methods of saliva collection also result in sampling error (Bhattarai, Kim e Chae, 2018; Erasmus *et al.*, 2010). In addition, there are treatments such as BoNT-A that could cause a decrease in salivary flow and changes in viscoelastic properties, which could interfere with an appropriate collection for the evaluation of any biomarker (Erasmus *et al.*, 2010). Considering the uncertainty of salivary cortisol values compared to serum values, it is important to expand scientific investigations that justify the correlation or not of these measures to assess any type of stress.

The aim of this systematic review was to investigate studies that evaluated and compared simultaneously blood and salivary cortisol levels, and identify whether there is a correlation between these biomarkers during basal and stressful situations.

## Material and Method

### *Search strategy*

This systematic review was registered on PROSPERO platform on 12/30/2022, and accepted under number CRD42022384684, and aimed to answer the following question: "During stressful situations, the level of salivary cortisol is correlated with the level of plasmatic cortisol?" To answer this question, an electronic search in PubMed, Scopus, VHL Regional Portal, Web of Science (WOS), and Embase databases was carried out. The searches were carried out up to January 2023, using descriptors and keywords as "salivary cortisol", "blood cortisol", "plasma cortisol", "serum cortisol" and "stress".

*Selection criteria*

Studies were selected after a careful reading of the title and abstract to verify whether they corresponded to the review question. After the initial selection, abstracts were read in full by two different examiners, and articles that met all the following inclusion criteria were chosen: studies that measured simultaneously blood and salivary cortisol to analyze stress level; research conducted in humans, regardless of the sample number; randomized controlled clinical trials published until January 2023. Exclusion criteria: animal research; studies with only one type of cortisol measurement; studies about cortisol that did not evaluate stress; studies in humans under 18 years of age (studies in infants, children's or adolescents) and any kind of review.

