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Nociception related biomolecules in the adult human saliva: a scoping review with additional quantitative focus on cortisol

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Abstract

Nociception related salivary biomolecules can be useful patients who are not able to self-report pain. We present the existing evidence on this topic using the PRISMA-ScR guidelines and a more focused analysis of cortisol change after cold pain induction using the direction of effect analysis combined with risk of bias analysis using ROBINS-I. Five data bases were searched systematically for articles on adults with acute pain secondary to disease, injury, or experimentally induced pain. 43 articles met the inclusion criteria for the general review and 11 of these were included in the cortisol-cold pain analysis. Salivary melatonin, kallikreins, pro-inflammatory cytokines, soluable TNFαreceptor II, secretory IgA, testosterone, salivary α-amylase and, most commonly, cortisol have been studied in relation to acute pain. There is greatest information about cortisol and sAA which both rise after cold pain when compared with other modalities. Where participants have been subjected to

both pain and stress, stress is consistently a more reliable predictor of salivary biomarker change than pain. In conclusion, there remain considerable challenges in identifying biomarkers that can be used in clinical practice to guide the measurement of nociception and treatment of pain. Standardization of methodology and researchers' greater awareness of the factors that affect salivary biomolecule concentrations are needed to improve our understanding of this field towards creating a clinically relevant body of evidence.

INTRODUCTION

Effective pain management is a humanitarian responsibility and is essential to recovery and rehabilitation after surgery and trauma.[1] Achieving it relies on robust methods for the assessment of pain and nociception. Pain is by nature subjective[2] and acute pain assessment methods rely on self-reporting, using either scales (predominantly in acute pain) or questionnaires (predominantly in chronic pain). These methods are unhelpful when patients cannot self-report, for example, infants and young children, people under anaesthesia, or those with cognitive disabilities and mobility impairments. In these circumstances assessments based on behavioural and physiological indicators are used [3,4] which rely on the expertise of healthcare professionals, limiting their reliability.[5] Further, they are not specific and may indicate other physiological or pathological processes.[6] The relationship between pain and nociception, that is the level of activity in noxious pathways, is not straightforward and can particularly be affected by stress. Nonetheless when pain self-reporting cannot be used, a reliable assessment method based on nociception, such as monitoring the bio-fluid levels of molecules related to nociceptive signaling

would enable clinicians to titrate analgesics more effectively. Saliva is a favorable bio-fluid because it can be obtained rapidly and non-invasively, when compared to, for example, blood or cerebrospinal fluid.

We aimed to collate the evidence on salivary nociception-related biomolecules in order to (1) identify potential biomarkers for acute pain, (2) determine whether change in biomolecule levels correlates with pain intensity and (3) whether this is different between the sexes. After article selection in line with inclusion criteria, it was evident that most of the studies in this field relate to change in salivary cortisol with experimentally induced cold pain and we have therefore done a more detailed review of this.

METHODS

Design

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR).[7] The protocol was registered with the Open Science Framework.[8] The review has one deviation from the registered protocol. This has been explained in the data synthesis section.

Search strategy

A preliminary search was conducted in Medline to develop the key search items. A systematic literature search was done in Ovid MEDLINE, Ovid EMBASE, Web of Science, CENTRAL and PubMed in July 2020. There were no limitations by study design, language, or publication year. E-mail alerts were set up until 31st December 2022. The final search strategy is reported in the registered protocol.

Study selection

Two reviewers removed duplicates and assessed titles and abstracts independently. The full texts of potentially relevant articles were screened against the inclusion and exclusion criteria (TABLE 1) and reasons for exclusion were recorded. Disagreements were resolved by consensus between all authors. The reference lists of included articles were hand-searched to identify additional relevant articles.

TABLE 1. Inclusion and exclusion criteria for selecting the sources of evidence.

Attribute	Inclusion criteria	Exclusion criteria
Study population	Human adults (≥18 years): - with experimentally induced acute pain on a background of no pain or a background of a chronic painful condition - with acute pain conditions (such as post-operative pain, burns pain, zoster pain, acute (spinal) disc prolapse, fracture pain, renal colic, or biliary colic) of less than 6 weeks duration	• Age <18 years • Animal studies • Studies with participants who: - had chronic pain, a chronic painful condition, or acute pain that is an exacerbation or flare-up of a chronic or recurrent pain problem (for example migrainous acute headache) - had labour pain or postnatal pain - had a condition that disrupts the normal physiological conditions in the oral cavity (e.g. oral mucositis, oral diseases, or acute dental pain)

		- had substance dependence or abuse
Type of article	• Peer-reviewed published observational studies in any setting • Studies in any language and with any publication date	Review articles, case reports, conference abstracts, publications without data, letters, and comments
Study outcomes	• Studies designed to detect and measure nociception-associated biomolecules in human saliva after pain induction AND • compared these to a baseline level before pain induction, either before the onset of acute pain or using a control group	• Studies that did not include a pain assessment method

Data extraction and data synthesis

Two reviewers independently charted data on article characteristics, study methodology and outcomes. Using a narrative synthesis approach,[9] included studies are grouped based on the type of biomolecule and the modalities of pain sensation. Variations in outcomes between sexes and correlation between biomolecule concentration change and pain severity are noted where data are

available. Papers appear in more than one category if more than one biomolecule was studied.

Differences between the registered protocol and this review

A more focused analysis was added to examine the evidence for consistent rise in cortisol after cold pain and the pattern of this change. We ran the papers on salivary cortisol and cold pain through an additional set of inclusion criteria where participants were healthy, took no analgesia, and underwent cold exposure shown to be painful as evidenced by increase in pain intensity using a validated tool. Studies were excluded if participants had intentional exposure to another stressor (for example a cognitive task) in the same experiment. If there was more than one arm to the study, only participants not exposed to additional stressors were included.

Cortisol-cold pain data synthesis: After contacting study authors, we were unable to obtain data on missing elements (e.g. precise *p*-values, effect size estimates) in a number of studies. This limited the options for data synthesis methodology. To try to determine time of maximum cortisol change after pain induction, we used vote counting based on direction of effect[10] where outcomes are classified as increase in salivary cortisol (positive direction of effect), decrease (negative direction) or no clear effect (NCE) in 3 defined outcome domains: a) ≤ 10 minutes, b) 10 - 20 min and c) ≥ 20 minutes after cold pain induction. In experiments that had multiple time points within a domain, the effect direction was determined using the method described by Hilton, Boon and Thomson (2020). The pre-CPT cortisol concentration at the time point closest to the onset of pain induction was taken as the baseline value.

Statistical significance and effect size were not considered in the categorization.[11]

The included studies in the cortisol cold pain analysis were assessed for methodological heterogeneity and risk of bias by two of the authors. Articles were assessed in all 7 domains of Risk of Bias In Non-randomized Studies of Interventions (ROBINS-I).[12] We added assessment of funding and conflict of interest. In each domain one of three categories (low, moderate, high) of risk of bias judgement was assigned.

RESULTS

Selection of sources of evidence

The PRISMA flow diagram appears in FIGURE 1. The initial search yielded 1886 records and 180 records came through e-mail alerts until 31st December 2022. Ninety-one articles were selected for full text assessment, which were all in English. Forty-three fulfilled the criteria for inclusion (TABLE S1 in supplementary material). No additional articles were identified by searching the reference lists.

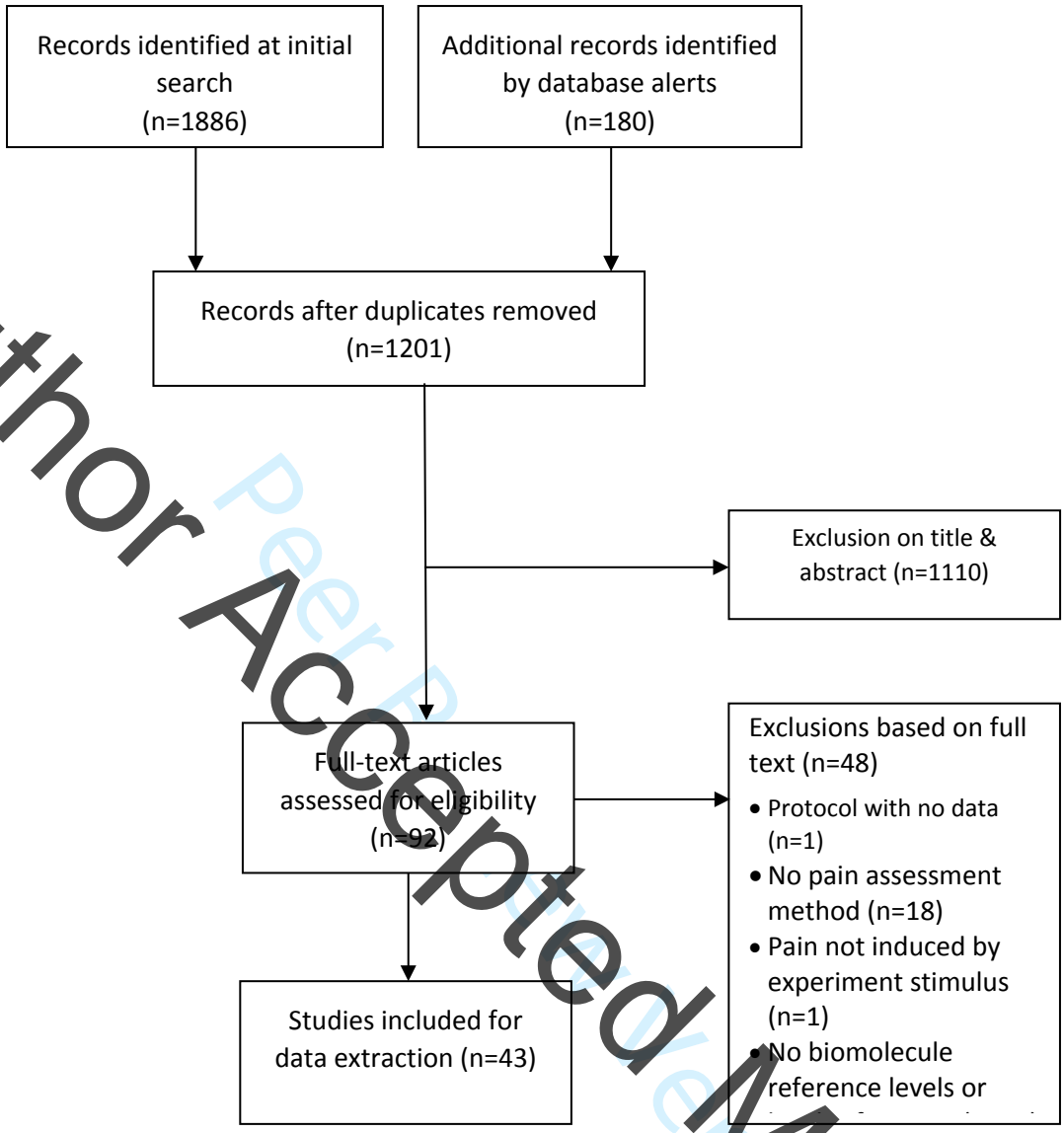

Fig 1: PRISMA flow diagram

Characteristics of included studies

Most studies (35 of 43) involved experimentally induced pain.[13–46] The rest involved acute pain after surgery or a medical procedure: hysterectomy,[47] thoracic surgery,[17] skin surgery,[48] corneal surgery,[49] breast surgery,[50] gastric or bronchial tube replacement[51] and drawing blood[52] or vascular access.[53] Experimental pain induction was done by cold, heat, ischemia and mechanical,

visceral, chemical or electrical stimulation (TABLE 2). Four studies used more than one pain induction method.[29,30,41,54] Twenty-eight researchers used standardized methods with citation.[13–16,19–22,24,25,27–32,34–39,42–46,54] Seven used either a novel technique or a known technique with no citation.[18,23,26,33,40,41,55] In 41 studies, baseline and post-pain biomolecule concentrations were measured from the same participant group in a before-after design. Two studies used measurements from a control experiment as reference.[40,54]

Long term analgesia intake interferes with the biomolecules involved in nociceptive pathways.[56] In 20 articles participants taking regular analgesia were excluded.[15,18–20,22,23,27–30,34,35,37,38,41,43,44,49,50,54] Four studies included occasional users of analgesics[16,21,31,33] and one included participants treated with regular analgesia including opioids[55]. Sixteen studies did not report on analgesia intake.[13,14,24–26,32,36,39,40,42,45,46,48,51–53] .

There is considerable overlap between nociception-related biomolecules and those associated with stress and chronic health conditions. Twenty articles excluded participants with psychiatric disorders[16,20–30,32–34,37,41,43,48,50] and two did not[49,55]. The remaining 19 did not report on psychiatric conditions.[13–15,17–19,31,35,36,38–40,42,44–47,51,52] People with chronic pain were excluded in 19 studies[1.3,17,19–22,24–30,35,38,43,47,49,50] whereas in 20 studies it was not clear if any participants had chronic pain[14–16,18,31–34,37,39,40,42,44–46,48,51–54]. Four studies enrolled participants with chronic conditions including back pain,[55] chronic fatigue,[36] fibromyalgia,[23] and temporomandibular disorder[41] as part of the study design.