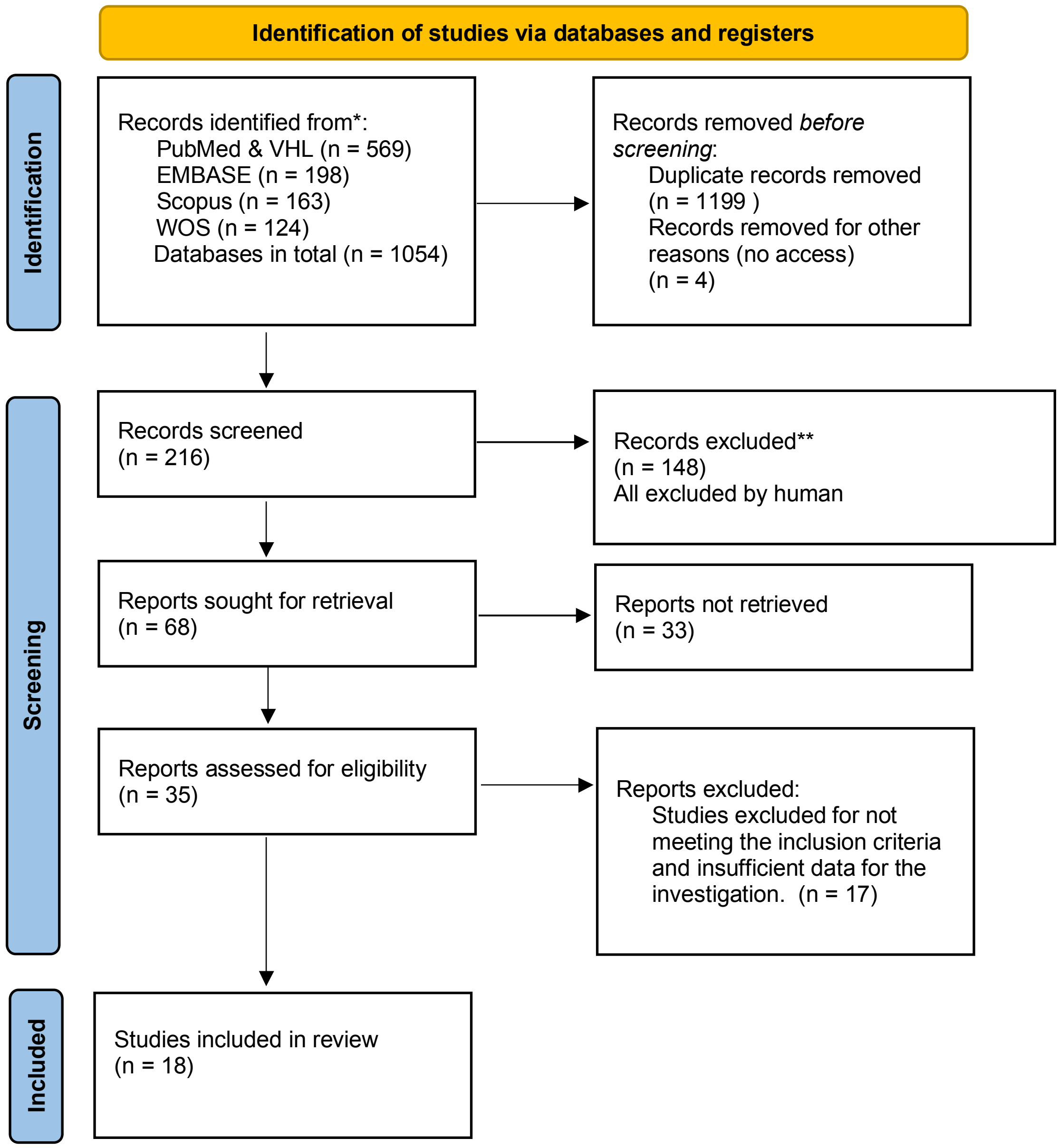
*Data collection*

After reading the articles, data from each study were organized into tables according to the authors names, year of publication, country where the study was conducted, type of study, type of procedure, gender, number of patients, method for assessing stress (objective and/or subjective), type of blood and saliva cortisol measurement, result of blood and saliva cortisol levels and result of each study. Subsequently, data were analyzed to answer the main question of this systematic review.

**Results**

This systematic review followed the criteria defined by the PRISMA system (Preferred Reporting Items for Systematic Reviews and Metanalyses), as shown in Figure 1.

Figure 1 – The PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only.




*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).
**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71 For more information, visit: http://www.prisma-statement.org/

In the initial search, 1054 articles were found in the databases selected for this review. 53.98% of the articles were retrieved from the PubMed & VHL database (569 articles), 18.79% from the EMBASE database (198 articles), 12.90% from the Scopus database (163 articles) and the remaining 11.76% from the WOS database (124 articles) (Table I).

After reading the titles and abstracts, 216 articles (20.49% of the total), that could meet the eligibility criteria for this review, were selected. Of this total, 117 articles (54.17 %) were retrieved from PubMed & VHL database; 54 (25 %) from EMBASE database; 30 from (13.89 %) Scopus database, and 15 (6.94%) from WOS database.

In our search, we grouped the PubMed & VHL databases because both are indexed in Medline. After the complete reading of the articles by two different examiners, 68 articles were considered for eligibility for this review. Forty-four were retrieved from PubMed & VLH; 20 from EMBASE; 2 from Scopus and two from WOS. However, after careful reading, considering the inclusion and exclusion criteria, only 18 articles met all the eligibility criteria proposed for this systematic review. Considering the 1054 studies initially found with the keywords and descriptors, only 1.71% of them were eligible for this study **(Table 1)**.

**Table 1: Distribution of the articles identified in the initial search in the different databases**

| Databases | Articles found | Title and abstract selection | General reading and selection | Selection according to inclusion and exclusion criteria |
|---|---|---|---|---|
| PubMed | 569 | 117 | 44 | 12 |
| VHL | | | 2 | 2 |
| EMBASE | 198 | 54 | 20 | 3 |
| Scopus | 163 | 30 | 2 | 0 |
| Web of Science | 124 | 15 | 2 | 1 |
| Total | 1,054 | 216 | 68 | 18 |

**Table 2: Articles included in this review classified as stress of a psychological nature**

| Article/Year of Publication | Title | Classification of the Study |
|---|---|---|
| Gaab 2005 | Stress-induced changes in LPS-induced pro-inflammatory cytokine production in chronic fatigue syndrome. | Psychological Stress |
| Ju Bae 2019 | Salivary cortisone, as a biomarker for psychosocial stress, is associated with state anxiety and heart rate. | Psychological Stress |
| Heinrichs 2001 | Effects of suckling on hypothalamic-pituitary-adrenal axis responses to psychosocial stress in postpartum lactating women. | Psychological Stress |
| Smeets 2021 | Validation of a new method for saliva cortisol testing to assess stress in first responders. | Psychological Stress |

**Table 3: Articles included in this review that show drug effects on cortisol level**

| Article/Year of Publication | Title | Classification of the Study |
|---|---|---|
| Bohringer 2008 | Intranasal insulin attenuates the hypothalamic-pituitary-adrenal axis response to psychosocial stress. | Drugs on Stress |
| Jezova 2005 | Subchronic treatment with amino acid mixture of L-lysine and L-arginine modifies neuroendocrine activation during psychosocial stress in subjects with high trait anxiety. | Drugs on Stress |
| Fries 2006 | Attenuation of the hypothalamic-pituitary-adrenal axis responsivity to the Trier Social Stress Test by the benzodiazepine alprazolam. | Drugs on Stress |
| Khalili-Mahani 2015 | Effect of subanaesthetic ketamine on plasma and saliva cortisol secretion. | Drugs on Stress |
| Hellhammer 2013 | Effects of a homeopathic combination remedy on the acute stress response, well-being, and sleep: a double-blind, randomized clinical trial. | Drugs on Stress |
| Walter 2013 | Acute effects of intravenous heroin on the hypothalamic-pituitary-adrenal axis response: a controlled trial. | Drugs on Stress |
| Hellhammer 2004 | Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the endocrine and psychological responses to mental stress. | Drugs on Stress |