Saliva sampling techniques:

Whole saliva is a mixture of secretions from salivary glands plus non-salivary components.[57] Oral mucosal transudate (OMT), collected from the tissues between the cheeks and gums, derives from passive movement of serum components through the oral mucosa into the mouth.[58]

Although salivary biomolecule concentration can be affected by the method of saliva collection and stimulation of flow,[59] reporting on these in research studies is inconsistent. Three studies provided no information[38,48,53] and eight did not clearly report whether saliva was stimulated[16,21,23,31,33,36,48,55], although this is of little consequence where the biomolecule concentration is independent of salivary flow (e.g. for cortisol). Seven studies included a restriction of 0.5–3 hours on tooth brushing and eating to avoid blood contamination from mucosal micro-injuries.[19,20,28–30,32,44]

Food, alcohol, nicotine and caffeine affect salivary flow.[60,61] In 9 of the studies no restrictions are reported.[13,17,33,38,42,48,50,53,55] In 11 studies restrictions were variably applied: food was restricted for 30-120 minutes (mode 60) and caffeine for 0.5-12 hours (mode 12). Smoking and alcohol were more variably restricted, sometimes as length of time, and sometimes as dose. One study was specifically designed for investigating pain in smokers[37] and three gave no information on smoking[27,35,53]. Alcohol was restricted as the number of units or "drinks" a day in 7 studies[14,15,21,22,31,37,51] or by asking participants to avoid intake for 0.5-24 hours[16,18–20,28–30,32,34–36,41,43–45,47,52]. Nineteen studies did not report on alcohol intake.[13,17,23–27,33,38–40,42,46,48–50,53–55]

TABLE 2: Summary of the experimental pain induction methods used

Type of pain	Pain induction method	Description of method	Articles
Cold pain	Cold Pressor Task n = 18	Immersion of a body region in a cold water bath for a pre-specified maximum time of cold exposure or for as long as the subject could tolerate it Water temperature [a] 0-5 [b] 8-10 Body region immersed [c] Hand or arm [d] Foot or feet Endpoint (sec) [e] 45 [f] 60 [g] 90 [h] 180 [i] 240 [j] 300	[4] al' Absi 2003 [a, c, g] [3] al' Absi 2002 [a, c, g] [6] Bachmann 2003 [a, d, h] [27] Goodin 2012 (1) [a, c, j] [31] Hengesch 2018 [a, d, h] [42] Larra-Cinisomo 2015 [a, c/d, h] [54] Nakajima 2011 [a, c, g] [57] Niedbala 2018 [a, c, h] [66] Serrano 2019 [a, c, f] [28] Goodin 2012 (2) [a, c, j] [29] Goodin 2012(3) [a, c, j] [62]Quartana (2010) [a, c, e] [79] Youssef (2018) [a, c, j] [1] Archey 2019 [a, c, j] [12] Burns (2004) [b, c, i] [16] Cruz-Almeida (2004) [b, d, f] [18] Finke (2021) [a, d, h] [47] Lukacs (2022) [a,c,g]
	Plunge test n = 1	Intermittent immersion of hand and forearm in a cold water bath of 5°C with duration of immersion and rest periods of 5, 10, or 15 sec	[80] Zimmer 2013
Heat pain	Heat pain threshold n = 10	Administration of heat stimuli on the ventral forearm with a thermal device at 35-52°C for 6-50 sec or until pain tolerance was reached	[19] Gaab 2016 [23] Geva 2014 [24]Geva 2017 [25] Geva 2018 [53] Muhtz 2013 [62]Quartana 2010 [76]Wittwer 2016 [9] Benson 2019 [26] Geva 2022 [67] Schneider 2022
	Hot water task n = 3	Immersion of hand or arm in a bath of circulating hot water (46-47°C) for 2 min, or for as long as could be tolerated, with a maximum pain exposure of 5 min	[51] Meeus 2008 [28] Goodin 2012 (2) [29] Goodin 2012 (3)

Mechanical or visceral pain	Mechanical pressure n = 6	Application of algometer on at increasing force rate until *pain threshold was reached or †at 10N force for 2 min	[21] Geiss 2012* [34] Hoeger Bement 2010† [62] Quartana 2010*
		Balloon rectal distensions at a pressure of 2-55 mmHg	[9] Benson [35] Icenhour 2020
		Lying on the back on a bed of sharp-edged plastic nails (Shakti-mat) for 20 min	[58] Olsson 2011
Ischemic pain	Ischaemic pain task n = 2	Modified submaximal effort tourniquet procedure, exercising the hand while blood flow to the arm is occluded for as long as tolerated, with a maximum task duration of 15 min	[28] Goodin 2012 (2) [29] Goodin 2012 (3)
Chemical stimulation	Saline muscle injection n = 1	Intra-muscular injection of 0,4 ml hypertonic saline solution into the masseter muscle over about 30 sec	[13] Christidis 2020
Electric stimulation	Electric shock n = 1	Electric shock stimuli using a bipolar cutaneous electrode stimulator on the volar forearm; 20-impulse train of electroshocks with a duration of 5 ms to produce pinching pain	[56] Nelson 2001

Synthesis of results:

Melatonin

Melatonin has anti-inflammatory properties and reduces hyperalgesia in animal models. Surgical and cancer patients report higher pain intensity and use more analgesia in the day time though melatonin's role in these phenomena is unproven.[62] In a study of healthy people salivary melatonin decreased within 5 minutes of painful electric stimulation followed by a rise.[38] Correlation with pain ratings or sex were not analyzed.

Kallikreins

Kallikreins are responsible for physiological functions including blood pressure regulation and inflammation.[63] Increase in salivary kallikrein was shown 2-6 hours after hysterectomy. The peak increase was at 4 hours but pain ratings did not follow this pattern, peaking one hour post-operatively before decline.[47] No analysis by participants' sex was done.

Secretory Immunoglobulin A (sIgA)

There is a relationship between stress, including that induced by CPT, and change in sIgA . There are no clear mechanisms to explain sIgA change in relation to pain.[64] In a study where pain intensity during CPT was measured, sIgA fell significantly after first exposure to CPT, but not after second exposure in the same participants' other arm. There is no correlation with pain intensity.[18]

Salivary sIgA was measured in thoracic surgery with or without regional anaesthesia. While there was no difference in pain intensity between the two groups, reduction in sIgA, only occurred in the regional anaesthesia group at the 6 hour time point.[17] Conversely, in patients who underwent corneal surgery, sIgA increased 1 hour post-surgery and this rise correlated with pain intensity.[49] Sex differences were not analysed in either study.

Testosterone

Animal studies show that testosterone may have a protective effect in the development of chronic pain.[65] No change in salivary testosterone was found after corneal surgery[49] but after thoracic surgery, testosterone increased regardless of whether regional anaesthesia was used for pain control. In a study on female pain perception, no difference in salivary testosterone was found between healthy males and females after CPT.[13]

Pro-inflammatory Cytokines and soluble tumour necrosis factor α receptor II (sTNF-αRII)

Pro-inflammatory cytokines have a role in the development of neuropathic pain.[66] In a study that measured change in four cytokines (IL-6, IL-8, IL-10, IL-4) in the saliva and blood of healthy participants, after CPT or a painless thermal task, cytokine concentrations peaked 45-60 minutes after CPT while no change occurred in the control experiments.[20] The time course of cytokine change was nearly identical in saliva and plasma. In another study, pressure pain thresholds were measured in defined anatomical points in women with

fibromyalgia and pain-free women. Salivary IL-6 (and cortisol) increased after pain pressure in patients with fibromyalgia but not in healthy subjects.[23]

TNFα receptor 2 (TNFαR-II) has a neuroprotective role. Soluable TNFαR-II is the circulating form of this membrane bound receptor. In all three studies that analyzed salivary sTNFαR-II, there was reduction in the levels after acute pain.[29,30,49] Two were studies in healthy volunteers after exposure to multiple pain modalities (cold, heat and ischaemic pain). sTNFαR-II fell either immediately after pain induction or 25-35 minutes later.[29,30] sTNFαR-II also fell one hour after corneal surgery.[49] There was no significant correlation with pain ratings in the 2 studies that analysed this.[29,30] None of the studies reported analysis by participants' sex.

Salivary alpha-amylase

Salivary alpha-amylase (sAA) increases in response to sympathetic over-activity.[67] We found 13 acute pain studies that assayed sAA.[17,19,22,34,42–45,49–51,53,54] No change was found in healthy participants after painful hypertonic saline muscle injection.[19] One heat pain experiment reported rise in sAA correlating with pain intensity[44] but in two studies designed to observe the impact of psychosocial stress on pain perception, heat pain alone was not associated with change in sAA, while psychosocial stress was.[22,43] Similarly, in a study that examined the effect of hydrocortisone *vs* placebo on heat and visceral pain, there was no rise in sAA (or cortisol) after pain induction in the control arm.[54]

Rise in sAA after cold pain in healthy participants was showed in 2 studies but correlation with pain intensity was not analysed.[34,45] Change in sAA after CPT is affected by catechol-O-methyltransferase (COMT) Val158Met polymorphism where greater change has been found in Met allele carriers though pain ratings were equal in the groups.[42] sAA rise also occurred in people with severe disabilities undergoing medical procedures, correlating with pain intensity,[51] and after thoracic surgery[17] but no rise was found after painful vascular access,[53] corneal or breast surgery[49,50].

Cortisol

Cortisol is the most studied salivary biomolecule in relation to nociception.[14–17,21–37,39–43,46,48,49,52,54,55] Thirty two studies in this review have measured salivary cortisol and in most (n= 26), pain was experimentally induced.[14–16,21,22,24–32,34,35,37,39–41,43,46,48,49,54,55] In the induced studies, six found no difference between men and women[14,27,28,31,32,34] and one reported a greater cortisol rise in men[46]. In 3 studies a positive correlation was found between cortisol change and pain intensity ratings,[28,29,46] while five studies found no such correlation [22,36,40,41,43,55]. The rest did not report any analyses with respect to sex or pain intensity.

Post-operative and Post-procedure Pain: Salivary cortisol levels were at the high end of the normal range immediately before drawing blood, thereafter declining (after venipuncture) or staying the same (after finger prick).[52] There was rise in salivary cortisol 30 minutes after skin surgery compared with 1 week before the operation, but not when compared to 30 minutes pre-operatively.[48]

Cortisol increased in the immediate pre-operative period compared to baseline in people having corneal surgery, with a further rise 1 hour post-surgery.[49]

After thoracic surgery, cortisol increased compared to a baseline taken at the time of qualification for surgery, regardless of the provision of regional anaesthesia.[17] Importantly, salivary cortisol was not measured in the immediate pre-operative period in this study. None of the surgical studies analyzed correlation with sex. Correlation with pain intensity after drawing blood and thoracic surgery were analyzed and were not significant.

Heat pain: Salivary cortisol (and sTNFαR-II) were measured in two studies, where healthy volunteers were exposed to multiple pain modalities including cold, heat and ischaemic pain tasks.[29,30] In one of these, salivary cortisol elevation occurred after a battery of painful tasks.[30] In the other biomolecule changes were analysed separately, with the finding that heat pain (and ischaemic pain) alone did not induce change in salivary cortisol while CPT did.[29] Similarly, in six studies designed to assess the effect of acute psychosocial stress on pain modulation, heat pain alone or in combination with a sham stress task, was not associated with change in cortisol.[22,24–27,43] It was psychosocial stress that predicted cortisol rise. Correlations with sex were not analyzed except in one study where the researchers found that women exhibited stress-induced anti-nociception and men exhibited stress-induced pro-nociception.[27] Correlation with pain intensity was not analysed in these experiments.