**Table 4: Articles included in this review in which the nature of stress was classified as smoking abstinence, intense physical exercise, sleep deprivation and patients undergoing surgical procedures**

| Article/Year of Publication | Title | Classification of the Study |
|---|---|---|
| Jen-Yu Hoa 2014 | Effects of resistance exercise on the HPA axis response to psychological stress during short-term smoking abstinence in men. | Smoking Abstinence/Exercise |
| Al`Absi 2021 | Blunted opioid regulation of the HPA stress response during nicotine withdrawal: therapeutic implications. | Smoking Abstinence |
| Kirschbaum 1994 | Pituitary and adrenal hormone responses to pharmacological, physical, and psychological stimulation in habitual smokers and nonsmokers. | Smoking Abstinence |
| Duclos 1998 | Corticotroph axis sensitivity after exercise in endurance-trained athletes. | Athletics & Exercise |
| Borchers 2022 | Salivary Diagnostic for Monitoring Strenuous Exercise—A Pilot Study in a Cohort of Male Ultramarathon Runners. | Athletics & Exercise |
| Matzner 2013 | Resilience of the immune system in healthy young students to 30-hour sleep deprivation with psychological stress. | Sleep Restriction |
| Bergmann 2001 | The influence of medical information on the perioperative course of stress in cardiac surgery patients. | Surgical Patients |

**Table 5: Articles included in this review classified as stress of a psychological nature (brief summary)**

| Article/Year of Publication | Brief summary of the study |
|---|---|
| Gaab 2005 | Verified responses to stressful stimuli in patients diagnosed with Chronic Fatigue Syndrome (by completing questionnaires). Results compared with control group. |
| Ju Bae 2019 | Investigated the adaptive response to stress in healthy adults, measuring several steroid hormones in plasma (including cortisol), cortisol and salivary cortisone. One group received a stressful stimulus (TSST) and the other, the control group, was not submitted to this test (TSST-Placebo). |
| Heinrichs 2001 | Verified the effect of the breast sucking stimulus on the HPA axis, after a stressful stimulus. The test group breastfed their children for 15 minutes and the control group just held the child in their arms, without breastfeeding. Test used = TSST. |
| Smeets 2021 | Verified ambulance service and medical emergency professionals, firefighters, police officers, elite military groups, submitted to two hours of simulation of a terrorist scenario (performed by a group of actors). |

**Table 6: Articles included in this review that show drug effects on cortisol levels (brief summary)**

| Article/Year of Publication | Brief summary of the study |
|---|---|
| Bohringer 2008 | The study sought to assess whether intranasal insulin administration could affect the HPA axis in a stressful situation. |
| Jezova 2005 | The study sought to analyze the possible influence of treatment with L-lysine and L-arginine (capsules orally) on neuroendocrine activation. Control group received placebo capsules |
| Fries 2006 | The study sought to verify the effect of alprazolam on the HPA axis and medullary sympathetic adrenal after application of TSST. |
| Khalili-Mahani 2015 | The study verified the effect of ketamine in subanesthetic doses intravenously, compared with the infusion of placebo. Study in a crossover model, where each patient received both types of treatment. Ketamine can be considered as an activator of the HPA axis, causing an increase in the cortisol level. Placebo reveals the circadian rhythm of hormone production |
| Hellhammer 2013 | Randomized, double-blind study, compared with placebo, which analyzed the effect of a homeopathic combination on stress after application of the TSST test. Participants were medicated with the test drug or placebo for 14 days, after which they underwent the TSST. |
| Walter 2013 | The study analyzed plasma and salivary cortisol levels in heroin-addicted patients, who received intravenous saline and/or heroin, in a crossover study. Withdrawal from heroin (or IV saline) can be considered as the stressful stimulus; IV heroin significantly reduced plasma and salivary cortisol levels, as well as ACTH levels. |
| Hellhammer 2004 | The study verified the effect of a dietary supplement containing soy lecithin and phosphatidylserine, administered for 21 days, on plasma levels of cortisol and ACTH, as well as on heart rate and salivary cortisol, after the stressful stimulus of the TSST. |

**Table 7: Articles included in this review in which the nature of stress was classified as smoking abstinence, intense physical exercise, sleep deprivation and patients undergoing surgical procedures**

| Article/Year of Publication | Brief summary of the study |
|---|---|
| Jen-Yu Hoa 2014 | The study sought to verify the effect of physical exercise on the HPA axis in chronic smokers during abstinence and stressful mental tasks. |
| Al`Absi 2021 | Article that studied the effect of naltrexone (opioid antagonist) on acute stressful stimuli applied to ad libitum smokers, abstinent smokers for 24 hours and non-smokers. |
| Kirschbaum 1994 | Study that recruited students submitted to two stressful stimuli: ergometric test until physical exhaustion and TSST. Comparison of cortisol levels in smokers and non-smokers. |
| Duclos 1998 | Study carried out with marathon runners, checking plasmatic and salivary cortisol before and after a training session (2 hours). Continuous training of runners is considered as chronic stress in humans. Thus, chronically stressed subjects were submitted to acute stress (two hours of exercise). |
| Borchers 2022 | Pilot study with male ultramarathon runners (160 km runners), measuring salivary and plasmatic cortisol from 4 to 13 days before the race, immediately after the race and in a third period of 8 to 11 days after the race. Only the measurement before and immediately after the race was used, considering the ultramarathon as the acute stressful stimulus. |
| Matzner 2013 | Study carried out with psychology students submitted to sleep deprivation for 30 hours, during which they also performed stressful activities of a psychological nature (performing computational tasks with one hand immersed in ice water, participating in social stress procedures and arithmetic mental tasks). Saliva and blood collected before sleep deprivation and at the end of stressful tasks. The article separates results for men and women, but there are no significant differences between the two genders. Gathered all the data. |
| Bergmann 2001 | Study carried out with patients who would undergo cardiac surgery, through the opening of the chest. The aim was to verify whether patients who received more information and attention from the surgeon would be less stressed with the procedure. Cortisol dosage served as a parameter for assessing the level of stress. |

**Table 8: Acute stress method used and participants of the studies included in this review**

| Article/Year of Publication | *Stress method used* | *Participants* |
|---|---|---|
| Gaab 2005 | TSST Test (Trier Social Stress Test) | Control Group: 20 (11 Men and 9 Women); SFC Group: 21 (10 Men and 11 Women). Age between 29 and 47 years |
| Ju Bae 2019 | TSST Test (Trier Social Stress Test) | 25 Men with an average age of 25.20 years |
| Heinrichs 2001 | TSST Test (Trier Social Stress Test) | 23 Women with an average age of 30.2 years |
| Smeets 2021 | Terrorist attack simulation | 36 (30 men and 6 women) with an average age of 39 years |
| Bohringer 2008 | TSST Test (Trier Social Stress Test) | 24 Men aged between 20 and 31 years |
| Jezova 2005 | TSST Test (Trier Social Stress Test) | 29 Men aged between 20 and 40 years |
| Fries 2006 | TSST Test (Trier Social Stress Test) | 46 Men aged between 18 and 31 years |
| Khalili-Mahani 2015 | IV injection of Ketamine in subanesthetic doses | 12 Men aged between 19 and 36 years |
| Hellhammer 2013 | TSST Test (Trier Social Stress Test) | 39 Women aged between 30 and 50 years |
| Walter 2013 | Heroin abstinence | 19 Men and 9 Women over 18 years old aged between 23 and 58 years old |
| Hellhammer 2004 | TSST Test (Trier Social Stress Test) | 10 Women and 9 Men with an average age of 28.2 years |
| Jen-Yu Hoa 2014 | Six endurance physical exercises in the morning and challenging math mental tasks in the evening. Smoking abstinence. | 8 Sedentary men with an average age of 20.1 years |
| Al`Absi 2021 | Public speaking, arithmetic mental tasks and hand immersion in ice water. Smoking abstinence | 85 Men and 64 Women with an average age of 34.9 years |
| Kirschbaum 1994 | Ergometric test to physical exhaustion and TSST | 22 Men with an average age of 24.65 years |
| Duclos 1998 | Two hours of marathon training | 8 Men with an average age of 41 years |
| Borchers 2022 | Ultramarathon (160 km run) | 9 Men aged 35 to 62 years |
| Matzner 2013 | Perform computational tasks with one hand immersed in ice water, participate in social stress procedures and mental arithmetic tasks | 9 Men and 14 Women with an average age of 24 years |
| Bergmann 2001 | Surgical procedure to which they would undergo | 26 Men and 34 Women aged between 55 and 65 years |

**Table 9: Plasma (PC) and salivary (SC) cortisol values at baseline and post-stress conditions. *Data obtained by analyzing the values shown by the graphs of the articles**