In comparisons of healthy subjects with participants who had chronic pain, fatigue or depression, no increase was found in salivary cortisol after heat pain induction in any of the groups.[36,55]

Mechanical & visceral pain: No change was observed in salivary cortisol in healthy participants lying on a bed of nails compared to lying on a soft bed, despite participants lying on nails reporting rapid rise in pain.[40] Similarly, salivary cortisol did not change from baseline after applying painful pressure to the index finger of healthy participants but there was rise in cortisol if they were due to do a cognitive 'stressor' mathematics task.[32]

In women with fibromyalgia salivary cortisol (and IL-6) increased after measuring pain pressure thresholds but this did not happen in pain-free women.[23] In a comparison of people with temporomandibular disorder with healthy controls, pain pressure thresholds were measured, along with heat and cold pain thresholds. There was no difference in cortisol response between the two groups.[41]

In a study of visceral pain induced by rectal distension in healthy individuals, the results of salivary cortisol change were analyzed according to whether participants had high or low perceived background stress. Cortisol levels were higher throughout the experiment in those with higher perceived stress but there was no rise in cortisol in either group.[33] Similarly, there was no rise in

salivary cortisol (or sAA) after visceral and heat induction the placebo arm of a trial examining the effect of hydrocortisone *vs* placebo on pain perception.[54]

Cold pain: In studies that measured salivary cortisol, cold pain was induced using CPT[14–16,21,28–31,34,35,37,39,41,42] or the plunge test[46]. Four of the cortisol-cold pain studies were excluded from the direction of effect analysis because either pain was induced by a combination of stimuli with no separate analysis of cold pain ,[30,41] or, the experimental design included an emotional or cognitive task not separated from cold induction[31,39]. In one of these, where participants were put in a situation that allowed positive appraisal of cold pain, the cortisol response was inhibited compared with controls, though pain intensity was the same.[39] The other study showed that the cortisol response to CPT combined with a cognitive task was blunted in people with early life adversity though they experienced the same pain intensity as controls.[31] In a study where half of the healthy participants were exposed to social stress and the others were not, salivary cortisol increased in both groups after CPT.[15] Participants exposed to social stress reported less pain but had greater cortisol rise. The 76 participants of this study who were not exposed to social stress met the inclusion criteria for the direction of effect analysis. Overall these results suggest a disconnection between the salivary cortisol response to cold and pain intensity.

Eleven articles met the inclusion criteria for a more focused review of the effect of cortisol on cold pain.[14–16,21,28,29,34,35,37,42,46] In one of these, hand CPT and foot CPT were done separately in the same participants.[34] This study was therefore entered as 2 experiments, giving 12 experimental study groups for

the analysis. Therefore a total of 576 individual cold pain experiments (mean participant age 23.2 years) were included in total, with 11 drop outs. The effect direction plot is presented in TABLE 3. Measurements after 20 minutes were only taken in six experiments. Ten experiments had data in the less than 10-minutes outcome domain and ten in the 10-20 minutes outcome domain. Increase in salivary cortisol is reported in most experiments 10-20 minutes after cold pain induction.

TABLE 3. Effect direction plot summarizing direction of change in salivary cortisol levels from studies of experimental cold pain induction

First author (Date)	Reference	Sample size	Cold stimulus	Cold limb	Cortisol levels from baseline (min after cold pain)		
					<10	10-20	=>20
al'Absi (2003)	[4]	76[a]	CPT	hand	▼	▲	No data
al'Absi (2002)	[3]	62	CPT	hand	▼	▲	No data
Bachman n (2018)	[6]	27	CPT	feet	No data	▲	▲[4]
Goodin (2012)1	[27]	40	CPT	hand	▲	▲[2]	▲[3]
Lara (2015)	[42]a*	22	CPT	hand	▼	▼	▼[4]
	[42]b*	22	CPT	feet	▲	▲	◀▶[4]
Nakajima (2011)	[54]	91	CPT	hand	▼	▲	No data
Serrano (2019)	[66]	86	CPT	hand	▼	No data	No data

Zimmer (2003)	[80]	76	Plunge test	hand & forearm	No data	▲	No data
Goodin (2012)2	[28]	10	CPT	hand	◀▶	▲[3]	◀▶[2]
Finke (2021)	[18]	14	CPT	feet	◀▶	▲	◀▶[2]
Lukacs (2022)	[47]	50	CPT	hand	◀▶	No data	No data
Included: 12 experiment groups, total of 576 cold pain experiments in 554 participants							
Positive Effect (upwards arrow)					2	9	2
Negative Effect (downwards arrow)					5	1	1
No Clear Effect (sideways arrow)					2	0	3
No Data in the Outcome Domain					2	1	5
Two-tailed p-value (sign test for positive effect direction)					0.453	0.022	1.000

Effect direction:
▲▲▲ = increased levels from baseline
▼▼▼ = decreased levels from baseline
◀▶◀▶◀▶ = no change/mixed effects/conflicting findings
Sample size in each group:
large arrow, ▲ ▼◀▶ (orange colour) >50;
medium arrow, ▲▼◀▶ (green arrow) 25–50;
small arrow, ▲▼◀▶ (blue colour) <25.
The sample size corresponds to the final number of participants included in the analysis.
Number of time points in each outcome domain is 1 unless indicated with a number beside the effect direction arrow.
'No Data' indicates no measurement in the time frame of the outcome domain
CPT: cold pressor task; NCE: no clear effect.

Risk of bias (ROB)

A ROB table is presented (TABLE 4). The important confounder would be co-exposure to psychological stress which would falsely create, or amplify rise in salivary cortisol. All experiments with no control (neutral or warm water) were

judged at least moderate in risk of confounding. A reasonable step to minimise stress would be participant awareness that they could withdraw at any time. If no steps were taken to minimise stress the risk was judged high. Where there was a control, risk was judged to be low but only if stress and anxiety were showed to be equal in cold water and control groups, and, did not rise in the control group after the task. If this was not shown, the risk was judged moderate.

Selection bias is principally related to recruitment being restricted to university communities. This was judged to be at least moderate in all the experiments. It was judged high when it was unclear whether all potential participants had an equal chance of inclusion. This bias creates issues of generalizability or transferability to other populations, and could be classified as sampling (rather than selection) bias. Nonetheless, we included it because of a concern that it is ignored by many: only 4 of the 11 papers mentioned this bias in their discussion section.

ROB related to classification of exposure was judged low in all experiments because the exposures to cold and control procedures were well defined prior to the outcome assessment.

We considered experimenter and participant interaction to be a co-exposure that could affect change in cortisol concentration. ROB due to departures from intended exposure was judged moderate when these interactions were not clearly standardised for example it was unclear whether the experimenter was in the room during CPT.

ROB due to missing data was judged low when there were no missing data in relation to salivary cortisol measurement or researchers accounted for this in the analysis. Where this was not accounted for, ROB was considered moderate whether there was no indication of differential loss related to prognostic factors. Overall 576 individual experiments were done, where 19 (3.3%) had missing data relevant to pain induction and salivary cortisol measurement with 11 (1.9%) not accounted for in study analyses.

Samples were stored at -20, -70 or -80°C before defrosting in bulk for analysis sometime later. Although none of the papers described blinding at the analysis stage, we considered performance bias unlikely and ROB in measurement of outcomes was judged low.

We were not able to access pre-specified protocols for any of the included studies. All researchers used only one method of cortisol measurement and results analysis and values were not selected from multiple outcomes. ROB in selection of the reported result was judged moderate for all the studies.

All studies were funded by non-profit organisations, university funding bodies or national institutes and were at low risk of funding bias. Two papers specified the role of the funders in the conduct of the research and its publication, both reporting no role.[29,34]

Overall the risk of bias was judged high in 6 studies (7 experiments) and moderate in 4. High risk was due to possible confounding in 4 studies and due to possible selection bias in two (3 experiments).

TABLE 4. Risk of bias in included studies

	Funding & Conflicts of interest	Confounding	Selection of participants	Classification of exposure	Departures from intended exposure	Missing data	Measurement of outcomes	Selection of reported result
Al Absi 2003 [4]	Low	High	Moderate	Low	Moderate	Low	Low	Moderate
Al Absi 2002 [3]	Low	High	Moderate	Low	Moderate	Low	Low	Moderate
Bachmann 2018 [6]	Low	Low	Moderate	Low	Low	Moderate	Low	Moderate
Goodin 2012 (1) [28]	Low	Moderate	Moderate	Low	Moderate	Low	Low	Moderate
Lara 2015a [42]	Low	Moderate	High	Low	Low	Moderate	Low	Moderate
Lara 2015b [42]	Low	Moderate	High	Low	Low	Moderate	Low	Moderate
Nakajima 2011 [54]	Low	High	Moderate	Low	Moderate	Low	Low	Moderate
Serrano 2019 [66]	Low	High	Moderate	Low	Moderate	Low	Low	Moderate
Zimmer 2003 [80]	Low	Moderate	Moderate	Low	Moderate	Moderate	Low	Moderate
Goodin 2012 (3) [27]	Low	Moderate	Moderate	Low	Moderate	Low	Low	Moderate
Finke 2021 [18]	Low	Low	Moderate	Low	Low	Low	Low	Moderate
Lukacs 2022 [47]	Low	Moderate	High	Low	Moderate	Low	Low	Moderate

Methodological heterogeneity in salivary cortisol cold pain studies

Differences in saliva collection

Timing of collection: In all but one article experiments[35] were conducted in a particular part of the day: three were done in the morning[14,15,37] and 7 in the afternoon[16,21,28,29,34,42,46]. In 4 articles, no reason was given for this choice[14,15,34,37], 2 stated that afternoon times are associated with greater cortisol responses[28,29] and others simply stated 'to control for diurnal variation'[16,21,42,46].

Collection method: Whole saliva was collected in 11 experiments and oral mucosal transudate in one. The device used was usually a cotton swab that was later centrifuged to release saliva.[14–16,21,28,29,35,37,46] This method can yield a different cortisol concentration compared to saliva obtained by passive drool.[68] As salivary cortisol closely follows free serum cortisol, this is unlikely to be significant for this data synthesis.

Participant preparation: Restrictions to food, alcohol, smoking and caffeine were variably applied. Most researchers placed restrictions on all of these.[14–16,21,28,29,34] One study placed no restrictions,[42] one restricted alcohol only[37] and one restricted smoking only[46]. Precautions to reduce the risk of blood contamination from gums were taken in four experiments.[16,28,29,34]

Differences in assays of salivary cortisol concentration

Immuno-assays with high sensitivity were used. Intra and inter-assay coefficients of variation were reported in 6 of the included articles with values ranging 4-12%.[16,28,29,34,37,46]

Differences in conducting cold pain induction tests

Overall the experiments had little heterogeneity with respect to the conduct of CPT. Water temperature was 0-5 °C. Nine experiments were of upper limb immersion and 3 were feet immersions.

DISCUSSION

Many salivary biomolecules have been studied in acute pain settings. Researchers' rationale for selecting these biomolecules varies. Melatonin, cytokines and testosterone were selected because of evidence for their involvement in modulation of noxious stimuli. Kallikriens, sAA and sIgA have been selected because they are stress biomarkers. Cortisol is a stress marker and is released in response to acute pain through HPA axis activation.[69] Some of these salivary biomolecules have been studied in very few experiments and the most commonly studied are sAA and cortisol. Regardless of the type of biomolecule, there is considerable methodological variation in the studies. Most researchers have chosen to induce pain under controlled conditions with cold pain induced by CPT being the most studied modality.

Salivary biomolecules that change with stress would be expected to change after acute pain in healthy individuals. This expectation is not consistently met, but when pain modalities have been compared in salivary cortisol experiments, change is encountered after controlled pain induction with cold rather than other modalities including heat, ischaemic, pressure or visceral pain.

In experiments designed to differentiate between responses to pain and stress, stress is found to be a better predictor of sAA and cortisol rise. This may explain the inconsistent relationship between the magnitude of cortisol or sAA rise and pain severity. Exposure to stressful cognitive or psychosocial tasks combined with heat or pressure pain, results in rise in sAA and cortisol while heat or pressure pain alone do not. In contrast, in a study involving healthy people, positive appraisal of cold pain reduced the stress response, including a lack of rise in salivary cortisol.

In the same vein, where salivary cortisol has been measured after surgery or procedures, regardless of the great variation in the physical nature of the painful interventions, its rise is timed more to pre-operative or pre-procedure stress than the ensuing trauma and pain.

There are complex relationships between gonadal hormones and pain processing.[70] and in women, menstrual cycle phase and pregnancy can influence cortisol concentration. Some researchers have circumvented these effects by recruiting only male participants.

Of 43 articles included, 17 analyzed the relationship between biomolecule concentrations and pain intensity and only 11 analyzed the relationship with sex. The groups are highly heterogeneous and it is not possible to draw reliable conclusions from them.

Looking more closely at 12 experiments where salivary cortisol was measured after experimentally induced cold pain in healthy people, it is possible to cautiously suggest that salivary cortisol rises 10-20 minutes after cold pain induction. This caution is advised because more than half of the experiments are judged to be at high risk of bias (though only one domain carries this high

risk in each of these) and most papers had missing elements such as precise p values and effect size estimates which precluded reliable quantitative analysis.

Heterogeneities in methodology influence the magnitude of change in cortisol. These include differences in the timing and method of saliva collection, blood contamination, restrictions on substances that blunt or enhance the cortisol response, exercise, the assay used, and conduct of cold pain induction. Alcohol, nicotine and caffeine are commonly used substances that affect salivary flow.[60,61] As salivary cortisol is not affected by flow, this would not influence the results of the cortisol–cold pain data synthesis. The effects of these substances, and also food and exercise, on cortisol secretion are potentially more important. The effect of exercise varies depending on whether it is regular or done in acute bouts.[71] Caffeine and nicotine are HPA stimulators[72–74] though the cortisol response is blunted in habitual smokers.[75] Alcohol consumption is associated with higher daily circulating cortisol levels but the stress response is suppressed with habitual high intake.[76] Therefore these substances either blunt or enhance the cortisol response, influencing how easily it would be detected.