| *Article/ Year of Publication* | *Groups* | *PC Basal (ng/mL)* | *PC Post-Stress (ng/mL)* | *PC Post-Stress/ Basal* | *SC Basal (ng/mL)* | *SC Post-Stress (ng/mL)* | *SC Post-Stress/ Basal* | *SC/ PC Basal* | *SC/ PC Post-Stress* |
|---|---|---|---|---|---|---|---|---|---|
| Gaab 2005* | Control | 160 | 187.7 | 1.173 | 7.97 | 10.87 | 1.363 | 0.049 | 0.057 |
| | Chronic fatigue | 143 | 185 | 1.293 | 7.25 | 9.28 | 1.28 | 0.05 | 0.05 |
| Ju Bae 2019* | TSST Test | 59.81 | 148.62 | 2.48 | 0.72 | 3.73 | 5.18 | 0.012 | 0.025 |
| Heinrichs 2001* | Control | 61.1 | 92.22 | 1.509 | 2.356 | 4.531 | 1.923 | 0.038 | 0.049 |
| | Breastfeeding | 56.67 | 64.44 | 1.137 | 2.29 | 3.14 | 1.37 | 0.04 | 0.049 |
| Smeets 2021 | Test | 93.52 | 126.87 | 1.356 | 0.71 | 1.58 | 2.233 | 0.007 | 0.012 |
| Bohringer 2008* | Control | 48.65 | 140 | 2.877 | 0.74 | 4.23 | 5.717 | 0.0152 | 0.03 |
| | Insulin | 52.51 | 115 | 2.19 | 0.94 | 2.78 | 2.957 | 0.018 | 0.024 |
| Jezova 2005* | Control | 90 | 116 | 1.288 | 2.81 | 3.62 | 1.288 | 0.031 | 0.031 |
| | Amino acids | 99.23 | 137.69 | 1.387 | 2.8 | 4.28 | 1.53 | 0.028 | 0.031 |
| Fries 2006* | Placebo | 103.33 | 170 | 1.645 | 2.32 | 10.15 | 4.375 | 0.022 | 0.059 |
| | Alprazolam | 76.66 | 105 | 1.369 | 2.32 | 3.335 | 1.437 | 0.03 | 0.031 |
| Khalili-Mahani 2015* | Placebo | 130 | 79.75 | 0.613 | 6.52 | 3.62 | 0.555 | 0.05 | 0.045 |
| | Ketamine | 112.37 | 192.12 | 1.709 | 8.33 | 12.68 | 1.522 | 0.074 | 0.066 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Hellhammer 2013 | Placebo | 29.79 | 46.62 | 1.564 | 0.94 | 2.055 | 2.186 | 0.031 | 0.044 |
| | Homeopathy | 30.88 | 48.7 | 1.577 | 1.033 | 2.363 | 2.287 | 0.033 | 0.048 |
| Walter 2013* | Saline | 190 | 207.5 | 1.092 | 23.84 | 28.84 | 1.21 | 0.125 | 0.139 |
| | Heroin | 190 | 110 | 0.578 | 22.11 | 15.57 | 0.7 | 0.116 | 0.141 |
| Hellhammer 2004* | Placebo | 122.45 | 198.45 | 4.197 | 8.039 | 1.62 | 1.915 | 0.034 | 0.04 |
| Jen-Yu Hoa 2014* | Control | 193.33 | 261 | 1.35 | 5.8 | 6.28 | 1.082 | 0.03 | 0.024 |
| | Exercise | 241.66 | 459.16 | 1.9 | 10.87 | 21.75 | 2 | 0.044 | 0.047 |
| | Exercise and mental task | 261 | 227.16 | 0.87 | 9.66 | 10.39 | 1.075 | 0.037 | 0.045 |
| Al`Absi 2021 | Non-smokers (Placebo) | 74.5 | 112 | 1.503 | 2.2 | 2.7 | 1.227 | 0.029 | 0.024 |
| | Non-smokers (Naltrexone) | 78.5 | 135 | 1.719 | 2.25 | 4.25 | 1.88 | 0.028 | 0.031 |
| | Smokers ad libitum (Placebo) | 72.5 | 98.5 | 1.358 | 1.95 | 2.4 | 1.23 | 0.027 | 0.024 |
| | Smokers ad libitum (Naltrexone) | 72.5 | 158.5 | 2.186 | 2.2 | 6.15 | 2.795 | 0.03 | 0.038 |
| | Abstinent smokers (Placebo) | 70 | 94 | 1.342 | 2.15 | 2.6 | 1.209 | 0.03 | 0.027 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Abstinent smokers (Naltrexone) | 77 | 122.5 | 1.59 | 2.15 | 3.6 | 1.674 | 0.028 | 0.029 |
| Kirschbaum 1994* | Smokers Ergometric test | 150 | 210 | 1.4 | 2.66 | 11.33 | 4.26 | 0.018 | 0.054 |
| | TSST Smokers | 161.62 | 189.53 | 1.173 | 3.12 | 3.73 | 1.196 | 0.019 | 0.019 |
| | Non-smokers Ergometric test | 220 | 296 | 1.345 | 3.8 | 7 | 1.844 | 0.017 | 0.023 |
| | Non-smokers TSST | 184.88 | 232.55 | 1.257 | 3.55 | 3.99 | 1.123 | 0.019 | 0.017 |
| Duclos 1998 | Physical exercise | 101.13 | 155.15 | 1.534 | 2.35 | 4.17 | 1.774 | 0.023 | 0.027 |
| Borchers 2022 | Ultramarathon | 97 | 307 | 3.164 | 7 | 18 | 2.52 | 0.072 | 0.058 |
| Matzner 2013 | Sleep deprivation | 13.74 | 13.37 | 0.973 | 0.87 | 0.62 | 0.718 | 0.063 | 0.046 |
| Bergmann 2001 | Surgical patients | 140.85 | 237.38 | 1.685 | 1.27 | 11.96 | 9.41 | 0.009 | 0.05 |
| Mean | | 112.783 | 160.5688889 | 1.593972222 | 4.66355556 | 6.922055556 | 2.167916667 | 0.036838889 | 0.043166667 |
| Standard deviation | | 61.7371 | 85.55740441 | 0.696460828 | 5.29743423 | 6.23112365 | 1.726586614 | 0.025895563 | 0.027382476 |
| Standard Error | | 10.2895 | 14.2595674 | 0.116076805 | 0.88290571 | 1.038520608 | 0.287764436 | 0.004315927 | 0.004563746 |

**Table 10: Plasma (PC) and salivary (SC) cortisol values in baseline and post-stress conditions, using only control patients as a reference, without the influence of drugs or chronic stress. *Data obtained by analyzing the values shown by the graphs of the articles**

| *Article/ Year of Publication* | *Groups* | *PC Basal (ng/mL)* | *PC Post-Stress (ng/mL)* | *PC Post-Stress / Basal* | *SC Basal (ng/mL)* | *SC Post-Stress (ng/mL)* | *SC Post-Stress / Basal* | *SC/ PC Basal* | *SC/ PC Post-Stress* |
|---|---|---|---|---|---|---|---|---|---|
| Gaab 2005* | Control | 160 | 187.7 | 1.173 | 7.97 | 10.87 | 1.363 | 0.049 | 0.057 |
| Ju Bae 2019* | TSST test | 59.81 | 148.62 | 2.48 | 0.72 | 3.73 | 5.18 | 0.012 | 0.025 |
| Heinrichs 2001* | Control | 61.1 | 92.22 | 1.509 | 2.356 | 4.531 | 1.923 | 0.038 | 0.049 |
| Bohringer 2008* | Control | 48.65 | 140 | 2.877 | 0.74 | 4.23 | 5.717 | 0.0152 | 0.03 |
| Jezova 2005* | Control | 90 | 116 | 1.288 | 2.81 | 3.62 | 1.288 | 0.031 | 0.031 |
| Fries 2006* | Placebo | 103.33 | 170 | 1.645 | 2.32 | 10,15 | 4.375 | 0.022 | 0.059 |
| Khalili-Mahani 2015* | Placebo | 130 | 79.75 | 0.613 | 6.52 | 3.62 | 0.555 | 0.05 | 0.045 |
| Hellhammer 2013 | Placebo | 29.79 | 46.62 | 1.564 | 0.94 | 2.055 | 2.186 | 0.031 | 0.044 |
| Hellhammer 2004* | Placebo | 122.45 | 198.45 | 4.197 | 8.039 | 1.62 | 1.915 | 0.034 | 0.04 |
| Jen-Yu Hoa 2014* | Control | 193.33 | 261 | 1.35 | 5.8 | 6.28 | 1.082 | 0.03 | 0.024 |
| Al`Absi 2021 | Non-smokers (Placebo) | 74.5 | 112 | 1.503 | 2.2 | 2.7 | 1.227 | 0.029 | 0.024 |
| Kirschbaum 1994* | Non-smokers Ergometric test | 220 | 296 | 1.345 | 3.8 | 7 | 1.844 | 0.017 | 0.023 |
| | Non-smokers TSST test | 184.88 | 232.55 | 1.257 | 3.55 | 3.99 | 1.123 | 0.019 | 0.017 |
| Mean | | 113.68 | 160.07 | 1.75392308 | 3.67423077 | 4.95353846 | 2.29061538 | 0.02901538 | 0.036 |
| Standard Deviation | | 60.7880924 | 73.5781053 | 0.9292275 | 2.60541942 | 2.87564229 | 1.67713484 | 0.01200152 | 0.01384437 |
| Standard Error | | 16.8621616 | 20.4100153 | 0.25776075 | 0.72272383 | 0.79768163 | 0.46522464 | 0.00332913 | 0.00384033 |