The effect of the circadian rhythm on the cortisol response to pain is not known. Regardless of the timing, most researchers did not explain the reason for their time choice clearly and may have been influenced by convenience factors such as participants' availability or lab space. Cortisol is not the only nociception related biomolecule with a circadian rhythm. An obvious other example is melatonin and there may be other, hitherto unrecognized, patterns of diurnal change.

There is considerable methodological variation in inducing pain experimentally under controlled conditions with unknown consequences on the magnitude of biomolecule changes. We found this to be the case in all modalities, even cold pain induced by CPT, where we expected a relatively standardized approach. Variations have developed to the original CPT design,[77] including immersion of the non-dominant hand, hand plus forearm, one or both feet[16,34] or single finger[78]. They all induce a physiological response with some evidence for a relationship between the response magnitude and the surface area of cooled skin.[34,78] Additionally differences have been found in sympathetic responses to lateralized cold stimuli.[79] Although the cortisol response has not been studied in this way, some researchers argue for bilateral feet cold stimulation to avoid laterality bias and to keep arms free for other purposes (e.g. blood sampling).[16,31,34] Additionally, we have found other variations in the conduct of cold pain induction for example the exact water temperature and test end points.

Participants' mean age in the included studies is relatively young. Daily cortisol output increases with age[80] but the effect on the cortisol stress response is unknown, representing an important gap in the evidence. There is less knowledge on age related effects for other biomolecules, an important gap in the literature.

A highly heterogeneous and complex landscape has developed in this research field. To be useful in clinical practice as a guide to acute pain treatment, the ideal salivary nociception biomarker would be one (or a panel of biomarkers) that changes reliably after noxious stimuli, within a short time interval of at most

a few minutes, in healthy people and in those with acute or chronic conditions. It should be either minimally or predictably affected by change in the organism's internal or external environment. There remain considerable challenges in identifying such biomarkers. Importantly, there are differences in salivary biomolecule responses to different pain modalities and none of the biomolecules studied to date are specific to nociceptive pathways.

Improvements in bioengineering will enable measurement of salivary biomolecules more easily and at lower cost. To advance this area of research, it is essential to standardize methodology in salivary sample collection and pain induction. Salivary biomolecule secretion is affected by a complex multitude of factors in both healthy individuals and those with physical and mental health disorders or chronic stress.

Researchers should be aware of the wider factors that can affect biomolecule concentration such as salivary flow, commonly used pharmacological substances, exercise, acute stress, chronic conditions including chronic pain and psychiatric conditions, use of analgesia, diurnal variations and participant demographics. Cortisol secretion in particular is influenced by many of these. There can therefore be large differences in biomolecule levels that are not merely due to measurement error or individual variation. This difficulty can be augmented by the lack of consistency between different assays. In experimental designs, measuring change in biomolecule levels is likely to be more informative than absolute levels. Less heterogeneous experimental designs should be agreed and implemented by the researchers in this field in order to create a more cohesive and clinically relevant research literature.

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Author contributions

RZ: Concept, literature search, design, data acquisition, analysis, manuscript writing.
AV: Literature search, data extraction, manuscript preparation.
QL: Literature search, data extraction.
SG: Concept, design, analysis, manuscript writing

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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TABLE S1: Characteristics of included studies (n = 43).
Risk of bias summary for articles included in the cortisol – cold pain quantitative synthesis
Abbreviations: Indirect antibody enzyme-linked immunosorbent assay (ELISA). Where mean age was not reported, any other information about age was inserted in the table. Only significant outcomes are inserted in the table.

Ref	First Author (publication year) Biomolecule(s)	Characteristics
15	Alabsi (2003) Cortisol	**AIM** To determine the extent to which hemodynamic and cortisol changes during acute psychological stress predict pain perception **METHODS** **Country:** United States of America **Participants** **Recruitment:** From the university community by posters and newspaper advertisements **Age:** Mean 20.3 **Sex:** 80 F, 72 M **Total number of participants:** 152 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 152 **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric conditions and chronic pain not specifically reported) **Restrictions:** Food, alcohol, smoking, caffeine **Study design:** Salivary cortisol measured before and after an induced noxious cold stimulus applied after a psychosocial stress (public speaking) or rest condition **Other measurements:** Pain intensity and quality, mood, blood pressure, stroke volume **Interventions:** Acute cold pain induced using CPT **Comparison:** Change from baseline compared between stress and rest states. **Salivary assay:** Time resolved immunoassay with fluorometric end point detection **Saliva type:** Unstimulated saliva **Collection method:** Swab **OUTCOMES**

		Salivary cortisol increased in both groups after CPT. Participants in the social stress arm of the experiment reported less pain but salivary cortisol rise was greater **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed **NOTES** **Inclusion in the cortisol- cold pain quantitative analysis:** Yes, for the 76 participants not exposed to the stress task **BIAS** **Bias Type:** High risk of confounding. Moderate risk in selection of participants, departures from intended exposure and selection of reported results **Author's judgement:** High ROB **Support for judgement:** No control arm and no reported measures to reduce participant anxiety, recruitment limited to university community, interactions between experimenter and participant not clearly defined and no published pre-specified protocol
[14]	Alabsi (2002) Cortisol	**AIM** To evaluate the extent to which cortisol concentrations, blood pressure and hemodynamic contribute to gender differences in pain sensitivity has not been investigated **METHODS** **Country:** United States of America **Participants** **Recruitment:** From the university community by posters and newspaper advertisements **Age:** 19.7 **Sex:** 34 F, 31 M **Total number of participants:** 65 **Dropouts:** 3 (2F, 1 M) **Reason for drop out:** Not applicable **Revised sample size:** 65 **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions and chronic pain not specifically recorded) **Restrictions:** Food, alcohol, smoking, caffeine **Study design:** Salivary cortisol measured before and after an induced noxious stimulus **Other measurements:** Blood pressure, heart rate, stroke volume, pain intensity, pain descriptors (MPQ), mood **Intervention:** Acute cold pain induced using CPT

		Comparison: Change from baseline **Salivary assay:** Time-resolved immunoassay with fluorometric end point detection **Saliva type:** Unstimulated **Collection method:** Swab **OUTCOMES** Salivary cortisol increased following the CPT **Correlation with pain ratings:** Not analysed **Sex effects:** No correlation found **NOTES** Women reported greater pain than men during and after CPT. Cortisol concentrations predicted lower pain reports during and after CPT in men only. **Inclusion in the cortisol- cold pain quantitative analysis:** Yes **BIAS** **Bias Type:** High risk of confounding. Moderate risk in selection of participants, departures from intended exposure and selection of reported results **Author's judgement:** High ROB **Support for judgement:** No control arm and no reported measures to reduce participant anxiety, recruitment limited to university community, interactions between experimenter and participant not clearly defined and no published pre-specified protocol
13	Archey (2019) Testosterone	**AIM** To examine the role of testosterone in female cold pain expression and perception **METHOD** **Country:** United States of America **Participant** **Recruitment:** Recruited from an undergraduate university using flyers and campus-distributed **Age:** Mean 21.61 **Sex:** 38 F, 16 M, 2 not self-identified on questionnaire **Total number of participants:** 56 **Dropouts:** 10 (8 medication use or medical conditions, 2 incomplete sex identification on question sheet) **Reasons for drop out:** Medication use, medical conditions known to affect hormone levels **Revised sample size:** 46 (32 F, 14 M) **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions not specifically reported, chronic pain part of exclusion criteria)

		Restrictions: None reported **Study design:** Salivary testosterone measured before and after an induced noxious stimulus **Other measurements:** Pain intensity **Intervention:** Cold pain induced by CPT **Comparison:** Change from baseline **Salivary assay:** ELISA **Saliva type:** Stimulated **Collection method:** Passive drool **OUTCOMES** No significant difference in salivary testosterone between males and females **Correlation with pain ratings:** No correlation found **Sex effects:** No significant difference between males and females
[48]	Augustin (1999) Cortisol	**AIM** To investigate the peri-operative stress reactions following skin surgery by assessing cortisol, anxiety, vital functions and immune parameters **METHOD** **Country:** Germany **Participants** **Recruitment:** Consecutive patients with nevi from a dermatology clinic **Age:** Mean 33.9 **Sex:** 25 F, 20 M **Total number of participants:** 58 **Dropouts:** 13 **Reason for drop out:** Not fully reported (most frequent reason stated to be “fear of blood-drawing”) **Revised sample size:** 45 **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions and chronic pain not specifically reported) **Restrictions:** None reported **Study design:** Salivary cortisol measured before noxious surgical stimulus, intraoperatively and after surgery **Other measurements:** Blood pressure, heart rate, respiratory rate, lymphocyte subpopulations, pain intensity **Intervention:** Skin surgery under local anaesthesia **Comparison:** Change from baseline (pre-operative) samples **Salivary assay:** Not reported

		Saliva type: Unclear **Salivary Collection Method:** Not reported **OUTCOMES** Significant rise in salivary cortisol 30 minutes post-surgery compared with 1 week before the operation, but not when compared to 30 minutes pre-operatively No significant difference between 30 minute preoperative and intra-operative levels Cortisol levels remained elevated one week after surgery **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed **NOTES** The reported pain intensity after the surgery was low-moderate in this study. On this basis the authors concluded that the cortisol rise is likely to be related to anxiety rather than pain or pain-induced stress
16	Bachmann (2018) Cortisol	**AIM** To examine the validity and feasibility of a fully automated bilateral feet CPT **METHOD** **Country:** Germany **Participants** **Recruitment:** Internet announcement posted at the university **Age:** 26 **Sex:** Male only **Total number of participants:** 28 **Dropouts:** 1 **Reason for drop out:** Missing samples for cortisol analysis (1), problems with haemodynamic data (2) **Revised sample size:** 27 for cortisol analysis, 26 for haemodynamic analysis **Analgesia intake:** Yes **Chronic conditions:** Healthy (psychiatric conditions part of exclusion criteria, chronic pain not specifically reported) **Restrictions:** Food, alcohol, smoking, caffeine. Precautions to reduce risk of contamination due to bleeding from gums **Study design:** Measurement of salivary cortisol before and after exposure to noxious cold stimulus or warm water control **Other measurements:** Pain intensity, stress, arousal, anxiety, haemodynamic data (blood pressure, heart rate, stroke volume, left ventricular ejection fraction, cardiac output, total peripheral resistance,

		Comparison: Change from baseline compared between CPT and control groups **Intervention:** Acute cold pain using CPT or warm water control **Salivary assay:** Time-resolved immunoassay with fluorometric detection **Saliva type:** Unclear **Saliva collection method:** Swab **OUTCOMES** **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed **NOTES** **Inclusion in the cortisol- cold pain quantitative analysis:** Yes **BIAS** **Bias Type:** Moderate risk in selection of participants, missing data missing data and selection of reported results **Author's judgement:** Moderate ROB **Support for judgement:** Recruitment only from the university community, missing data excluded in the analysis (but no indication that there was differential loss of data) and no published pre-specified protocol
54	Benson (2019) Salivary alpha amylase	**AIM** To test the effects of oral hydrocortisone on pain thresholds and explore the sex differences as well as the effects of hydrocortisone on pain related fear **METHOD** **Country:** Germany **Participants** **Recruitment:** By local advertisement **Age:** hydrocortisone arm: mean 24.8, placebo arm: mean 25.1 **Sex:** 50 F, 50 M **Total number of participants:** 108 **Dropouts:** 8 **Reasons for drop out:** medical condition or medication intake (5), technical issues on the study (3) For rectal distension: distention pressure limit precluded measurement of the threshold (8 in hydrocortisone group, 7 in placebo group and in the heat pain 4 excluded (no reliably determined heat thresholds 3 shared with the other 15. **Revised sample size:** 100 **Analgesia intake:** None (irregular use of over the counter pain medications permitted) **Chronic conditions:** Healthy (psychiatric conditions and chronic pain not reported)