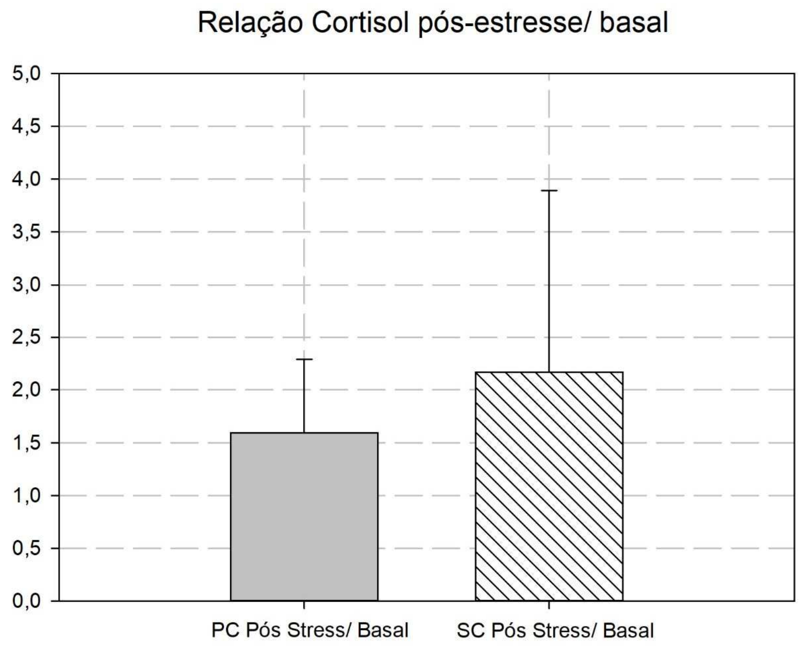



**Figure 2: Relationship between post-stress cortisol concentration and baseline concentration in plasma and saliva, based on all groups of all studies included in this review. Average of the values in the bars**

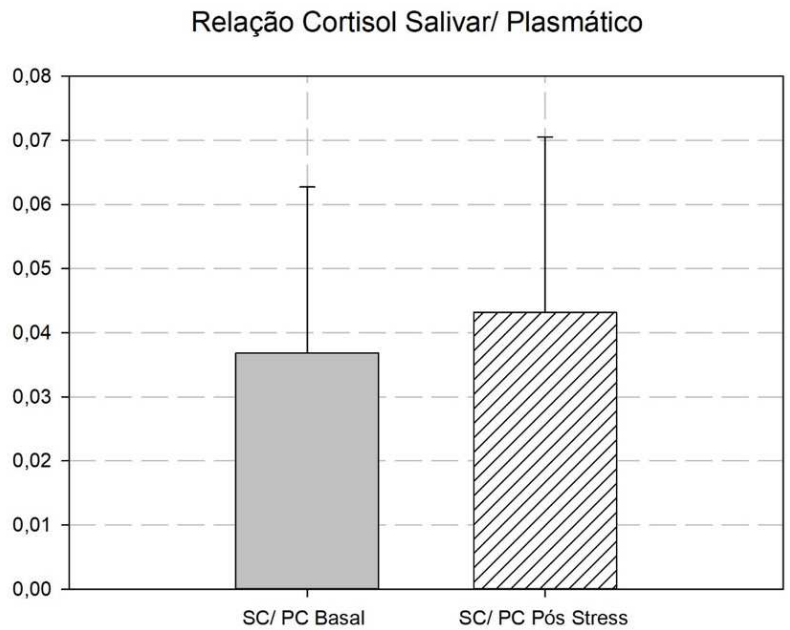



**Figure 3: Relationship between salivary and plasmatic cortisol concentrations at baseline and after application of the stressful stimulus, based on all groups of all studies included in this review. Average of the values in the bars.**

Tables 2, 3, 4, 5, 6, 7 and 8 briefly describe the general aspects of the articles included in this review. In 13 of the 18 studies (approximately 72%), the acute stress method applied was the TSST (Trier Social Stress Test) or slight modifications of it. This test, in general, corresponds to a short time preparation of a presentation, which will be exposed in public to a panel (generally a job application), followed by arithmetic calculation activities (also performed in public). This test has been pointed out by several authors as a very effective stressful stimulus (Kirschbaum e Hellhammer, 1994).

Although we have grouped the articles according to the nature of the study (4 studies - psychological stress; 7 - effects of drugs on stress; 3 - abstinence from smoking; 2 - intense physical exercises; 1 - sleep deprivation and 1 - patients who would undergo surgery), all data regarding plasma and salivary cortisol levels, obtained before and after the acute stressful stimulus, were considered in Table 9, regardless of the nature of the stress or the classification of the study. In this table 9, we observe that only 7 articles presented plasma and salivary cortisol values (before and after the stressful stimulus) in the form of tables or described in the Results chapter. In 11 of the studies, data were extrapolated from the graphs shown. All cortisol concentration values were expressed in ng/mL, based on the value of a gram of cortisol molecule (362.5g), so that relationships between plasma and saliva could be established.

In the 36 study groups of the 18 selected articles, the mean post-stressful stimulus plasma cortisol values were about 59% higher than at baseline (Post-stress/Baseline PC ratio 1.59 $\pm$ 0.69). Salivary cortisol seems to show a proportionally even greater increase after applied stress (about 116% - SC Post-Stress/Base ratio 2.16 $\pm$ 1.72) (Table 9). However, as we can see from the graph in Figure 2, there is no significant difference when comparing these relationships between salivary and plasmatic cortisol dosages.