		Restrictions: smoking **Study design:** Measurement of salivary cortisol and alpha-amylase before and after induced noxious visceral and heat stimuli with pain induction taking place before and after hydrocortisone or a placebo control pill, in double blind randomized trial **Other measurements:** pain intensity **Interventions:** Combined pressure-controlled rectal distension (barostat system) & heat pain **Comparison:** Comparison of biomolecule levels at different experimental time points between hydrocortisone and placebo arms **Salivary assay:** Cortisol: ELISA. Alpha amylase: Saliva Enzymatic Assay **Saliva type:** Not recorded **Salivary Collection method:** swab **OUTCOMES** No rise in amylase or cortisol after pain stimuli in the placebo arm of the trial **Correlation with pain ratings:** Not analysed in the control arm **Sex effects:** Not analysed in the control arm **NOTES** Heat pain thresholds were not affected by hydrocortisone Hydrocortisone decreased the pain threshold for visceral pain and this was primarily driven by women
17	Bialka 2021 Cortisol Testosterone sIgA Alpha-amylase	**AIM** To assess the effectiveness of thoracic paravertebral regional block for post-operative pain after video-assisted thoracic surgery (VATS) compared with no block **METHOD** **Country:** Poland **Participants** **Recruitment:** Details not reported **Age:** Mean age of paravertebral block group: 64, control: 61 **Sex:** paravertebral block group: F 21, M 16. Control: F 16, M 17 **Total number of participants:** 119 **Dropouts:** 49 **Reason for drop out:** 7 did not meet inclusion criteria, 2 declined participation, 19 conversion to open procedure, 7 re-operation, 6 ineffective block, 8 data lost **Revised sample size:** 70 (study group: 37, control: 33) **Analgesia intake:** post-operative patient-controlled analgesia with oxycodone, no preoperative analgesia

		Chronic conditions: Participants could have a range of health conditions within American Society of Anesthesiology physical status I-III (psychiatric conditions not specifically excluded, chronic pain part of exclusion criteria) **Restrictions:** None reported **Study design:** Salivary cortisol, testosterone, sIgA & alpha-amylase measured before and after surgery in a randomised study comparing thoracic paravertebral block and a control group with no block **Other measurement:** Pain intensity, blood pressure, heart rate **Interventions:** VATS **Comparison:** Change from baseline compared between study (regional block) and control groups **Salivary assay:** Cortisol & testosterone : Commercial ELISA. sIgA: Commercial ELISA kits. Alpha-amylase: static method with an AMYLAZA kit **Saliva type**: Mixed stimulated and unstimulated **Salivary Collection method:** Swab **OUTCOMES** There was increase in all the biomolecules measured in the study 6 hours and also 24 hours after surgery compared with pre-operatively with the exception of sIgA which fell in the regional block group between at the 6 hour time point. There was no significant difference in this change between the intervention and control groups. **Correlation with pain ratings:** Alpha-amylase levels were significantly associated with higher one month pain intensity score **Sex effects:** Not analysed
18	Burns (2004) sIgA	**AIM** To investigate whether differences in the timing of saliva are the explanation for discrepant results in change in salivary IgA after acute stress tasks **METHOD** **Country:** United Kingdom **Participants** **Recruitment:** No details reported **Age:** 22 **Sex:** 20 F, 20 M **Total number of participants:** 40 **Dropouts:** 4 **Reason for drop out:** Failed to produce sufficient saliva for analysis **Revised sample size:** 36 **Analgesia intake:** None

		Chronic conditions: Healthy (psychiatric conditions and chronic pain not specifically reported) **Restrictions:** Alcohol, vigorous exercise, food, caffeine **Study design:** Salivary sIgA measured before and after an induced noxious stimulus (first and second exposures in different arms) **Other measurements:** Blood pressure and heart rate, pain intensity **Intervention:** Cold pain induced by CPT **Comparison:** Change from baseline. Changes compared between first and second exposures. **Salivary assay:** Radial immunodiffusion (RID) assay (Bind A Rid, The Binding Site Ltd, Winzer 1999) **Saliva type:** Stimulated saliva **Collection method:** Swab **OUTCOMES** sIgA levels fell significantly after first exposure to CPT, but not after second exposure in the same participants' other arm **Correlation with pain ratings:** No significant difference found **Sex effects:** No significant difference found
19	Christidis (2020) Alpha-amylase	**AIM** To investigate whether the levels of sAA are influenced by experimentally induced muscle pain **METHOD** **Country:** Sweden **Participants** **Recruitment:** Advertisement on social media and among undergraduate dental students at Karolinska Institute **Age:** 23.8 **Sex:** 13 F, 13 M **Total number of participants:** 26 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 26 **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric conditions not specifically reported, chronic pain part of exclusion criteria) **Restrictions:** All of food, alcohol, smoking, caffeine and precautions to reduce risk of contamination due to bleeding from gums **Study design:** Salivary AA measured before and after an induced somatic noxious stimulus

		Other measurements: Depression and anxiety, somatic symptoms, pain intensity **Interventions:** Hypertonic saline muscle injection **Comparison**: Change from baseline **Salivary assay:** Commercially available enzymatic assay kit **Saliva type:** Stimulated saliva **Collection method:** Passive drool **OUTCOMES** No change in sAA **Pain intensity:** Not analysed **Sex differences:** No difference
20	Cruz-Almeida (2017) Panel of cytokines (IL-6, IL-8, IL-10, IL-4)	**AIM** To characterize the time course, duration and magnitude of changes of commonly measured pro- (interleukin [IL]-6, IL-8) and anti-inflammatory (IL-10, IL-4) cytokines in saliva samples and to test for age-related differences **METHODS** **Country:** United States of America **Participants** **Recruitment:** Details not reported **Age:** 8 younger participants: mean 21.4; 9 older participants: mean age 68.1 **Sex:** 8 F, 9 M **Total number of participants:** 17 **Dropouts:** 1 **Reason for drop out:** Vigorous physical activity before arriving for the session **Revised sample size:** 16 **Analgesia intake:** None **Chronic conditions:** Healthy (chronic pain and psychiatric conditions part of exclusion criteria) **Restrictions:** All of food, alcohol, smoking, caffeine **Study design** A panel of salivary cytokines (IL-6, IL-8, IL-10, IL-4) measured before and after an induced noxious stimulus or a non-noxious control in saliva and blood Non-painful task was done on the same participants with and without venepuncture **Other measurements:** Blood pressure, pain intensity, **Intervention:** Cold pain induced by CPT with a non-painful thermal water task as control

		Salivary Assay: MILLIPLEX XMAP human cytokine/chemokine-premixed 13-Plex assay **Comparisons:** Change from baseline. Changes compared (1) between the CPT and control groups, (2) between the two age groups, (3) with or without venipuncture in control group **Saliva type:** Unstimulated saliva **Salivary collection method:** Swab **OUTCOMES** IL-6, IL-10 & IL-4 concentrations increased from baseline, peaking at 60 minutes after CPT IL-8 peaked at 45 minutes after CPT No significant changes reported in control group Venepunture had no significant effect on the cytokine levels **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed **NOTES** The time course of the peak levels of cytokines in the CPT session was nearly identical in saliva and plasma Older adults experienced greater salivary changes in all cytokines during the cold pressor session compared to younger adults in the non-painful sessions
21	Finke (2021) Cortisol	**AIM** To assess how concurrent administration of a cognitive and physical stressor affects stress response patterns on subjective and physiological dimensions **METHOD** **Country:** Germany **Participants** **Recruitment:** University's email newsletter **Age:** Mean 23 **Sex:** 28 F, 28 M **Total number of participants:** 56 **Dropouts:** From CPT: 0. From cognitive stress task: 0. From cardiovascular parameters heart rate variation data analysis: 2. From respiration breathing pattern analysis: 3. From saliva sampling: 0, From pain intensity rating: 1, From voice frequency analysis 3, **Reason for drop out:** Artefacts in ECG (2), poor quality respiratory data (3), technical failure in recording subjective rating (1), technical failure in speech recording (3) **Revised sample size:** 56

		Analgesia intake: Occasional use of simple analgesics allowed **Chronic conditions:** Healthy (psychiatric conditions and chronic pain part of exclusion criteria) **Restrictions:** All of food, alcohol, smoking, caffeine **Study design:** Four conditions (fully crossed interventions, evenly divided across sexes): (i) CP with simultaneous PASAT; (ii) CP without PASAT; (iii) PASAT during warm-water exposure; (iv) warmwater procedure without PASAT (control) **Salivary assay:** Time-resolved immunoassay with fluorescence detection **Saliva type:** Unclear **Salivary collection method:** Swab **OUTCOMES** Intervention: Cold pain induction or a warm-water control condition In half of the sample, the Paced Auditory Serial Addition Task (PASAT) was performed simultaneously (fully crossed interventions). Salivary cortisol, cardiovascular parameters, and subjective ratings as well as voice pitch (F0) were assessed Comparison with **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed **NOTES** **Inclusion in the cortisol- cold pain quantitative analysis:** Yes **BIAS** **Bias Type:** Moderate in selection of participants and selection of reported results **Author's judgement:** Moderate ROB **Support for judgement:** Recruitment limited to the university community, no published pre-specified protocol
[22]	Gaab (2016) sAA, cortisol	**AIM** To investigate the effects of acute psychosocial stress on heat pain perception and salivary cortisol levels and α-amylase activity **METHODS** **Country:** Germany **Participants** **Recruitment:** E-mail or oral promotion in lectures of the Department of Psychology, University of Basel **Age:** Mean 24.6 **Gender:** Male only **Total number of participants:** 29

		Dropouts: 0 **Reason for drop out:** Not applicable **Revised sample size:** 29 **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric conditions and chronic pain part of exclusion criteria) **Restrictions:** Smoking, alcohol **Study design:** Salivary sAA and cortisol measured before and after exposure to noxious heat first before and after the Trier social stress test compared with a control condition with cross over design **Other measurements:** Pain intensity, anxiety **Interventions:** Acute heat pain induced using the Peltier device **Comparison:** Change in cortisol and sAA from baseline before and after heat pain. Comparison of change before and after stress test. **Salivary assay:** Cortisol: Highly sensitive liquid chromatography-tandem mass spectrometry. sAA: kinetic colorimetric test assay **Saliva type:** Unstimulated **Salivary collection method:** Swab **OUTCOMES** Heat pain alone was not associated with significant change in sAA and cortisol, while psychosocial stress was **Correlation with pain ratings:** No significant correlation for cortisol **Sex effects:** Not analysed **NOTES** The pattern of cortisol change was similar to sAA
23	Geiss (2012) IL-6 Cortisol	**AIM:** To investigate the pathophysiologic relevance of cortisol levels for manifestation of fibromyalgia syndrome **METHODS** **Country:** Germany **Participants** **Recruitment:** Fibromyalgia patients from a local support group. Pain-free participants matched for age and education from local newspaper and bulletin board advertisements. **Age:** Fibromyalgia patients: mean 50, Controls: mean 41 **Sex:** Female only **Total number of participants:** 27 (12 with fibromyalgia, 15 healthy controls) **Dropouts:** 0 **Reason for drop out:** Not applicable

		Revised sample size: 27 **Analgesia intake:** None **Chronic conditions:** Psychiatric conditions part of exclusion criteria. **Restrictions:** Food, exercise **Study design** Salivary IL-6 and cortisol measured before and after an induced noxious stimulus **Other measurements:** Blood white cell count, free cortisol, ACTH, catecholamines, IL-6, pain intensity, fatigue levels, chronic stress levels **Intervention:** Mechanical pressure pain thresholds induced by algometry in 8 defined anatomical points **Saliva Assays:** IL-6: ELISA. Cortisol: time resolved fluorescence immunoassay **Comparisons:** Change from baseline. Change compared between patients with fibromyalgia and healthy pain free women. **Saliva Type:** Unclear if stimulated or unstimulated **Salivary collection method:** Swab **OUTCOMES** In women with fibromyalgia, IL-6 and cortisol increased significantly 10 minutes after measuring pain pressure thresholds but this did not happen in the healthy subjects. **Correlation with pain ratings:** Not analysed **Sex effects:** Not applicable (female only study)
[24]	Geva (2014) Cortisol	**AIM** To explore the effects of acute stress on pain perception, pain intensity and the associated stress response. **METHOD** **Country:** Israel **Participants** **Recruitment:** Advertisements posted at the university **Age:** 33 **Sex:** Male only **Total number of participants:** 29 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 29 **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric and chronic pain part of exclusion criteria) **Restrictions:** Exercise, food, caffeine **Study design:**

		Salivary cortisol measured before and after exposure to induced noxious heat twice; before and during the Montreal Imaging Stress Task (MIST) **Other measurements:** Perceived stress, anxiety, heart rate, blood pressure, respiratory rate, skin conductance, pain intensity **Interventions:** Heat pain using Peltier device **Comparison:** Change from baseline before and after two noxious heat stimulus sessions performed on either side of stress manipulation **Salivary assays:** Commercial ELISA **Saliva type:** Unstimulated **Salivary collection method:** Swab **OUTCOMES** Change in salivary cortisol after heat pain was not significant before the stress task. In contrast, cortisol levels increased significantly in response to heat pain after the participants had done the stress task. In contrast, cortisol levels increased significantly in response to heat pain after the participants had done the stress task. **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed
[25]	Geva (2017) Cortisol	**AIM** To test pain inhibition capabilities of triathletes under acute, controlled psychological stress manipulation **METHOD** **Country:** Israel **Participants** **Recruitment:** Advertisements posted at the university and internet sites of triathlon **Age:** Mean 35.9 **Gender:** Male **Total number of participants:** 25 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 25 **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions and chronic pain part of exclusion criteria) **Restrictions:** Exercise, food, caffeine **Study design:** Measurement of salivary cortisol before and after noxious heat stimulus twice; before and after the application of the stress manipulation (using the Montreal Imaging Stress Task) **Other measurements:** Skin conductance, perceived stress, anxiety, heart rate and heart rate variation, pain intensity

		Interventions: Acute heat pain induced by Peltier-based computerized thermal stimulators **Comparison:** Change from baseline before and after two noxious heat sessions performed on either side of stress manipulation **Salivary assay:** ELISA **Saliva type:** Unstimulated **Salivary collection method:** Swab **OUTCOMES** Change in salivary cortisol after heat pain was not significant before the stress task. In contrast, cortisol levels increased significantly in response to heat pain after the participants had done the stress task In contrast, cortisol levels increased significantly in response to heat pain after the participants had done the stress task. **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed
26	Geva (2018) Cortisol	**AIM** To study the effect of acute psychosocial stress manipulation on pain modulation **METHOD** **Country:** Israel **Participants** **Recruitment:** By advertisements posted around the university campus **Age:** Mean 34 **Gender:** Male only **Total number of participants:** 31 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 31 **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions and chronic pain part of exclusion criteria) **Restrictions:** Exercise, food, caffeine **Study design:** Measurement of salivary cortisol after heat pain induction twice; before and after the application of the stress manipulation (using the Montreal Imaging Stress Task) **Other measurements:** Skin conductance, anxiety, heart rate and heart rate variation, blood pressure, respiration, heart rate variability, perceived stress, pain intensity **Comparison:** Change from baseline before and after two testing sessions performed on either side of stress manipulation **Interventions:** Heat pain using Peltier thermal stimulator

		Salivary assay: Cortisol: ELISA **Saliva type:** Unstimulated **Salivary collection type:** Swab **OUTCOME** Change in salivary cortisol after heat pain was not significant before the stress task. In contrast, cortisol levels increased significantly in response to heat pain after the participants had done the stress task. **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed
[27]	Geva (2022) Cortisol	**AIM** To examine the effects of psychosocial stress on pain perception and modulation of women and men **METHOD** **Country:** Israel **Aim:** To examine the effect of psychosocial stress on pain perception and modulation **Participants** **Recruitment:** Recruited by advertisements posted around the university campus **Age:** F: Mean 30.9, M: Mean 28.3 **Sex:** F 82, M 66 **Total number of participants:** 148 **Dropouts:** 0 **Reasons for drop out:** Not applicable **Revised sample size:** 148 **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric conditions and chronic pain part of exclusion criteria) **Restrictions:** Food, caffeine, exercise **Study design:** Salivary cortisol measured before and after induced noxious heat stimulus followed by MIST (n=133) or sham task (n=15) and then also after a second episode of induced noxious stimulation. **Other measurements:** Pain intensity, heart rate, heart rate variability, galvanic skin response **Interventions:** Heat pain using Peltier-based computerized thermal stimulators **Comparison:** Change in cortisol from baseline **Salivary assay:** ELISA **Saliva type:** Stimulated **Salivary Collection method:** Swab

		OUTCOMES There was no change in salivary cortisol in the sham group **Correlation with pain ratings:** Not reported in the sham group **Sex effects:** No difference in the sham group **NOTES** In participants who were exposed to MIST, cortisol levels increased in men and fell back down during the recovery phase. In women this increase did not reach significance. Among men, temporal summation of pain increased following the MIST but was not predicted by the stress variables. The authors concluded that acute stress manipulation affects stress and pain responses in women and men differently: women exhibited stress-induced anti-nociception and men exhibited stress-induced pro-nociception.
29	Goodin (2012)-1 sTNFαR-II, Cortisol	**AIM** To characterize the neuroendocrine and inflammatory responses to multiple experimental pain modalities **METHODS** **Country:** United States of America **Participants** **Recruitment:** College students recruited, details not reported **Age:** 20.2 **Sex:** F 24, M 22 **Total number of participants:** 46 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 46 **Analgesia intake:** None **Chronic conditions:** Healthy (chronic pain and psychiatric conditions part of exclusion criteria) **Restrictions:** All food, alcohol, smoking, caffeine **Study design** Salivary sTNFαR-II and cortisol measured before and after an induced noxious stimulus **Other measurements:** Pain intensity, pain unpleasantness **Intervention:** Exposure to multiple pain modalities (cold, heat and ischaemic pain) induced by CPT, HWT IPT or room temperature water (control) **Comparisons:** Change from baseline. Changes compared between the painful pain modalities and the control group **Salivary assays:** Cortisol: High sensitivity immunoassay. sTNFαRII: Human sTNFαRII enzyme immunoassay **Saliva type:** Stimulated saliva

		Salivary collection method: Swab **OUTCOMES** Cortisol: Cold pain but not heat or ischaemic pain produced significant time-dependent elevation, whereas cortisol significantly decreased for the neutral water task sTNFαRII: The cold pressor, hot water, and ischemic modalities were associated with significant reduction over time, especially 25-35 minutes after pain induction. Response to neutral water initially decreased but returned to approximate baseline. **Correlation with pain ratings:** Significant positive correlation between cortisol change from baseline and pain intensity ratings **Sex effects:** Not analysed **NOTES** Researchers were aiming to assess salivary pro-inflammatory cytokines after acute pain induction and chose to measure sTNFαR-II, because it is more stable than TNFα and can be measured more reliably Cortisol response was negatively associated with the overall sTNFαRII response **Inclusion in cortisol-cold pain quantitative analysis:** Yes, for healthy participants (n=10) **RISK OF BIAS FOR CORTISOL-COLD PAIN ANALYSIS** **Bias Type:** Moderate for risks of confounding, selection of participants, and departures from intended exposure and measurement of outcome **Author's judgement:** Moderate risk of bias **Support for judgement:** No control but steps taken to reduce stress and anxiety in participants, recruitment limited to university community, interactions between participants and experimenters not fully described and no published pre-specified protocol.
30	Goodin (2012)-2 sTNFαR-II Cortisol	**AIM** To examine the association between cortisol awakening response (CAR) and acute pain stimulation and whether CAR was related with salivary cortisol and soluble tumour necrosis factor-α receptor II (sTNFαRII) responses to acute pain induction **METHOD** **Country:** United States of America **Participants** **Recruitment:** Recruited from an urban university setting, no further details **Age:** Mean 36 **Sex:** 17 F, 19 M **Total number of participants:** 36

		Dropouts: 0 **Reason for drop out:** Not applicable **Revised sample size:** 36 **Analgesia intake:** None **Chronic conditions:** Healthy (chronic pain and psychiatric conditions part of exclusion criteria) **Restrictions:** All of food, alcohol, smoking, caffeine **Study design:** Salivary sTNFαR-II and cortisol measured before and after noxious stimuli **Other measurements:** Pain intensity, perceived stress, morning salivary cortisol levels for CAR, **Interventions:** Exposure to multiple pain modalities (cold, heat, ischaemic pain induced by CPT, hot water task and ischaemic pain task **Comparisons:** Change from baseline **Salivary assays:** Cortisol: High sensitivity salivary cortisol immunoassay. sTNFαRII: Human sTNFαRII enzyme immunoassay **Saliva type:** Stimulated saliva **Collection method:** Swab **OUTCOMES** sTNFαR-II: Significant reduction immediately after pain induction Cortisol: Significant elevation after pain induction **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed **NOTES** Researchers were aiming to assess salivary pro-inflammatory cytokines after acute pain induction and chose to measure sTNFαR-II, because it is more stable than TNFα and can be measured more reliably **Inclusion in cortisol-cold pain quantitative analysis:** No. Reason: Results were not separated for different pain modalities
28	Goodin (2012)-3 Cortisol	**AIM** To investigate the effect of sleep quality on pain intensity and cortisol reactivity **METHOD** **Country:** United States of America **Participants** **Recruitment:** Recruited from a college campus using posted advertisements **Age:** Mean 20.2 **Sex:** 20 F, 20 M **Total number of participants:** 40 **Dropouts:** 0

		Reason for drop out: Not applicable **Revised sample size:** 40 **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric conditions and chronic pain part of exclusion criteria) **Restrictions:** Food, alcohol, smoking, caffeine. Precautions to reduce risk of contamination due to bleeding from gums **Study design** Salivary cortisol measured before and after an induced noxious stimulus **Other measurements:** Sleep quality, pain intensity, pain characteristics, affect **Intervention:** Cold pain induced using CPT **Comparison:** Change from baseline **Salivary assay:** High sensitivity immunoassay kits **Saliva type:** Stimulated **Salivary collection method:** Swab **OUTCOMES** Poor sleep quality was significantly associated with greater reports of CPT-induced pain severity and greater cortisol increase from baseline **Correlation with pain ratings:** Significant positive correlation **Sex effects:** No significant difference **NOTES** **Inclusion in the cortisol- cold pain quantitative analysis:** Yes **BIAS** **Bias Type:** Moderate for risks of confounding, selection of participants, and departures from intended exposure and measurement of outcome **Author's judgement:** **Support for judgement:** No control but steps taken to minimise participant stress, recruitment from the university community, interactions between participants and experimenter not fully described, no published pre-specified protocol
31	Hengesch (2018) Cortisol	**AIM** To investigate the association of exposure to early life adversity (ELA) and adult stress reactivity **METHOD** **Country:** Luxembourg **Participants** **Recruitment:** From Luxembourg and the greater region Saar-Lor-Lux **Age:** Controls: 21.8. Participants with ELA: 22.5 **Sex:** Healthy participants: 11 F, 11 M. ELA participants: 14 F, 8 M

		Total number of participants: 44 (22 healthy and 22 ELA) **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 44 **Analgesia intake:** Yes **Chronic conditions:** ELA or healthy (psychiatric conditions and chronic pain not specifically recorded in either group) **Study design:** Salivary cortisol measured before and after an induced noxious cold stimulus combined with a stressful cognitive task in people with ELA and healthy matched controls **Other measurements:** Stress, arousal, anxiety, pain intensity, heart rate, blood pressure **Interventions:** Acute cold pain using CPT combined with stress using the Paced Auditory Serial Addition Task (PASAT) **Comparison:** Change from baseline. Comparison between change in those with ELA and the control group **Salivary assay:** Time resolved immunoassay with fluorescence detection **Saliva type:** Unclear **Collection method:** Swab **OUTCOMES** In people with early life adversity (ELA) the cortisol response after CPT combined with PASAT was blunted compared with people who had not experienced ELA (even though there was no difference in reported pain intensity between the groups) **Correlation with pain ratings:** Not analysed **Sex effects:** No significant difference **NOTES** Inclusion in the cortisol- cold pain quantitative analysis: No **Reason:** Experimental design included stress induced by a cognitive task as well as noxious stimulus for participants
[32]	Hoeger-Bement (2010) Cortisol	**AIM** To investigate the influence of exposure to a cognitive stressor on pain perception and determine the individual characteristics that may be predictors of the pain response We examined pain perception to a mechanical noxious stimulus before and after exposure to a cognitive stressor across a range of pain responses. Mental math was used as the cognitive stressor because it is an established and effective psychosocial technique to induce stress [1]. Changes in stress and anxiety were assessed with both self-reported and physiological measures including questionnaires, visual analogue scales

		(VAS), mean arterial pressure (MAP), heart rate, and salivary cortisol levels. **METHOD** **Country:** United States of America **Participants** **Recruitment:** No details **Age:** 20.2 **Sex:** 13 F, 12 M **Total number of participants:** 25 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 25 **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions part of exclusion criteria, chronic pain not reported) **Restrictions:** Food, alcohol, smoking, mouth **Study design:** Salivary cortisol measured before and after two induced mechanical pressure noxious stimulus tests that were done on either side of 1) a mental math task (stressor) and 2) a rest (control) session in a cross over design **Other measurements:** Pain intensity, blood pressure, heart rate, state anxiety, perceived stress **Interventions:** Acute pressure pain induced using a pain pressure device **Comparison:** Change from baseline after pain tests compared between the stressor session and the rest session **Salivary assay:** Enzymatic immune-assay **Saliva type:** Stimulated **Salivary collection method:** Salivary Swab **OUTCOMES** Salivary cortisol did not change from baseline when the participants did not do the stressor task. There was significant rise in cortisol after pain induction when participants were due to do the stress task. **Correlation with pain ratings:** Not analysed **Sex effects:** No significant difference **NOTES** The authors concluded that rise in cortisol is related to anticipation of the stressor and not to pressure pain induction
33	Icenhour (2020) Cortisol	**AIM** To elucidate the role of chronic stress in visceral nociception