Analyzing only the groups of subjects who did not experience any type of chronic stress or were not dependent on any substance (Table 10), we see that this proportion remains similar (Plasma: about 75% - CP Post-Stress/Base ratio of 1 .75 $\pm$ 0.92; Saliva: about 129% - SP Post Stress Ratio/Baseline 2.29 $\pm$ 1.67; Mean $\pm$ standard deviation), with no significant difference between the values compared for plasma and saliva.

Mean data on the salivary cortisol/plasma cortisol ratio at baseline and post-stress conditions, from the 36 study groups, are very similar (0.0368 ± 0.0258 - Baseline; 0.0431 ± 0.0273 - Post-stress; 3.6% vs 4.3%) (Table 9 and Figure 3). A similar proportion is found in Table 10 (study groups that have not gone through any previous situation of chronic stress and that do not have any pharmacological dependence): 0.029 ± 0.012 - Baseline; 0.0360 ± 0.0138 - Post-stress (2.9% vs 3.6%). These data show that salivary cortisol seems to correspond to approximately 3 - 4% of the plasmatic cortisol concentration, whether in baseline conditions or immediately after acute stress.

**DISCUSSION**

The fundamental idea of this systematic review was to investigate in the scientific literature whether there was a constant and reliable correlation between salivary and plasma cortisol values in different stress situations. For this analysis, we only considered articles that submitted the research subjects to some type of acute stressful stimulus, since many physiological and adaptive variables can play an important role in long-term stress situations. (McEwen, 2007).

Clinical and surgical procedures in dentistry are considered by most people to be extremely stressful situations (Armfield, 2013a; b; Armfield and Heaton, 2013; Gatchel et al., 1983; Pohjola et al., 2007). Thus, the prior administration of anxiolytic agents in dental care seems to be of great importance. In scientific investigations in this field, many subjective tests have been used to assess the level of stress (Corah, Gale and Illig, 1978), while few are the articles that use objective methods for this assessment (Miller and Shakes, 1995). Thus, objective measurements of the level of stress would be of fundamental importance for the study of drugs that reduce anxiety and fear before and after surgery in dentistry. Plasma cortisol concentration has been used as one of the objective measurements, in addition to measurements of heart rate, blood pressure, measurement of plasma levels of adrenaline, noradrenaline and ACTH (Duclos et al., 1998; Hellhammer et al., 2004; Jezova et al., 2005). However, these blood measurements involve technical difficulties, in addition to causing a certain degree of stress in themselves. For this reason, the correspondence between salivary and plasma cortisol correlation would be so important, in control and stress situations, since saliva collection is an extremely simple and non-invasive procedure. This was the main objective of this systematic review.

We found only 1 article that met all inclusion criteria analyzing plasma and salivary cortisol in patients who would undergo cardiac surgery (Bergmann et al., 2001). This fact shows the importance of future investigations, randomized and controlled, that use simple and non-invasive objective methods, to investigate the level of pre, trans and postoperative stress.

This work has some limitations and inaccuracies, as only 7 studies showed real values (mean ± standard deviation) of cortisol concentrations in saliva and plasma, at baseline and post-stress. A large part of the data used in this analysis was obtained by extrapolating the points shown by the graphs in their respective value scales, which brings a certain degree of imprecision, but which does not invalidate the results obtained.

Our review also did not analyze populations living under constant stress compared to individuals under non-stress, since the main objective of this study was to bring consistent data, which would allow the use of salivary cortisol levels in the assessment of acute stress in dental surgeries.

Another interesting point shown in this review was the proportion of 4% of salivary cortisol compared to plasmatic cortisol, a ratio always constant both in baseline and post-acute stress situations. Khalili-Mahani and collaborators (2015), using pharmacokinetic analysis, also reported a significant correlation between salivary and plasma cortisol, around 4%, after consecutive measurements at different times. In this way, our studies corroborate, in a more systematic way, results shown in the scientific literature. This fraction of 4% (SC/PC ratio around 0.04) can be explained by the fact that the cortisol present in saliva is the one that was not bound to plasma proteins (free fraction), thus passing through the epithelial barrier and being secreted along with this one. Plasma cortisol dosage shows the total cortisol, bound or not to plasma proteins (Khalili-Mahani et al., 2015).

The similar percentage increase in cortisol concentration after acute stress, both in plasma and in saliva, demonstrates that the two dosages can be used as objective measurements in the assessment of anxiety and stress level, in different clinical situations. The consistency and similarity in the relationship between salivary/plasma concentration, in baseline situations and after the application of the acute stressful stimulus, allow us to state, based on the data analyzed in this review, that salivary cortisol is a reliable parameter for objectively assessing the level of stress , as it constantly and consistently reflects the concentration of this hormone in the plasma.

## CONCLUSION

The concentration of salivary cortisol consistently correlates with the plasmatic concentration of this hormone in all situations analyzed and, due to simplicity and practicality, its dosage in saliva can be indicated as a parameter for evaluating the level of stress in dental surgical procedures.

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