		METHOD **Country:** Germany **Participants** **Recruitment:** Local advertisements **Age:** Mean 26.38 **Sex:** 90 F, 90 M **Total number of participants:** 180 (tertiles based on Trier Inventory for Chronic Stress: 61 high stress, 57 low stress) **Dropouts:** 62 in the mid-tertile not included in analysis **Reason for drop out:** Not applicable **Revised sample size:** 118 **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric conditions part of exclusion criteria, chronic pain not specifically reported) **Restrictions:** None **Study design:** Differences in response to noxious visceral stimulus compared between participant groups of elevated perceived chronic stress and low perceived chronic stress **Other measurements:** Pain intensity, state anxiety, general self-efficacy **Intervention:** Acute visceral pain induced by balloon rectal distensions **Comparison:** Change from baseline compared between high and low chronic stress groups **Salivary assay:** Cortisol: ELISA **Saliva type:** Unclear **Collection method:** Swab **OUTCOMES** Cortisol levels were significantly higher throughout the experiment in those with higher perceived stress but there was no rise in cortisol in either group on measuring visceral pain thresholds **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed **NOTES** Significantly elevated state anxiety and cortisol concentrations were observed in the cohort with higher perceived chronic stress across experimental time points
53	Inayama (2022) Alpha-amylase	**AIM** To examine the hypothesis that listening to music decreases the pain of vascular access cannulation for haemodialysis **METHOD** **Country:** Japan

		Participants **Recruitment:** Dialysis patients in 5 centres who reported cannulation pain in a preliminary questionnaire **Age:** median 64 (mean not stated) **Sex:** F 35, M 86 **Total number of participants:** 121 **Dropouts:** 4 **Reasons for drop out:** patient withdrawal (4), protocol violations (17) **Revised sample size:** 99 **Analgesia intake:** Not reported **Chronic conditions:** Renal impairment needing regular dialysis (psychiatric conditions and chronic pain not reported) **Restrictions:** none reported **Study design:** Salivary amylase measured before and after vascular cannulation during classical music intervention compared to a white noise control group in a cross-over, single blind, randomized trial **Other measurements:** pain intensity, anxiety, blood pressure **Interventions:** Painful cannulation for haemodialysis vascular access **Comparison:** Difference between the intervention and control groups **Salivary assay:** Not stated **Saliva type:** Not stated **Salivary collection method:** Unknown **OUTCOMES** There were no significant differences in salivary amylase **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed
34	Larra (2015) Alpha-amylase Cortisol	**AIM:** To compare the neuroendocrine stress response elicited by bilateral feet CPT and the classical dominant hand CPT **METHODS** **Country:** Germany **Participants** **Recruitment:** Details not reported **Age:** Mean age 22.5 **Sex:** 12 F, 12 M **Total number of participants:** 24 **Dropouts:** 2 **Reason for drop out:** CPT terminated prematurely (1), baseline saliva sample could not be analysed (1) **Revised sample size:** 23 for sAA, 22 for cortisol analysis

		Analgesia intake: None **Chronic conditions:** Healthy (psychiatric conditions part of exclusion criteria, chronic pain not specifically reported) **Restrictions:** Food, alcohol, smoking, caffeine, precautions to reduce risk of contamination due to bleeding from gums **Study design:** Salivary cortisol and sAA measured before and after an induced noxious cold stimulus to hand and then separately to both feet in a crossover design **Other measurements:** Heart rate, blood pressure, stress levels, pain intensity **Comparison:** Change from baseline compared between hand and feet CPT **Interventions:** Acute cold pain induced by CPT **Salivary assays:** Cortisol: time-resolved immunoassay with fluorescence detection. sAA: Quantitative enzyme kinetic method **Saliva type:** Stimulated saliva **Collection method:** Spitting **OUTCOMES** sAA: Significant rise after both feet and hand CPT Cortisol: Decreased after hand CPT but increased after foot CPT **Correlation with pain ratings:** Not analysed **Sex effects:** No significant differences found for cortisol or sAA **NOTES** **Inclusion in the cortisol- cold pain quantitative analysis:** Yes Hand CPT experiment and feet CPT experiment entered in quantitative analysis as 2 separate experiments. **BIAS** **Bias Type:** High in selection of participants. Moderate in confounding, missing data and selection of reported result. **Author's judgement:** High ROB **Support for judgement:** Method of participant recruitment not reported. No neutral control but steps taken to minimise participant stress. Missing data excluded from all analysis and not accounted for but no indication of differential loss related to prognostic factors. No published pre-specified protocol.
52	Lorenz (2021) Cortisol	**AIM** To compare the physical and psychological stress responses to finger prick and venepuncture **METHOD** **Country:** United States of America

		Participants **Recruitment:** Flyers and e-mail announcements to the university list reserve and psychology participant pool **Age:** Mean 21.93 **Sex:** F only **Total number of participants:** 45 **Dropouts:** 5 **Reasons for drop out:** Did not complete both experimental sessions **Revised sample size:** 40 **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions and chronic pain not specifically recorded) **Restrictions:** food, alcohol, smoking **Study design:** Measurement of salivary cortisol before and after finger prick or venepuncture with a cross over design **Other measurements:** Pain intensity, heart rate, heart rate variability, stress, affect, **Interventions:** Acute pain after drawing blood by venepuncture or by finger prick **Comparison:** Change from baseline with comparison between the 2 procedures. **Salivary assay:** ELISA kits **Saliva type** Unstimulated **Salivary Collection method** Passive drool **OUTCOMES** Significant decline in cortisol at 10 and 20 minutes after drawing blood with venepuncture but no change with finger prick **Correlation with pain ratings:** No correlation **Sex effects:** Not applicable (all F) **NOTES** Psychological measures of stress such as negative emotion and perceived stress, were stronger predictors of reported pain than physical stress measures such as blood pressure and heart rate Pre-procedure mean cortisol levels were at the high end of the normal range for sex and time of day regardless of which procedure was being done. The authors concluded that anticipatory anxiety leads to cortisol rise that subsides after the event.
35	Lukacs (2022) Cortisol	**AIM** To examine the relationship between conditioned pain modulation and SNS and HPA reactivity where pressure pain was studied before and after CPT

		METHOD **Country:** Canada **Participants** **Recruitment:** university-level participants, purposive recruitment to ensure equal representation of sexes **Age:** 24.5 **Gender:** 25 F, 25 M **Total number of participants:** 50 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 50 **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric conditions not specifically reported, chronic pain part of exclusion criteria) **Restrictions:** food, alcohol, exercise **Study design:** Salivary cortisol measured before and after an induced noxious cold stimulus **Other measurements:** Pain intensity, galvanic skin response **Interventions:** Acute cold pain induced by CPT as the noxious conditioning stimulus. Pressure pain detection threshold measured before and after CPT. **Comparison:** Change from baseline **Salivary assay:** ELISA **Saliva type** Unstimulated **Salivary Collection method** Swab **OUTCOMES** No significant change in salivary cortisol 30 seconds after exposure to noxious cold **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed **NOTES** **Inclusion in the cortisol- cold pain quantitative analysis:** Yes
36	Meeus (2009) Cortisol	**AIM** To evaluate endogenous pain inhibition and the cortisol response in chronic fatigue syndrome patients with chronic widespread pain compared with a healthy control group using spatial summation of thermal noxious stimuli **METHODS** **Country:** Belgium

		Participants **Recruitment:** Study patients: Random selection from the medical files available at the university-based chronic fatigue clinic. Control subjects: From the staff and students of the university physiotherapy department and among friends and family of the researchers (age and gender-matched) **Age:** Mean: 44.4 **Sex:** CSF: 21 F, 10 M**.** Healthy: 21 F, 10 M **Total number of participants:** 62 (31 CFS-patients with chronic pain, 31 controls) **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 62 **Analgesia intake:** Not reported **Chronic conditions:** Healthy control group (psychiatric conditions not specifically recorded, chronic pain excluded in control group) **Restrictions:** Exertion, caffeine, alcohol, smoking **Study design** Measurement of salivary cortisol before and after induced noxious heat stimulus in people with chronic fatigue syndrome and widespread pain compared with controls **Other measurements:** Pain intensity, **Interventions:** Acute heat pain induced by hot water immersion **Comparison:** Change from baseline compared in people with chronic fatigue and widespread pain and healthy controls **Salivary assay:** Radioimmunoassay **Saliva type:** Unclear **Salivary collection method:** Swab **Restrictions:** Exertion, caffeine, alcohol, smoking **OUTCOMES** No significant change in salivary cortisol either group **Correlation with pain ratings:** In people with chronic fatigue syndrome (CFS) there was significant negative correlation between change in cortisol and pain intensity **Sex effects:** Not analysed
[55]	Muhtz (2013) Cortisol	**AIM** To examine the effects of pain stimuli on cortisol levels in patients with chronic pain and patients with depression **METHOD** **Country:** Germany **Participants** **Recruitment:** From an outpatient clinic for patients with chronic pain

		Age: Chronic pain: Mean 44.9. Depression: Mean 36.3. Controls: Mean 33.3 **Sex:** Chronic pain: 12 F, 8 M. Depression: 6 F, 16M. Controls: 21 F, 12 M **Total number of participants:** 75 (22 depression, 20 chronic low back pain, 33 controls) **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** As above **Analgesia intake:** None in the healthy control group. Medication for depression and chronic pain in experiment group. **Chronic conditions:** Controls: psychiatric conditions and chronic pain part of exclusion criteria. Depression group & chronic back pain group: no other psychiatric disorders as part of exclusion criteria **Restrictions:** None stated **Study design:** Measurement of cortisol before and after heat pain in 3 groups (1) healthy taking no analgesia, (2) chronic pain (3) depression **Other measurements:** Pain intensity **Interventions:** Acute heat pain induced using the peltier device **Comparison:** Change from baseline compared between the three groups **Salivary assay:** Radioimmunoassay **Saliva type:** Unclear **Salivary collection method:** Swab **OUTCOMES** No statistically significant change in cortisol levels observed in any of the three groups **Correlation with pain ratings:** No significant correlation **Sex effects:** Not analysed
37	Nakajima (2011) Cortisol	**AIM** To examine the extent to which pain perception prior to smoking cessation predicts early relapse **METHOD** **Country:** United States of America **Participants** **Recruitment:** Newspaper advertisements in the community and flyers in the university and participants completed a phone interview **Age:** Mean in abstinent group 36.7. Mean in relapsed group 35.4 **Sex:** 46 F, 45 M **Total number of participants:** 91 **Dropouts:** 0 for cortisol-CPT, 20 through the rest of the study **Reason for drop out:** Follow up non-attendance

		Revised sample size: 91 (all participants underwent CPT with saliva samples collected at the outset; dropout occurred later in the study) **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric disorders part of exclusion criteria, chronic pain not specifically reported) **Restrictions:** Alcohol, smoking **Study design:** Salivary cortisol measured before and after an induced noxious cold stimulus prior to smoking cessation **Other measurements:** Heart rate, blood pressure, pain intensity, pain characteristics, withdrawal symptoms, mood states, smoking status **Interventions:** Acute cold pain induced using CPT **Comparison:** Change from baseline. Changes compared between smokers who remained abstinent and smokers who relapsed **Salivary assay:** Time-resolved fluorescence immunoassay with a cortisol-biotin conjugate as a tracer **Saliva type:** Stimulated **Saliva collection method:** Swab **OUTCOMES** Increase in salivary cortisol after CPT with no difference between the 2 groups **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed **NOTES** **Inclusion in the cortisol- cold pain quantitative analysis:** Yes **BIAS** **Bias Type:** High in confounding. Moderate in selection of participants, departures from intended exposure and selection of reported results. **Author's judgement:** High ROB **Support for judgement:** No control and unclear if steps were taken to minimise participant stress. Unclear whether all potential participants had equitable opportunity to be included. Interactions between participants and experimenters not fully described. No published pre-specified protocol.
38	Nelson (2001) Melatonin	**AIM** To examine the response of salivary melatonin to acute pain stimuli (electric stimulation) **METHOD** **Country:** United States of America **Participants**

		Recruitment: Details not reported **Age:** Mean not reported (range: 19-55 years) **Sex:** 7 F, 11 M **Total number of participants:** 18 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 18 **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric conditions not specifically reported, chronic pain part of exclusion criteria) **Restrictions:** None reported **Study design** Salivary melatonin measured before and after an induced noxious stimulus **Other measurements:** Pain intensity **Intervention:** Acute pain induced by electric stimulation **Salivary Assay:** Salivary melatonin by direct radioimmunoassay **Comparison:** Change from baseline **Saliva type:** Unstimulated saliva **Saliva collection method:** Not recorded **OUTCOMES** Melatonin levels changed less than 5 minutes after the pain stimulus with initial decrease followed by a rise and then a reduction until levels similar to those anticipated for the time of day reached **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed
39	Niedbala (2018) Cortisol	**AIM** To determine whether retaliating against a threatening outgroup, enables individuals in a group endure more pain and actually feel less pain intensity **METHOD** **Country:** United States of America **Participants** **Recruitment:** Introductory psychology students (no further details) **Age:** Mean 19.32 **Sex:** 48 F, 26 M **Total number of participants:** 74 **Dropouts:** 0 **Reason for dropouts:** Not applicable **Revised sample size:** 74 **Analgesia intake:** Not reported

		Chronic conditions: Healthy (psychiatric conditions and chronic pain not specifically reported) **Restrictions:** Smoking, caffeine, mouth rinse before commencement **Study design:** Salivary cortisol measured before and after an induced noxious cold stimulus compared between retaliation and non-retaliation groups, where completing CPT was a way of subtracting points from the rival (i.e. positive appraisal of pain) **Other measurements:** Skin conductance, anger, approach motivation, pain intensity **Intervention:** Acute cold pain induced using CPT **Comparison**: Change from baseline. Change compared between different behaviour manipulation groups. **Salivary assay:** Commercially available enzyme immunoassay **Saliva type:** Stimulated **Salivary collection method:** Passive drool **OUTCOMES** The cortisol response was inhibited in participants with positive appraisal of pain compared with controls, even though they did not report less pain. **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed **NOTES** **Inclusion in the cortisol- cold pain quantitative analysis:** No **Reason:** Experimental design included emotional manipulation or a cognitive task as well as CPT
40	Olsson (2011) Cortisol	**AIM** To investigate the differences in physiologic and subjective parameters between lying on a bed of nails compared to a soft bed, and, whether there are any differences between listening to relaxation instructions on a CD versus no CD-instructions **METHOD** **Country:** Sweden **Participants** **Recruitment:** Internet advertisements and by posters at work places near the study location **Age:** Mean 39.7 **Sex:** 20 F, 12 M **Total number of participants:** 32 **Dropouts:** 3 **Reason for drop out:** Saliva samples turned up dry to the lab and could not be analyzed

		Revised sample size: 29 **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions and chronic pain not specifically recorded) **Restrictions:** Caffeine, nicotine and any medication 12 hours prior to participation **Study design:** Salivary cortisol measured before and after a noxious mechanical stimulus compared with a soft stimulus **Other measurements:** End-tidal carbon dioxide, oxygen saturation, respiration rate, heart rate, heart rate variability, skin conductance level, blood pressure, ECG, pain intensity **Intervention:** Acute pain induced by mechanical pressure pain (lying on a Shakti-mat), compared with lying on a soft bed, both with and without listening to a relaxing music CD **Comparison:** Change from baseline, compared in 4 groups: A—lying on nails in silence, B—lying on nails with CD at comfortable volume, C—lying on soft bed in silence, D—lying on a soft bed listening to CD **Salivary assay:** Spectria [125I]-Coated Tube Radioimmunoassay **Saliva type:** Stimulated **Salivary collection method:** Swab **OUTCOMES** No effects of either bed type or relaxing instructions on saliva cortisol **Correlation with pain ratings:** No correlation; no rise though participants reporting rapid and significant rise in pain at the start of lying on nail bed **Sex effects:** Not analysed **NOTES** Healthy participants habituated to the induced pain on the nail bed and were able to subjectively relax. When on the nail bed, signs of both sympathetic and parasympathetic nervous system activity were observed.
41	Quartana (2010) Cortisol	**AIM** To examine the relationship between trait pain catastrophizing and morning salivary cortisol levels before and after pain induction in pain free and temporomandibular disorder (TMD) participants and whether TMD patients had greater hyperalgesia and hypercortisolism **METHOD** **Country:** United States of America **Participants** **Recruitment:** TMD patients: from a dental school-based, orofacial pain clinic and media advertisements for a larger prospective study concerning

		sleep disturbance and TMD pain and function. Healthy controls: from fliers posted at a major teaching hospital and medical school **Age:** Mean: TMD 33.79 and controls 25.91 **Sex:** TMD: 32 F, 7 M. Healthy: 21 F, 1 M **Total number of participants:** 39 TMD, 22 healthy controls **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 61 **Analgesia intake:** None **Chronic conditions:** Healthy or TMD (psychiatric conditions part of exclusion criteria, chronic pain excluded in healthy group **Restrictions:** Smoking, food, caffeine, exercise **Study design:** Salivary cortisol measured before and after noxious cold, pressure and heat stimuli in TMD and control participants **Other measurements:** Pain catastrophizing, psychological distress, pain intensity **Interventions:** Acute pain induced by a combination of pressure, heat and cold stimuli induced using pressure algometry at defined anatomical sites, peltier stimulator, CPT **Comparison:** Change from baseline compared between TMD and healthy participants **Salivary assays:** Commercially available enzyme immunoassay (EIA) **Saliva type:** Unstimulated **Salivary collection method:** Swab **OUTCOMES** No difference in cortisol response from baseline to post-pain between the people with TMD and healthy people. In a separate analysis of the same experiment, in people who had a tendency to catastrophizing there was a reduction in salivary cortisol immediately and 20 minutes after pain compared to baseline **Correlation with pain ratings:** No significant correlation **Sex effects:** Not analysed **NOTES** **Inclusion in cortisol-cold analysis:** No **Reason:** Pain induced by a combination of noxious stimuli with no separate analysis of cold pain
43	Schneider (2022) Cortisol Alpha-amylase	**AIM** To evaluate the effect of psychosocial stress (Trier Social Stress Test, TSST) combined with performance feedback on changes in pain perception and their association with neuroendocrine stress parameters **METHOD**

		Country: Germany **Participants** **Recruitment:** A web based software for recruiting participants (SONA Systems), postings in a university department of psychology, advertisements in a local on-line newspaper **Age:** 23.83 **Sex:** F only **Total number of participants:** 186 **Dropouts:** 5 **Reason for drop out:** Experiment was uncomfortable or did not meet exclusion criteria (5), profound high cortisol levels (2), unaccounted for (2) **Revised sample size:** 177 **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric disorders and chronic pain part of exclusion criteria) **Restrictions:** Smoking, food, alcohol, caffeine **Study design:** Salivary cortisol and alpha- amylase measured before and after induced noxious heat combined with one of: TSST followed by positive feedback (43), negative feedback (46) or no feedback (45) or TSST placebo version (43) **Other measurements:** pain intensity, anxiety **Interventions:** Acute phasic heat pain induced by thermal stimulator, tonic heat pain (water bath) **Comparison:** Change after psychosocial stress induction and differences between the 3 study groups and placebo **Salivary assay:** Cortisol: chemi-luminescence immunoassay with high sensitivity. Alpha-amylase: enzyme kinetic method **Saliva type:** Not stated **Salivary Collection method:** swab **OUTCOMES** In the group who received TSST placebo (n=43), cortisol levels dropped during the course of the experiment with a significant drop after the first experimental heat pain. There was no change in alpha amylase. There was a rise in both biomolecules in response to TSST induced stress **Correlation with pain ratings:** No correlation **Sex effects:** Not applicable (female only study) **NOTES** Experimentally induced social stress did not influence pain in women with or without performance feedback.
42	Serrano (2019)	**AIM**

	Alpha-amylase Cortisol	To examine the association between the catechol-O-methyltransferase (COMT) allele and perceived pain, anxiety, cortisol and sAA levels **METHOD** **Country:** United States of America **Participants** **Recruitment:** Flyers distributed on university campus **Age:** 21.12 years **Sex:** 45 F, 41 M **Total number of participants:** 86 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 86 **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions and chronic pain not specifically reported) **Restrictions:** None reported **Study design:** Salivary cortisol and sAA measured before and after an induced noxious cold stimulus **Other measurements**: Anxiety, pain intensity **Interventions:** Acute cold pain induced by CPT **Comparison:** Change from baseline before and after CPT, compared between COMT Met allele carriers and Val homozygotes **Salivary assay:** Cortisol: Human cortisol enzyme immunoassay (EIA). sAA: Kinetic Enzyme Assay **Saliva type:** Unstimulated **Collection method:** Passive drool **OUTCOMES** Significantly greater change in sAA in COMT Met allele carriers compared with Val homozygotes at the 20 minute post-CPT time point **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed **NOTES** Pain ratings increased significantly immediately after CPT but were not affected by COMT polymorphism. The authors concluded that the COMT genotype influences the stress response to painful stimuli. **Inclusion in the cortisol- cold pain quantitative analysis:** Yes **BIAS** **Bias Type:** High in confounding. Moderate in selection of participants, departures from intended exposure and selection of the reported result. **Author's judgement:** High ROB

		Support for judgement: No control and unclear if steps were taken to minimise participant stress. Recruitment limited to university campus. Interactions between participants and experimenters not fully described. No published pre-specified protocol.
47	Smith-Hanrahan (1997) Kallikreins	**AIM:** To examine change in salivary kallikriens in association with the stress response to abdominal surgery **METHOD** **Country:** Canada **Participants** **Recruitment:** Patients scheduled for surgery at Montreal General Hospital. No further details. **Age:** Mean 43.9 **Sex:** Mixed, distribution not recorded **Total number of participants:** 19 **Dropouts:** 3 **Reason for drop out:** Insufficient data **Revised sample size:** 16 **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions not specifically reported and chronic pain excluded) **Restrictions:** Food, alcohol **Study design** Salivary kallikreins measured before and after gynaecological surgery **Other measurements:** Plasma cortisol, pain intensity **Intervention:** Elective hysterectomy with or without oophorectomy for benign disease **Salivary Assay:** ELISA **Comparison**: Change from baseline **Saliva type:** Stimulated saliva **Salivary collection method:** Swab **OUTCOMES** Kallikreins increased significantly at 2, 4, and 6 hours after surgery, but not at 1 hour Peak increase in kallikreins was at the 4 hour time point (8x higher than pre-operative levels) **Correlation with pain ratings:** Reported pain levels did not follow the pattern of change in kallikreins (pain intensity peaked at one hour and declined after this point) **Sex effects:** Not analysed
49	Sobas (2020)	**AIM**

	sTNFαR-II sIgA Alpha-amylase Testosterone Cortisol	To evaluate change in pain biomarkers in the saliva following Advanced Surface Ablation eye surgery, in order to determine their validity as objective pain measures **METHOD** **Country:** Spain **Participants** **Recruitment:** Consecutive patients listed for corneal surface ablation surgery from ophthalmology clinic **Age:** 28.78 ± 6.93 **Sex:** F 13, M 19 **Total number of participants:** 32 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 32 **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric conditions not reported, chronic pain part of exclusion criteria) **Restrictions:** None reported **Study design:** Salivary sTNFαR-II, sIgA, sAA, testosterone & cortisol measured before and after eye surgery **Comparison:** Change from pre-operative levels **Interventions:** Acute pain after corneal surgery **Salivary assays:** Cortisol: DRG® Salivary Cortisol ELISA, DRG® Instruments GmbH, Marburg, Germany.). Testosterone (DRG®Salivary Testosterone ELISA, DRG Instruments GmbH, Marburg, Germany). sAA (DRG Salivary Alpha Amylase ELISA, DRG Instruments GmbH, Marburg, Germany). sTNFαRII (Quantikine®, Human sTNF RII/TNFRSF1B Immunoassay, R&D Systems, Minneapolis, MN, USA). sIgA (Salimetrics® Salivary Secretory IgA ELISA, Pennsylvania, USA) **Saliva type:** Stimulated saliva **Collection method:** Passive drool **OUTCOMES** IgA: Significant increase one hour after surgery sTNFαR-II: Significant reduction one hour after surgery Cortisol: Rise in the immediate pre-operative period compared to baseline with a further rise 1 hour after surgery sAA: No significant rise in sAA Testosterone: No significant change **Correlation with pain ratings:** IgA: Significant positive correlation one hour after surgery. sTNFαR-II: No correlation. Cortisol: No correlation **Sex effects:** Not analysed

50	Tanaka (2021) Alpha-amylase	**AIM** To compare the effect of Yokucansan (YKS), a traditional Japanese herbal (Kampo) medicine, vs placebo treatment on women undergoing breast cancer surgery without axillary clearance **METHOD** **Country:** Japan **Participants** **Recruitment:** Not specified **Age:** range 20-60 **Sex:** F only **Total number of participants:** 100 **Dropouts:** 23 **Reason for drop out:** Protocol violation (1), refused participation (2), positive sentinel node (12), steroid treatment needed (1), incomplete data (7) **Revised sample size:** 77 (35 YKS, 42 control) **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric conditions and chronic pain part of exclusion criteria) **Restrictions:** none reported **Study design:** Salivary alpha amylase measured before and after breast surgery in women given YKS or placebo in a single blind randomised controlled trial **Other measurements:** pain intensity, anxiety, depression, quality of life **Interventions:** Breast surgery **Comparison:** Change from pre-operative baseline within each group and also compared between the active treatment and placebo group **Salivary assay:** Handheld monitor (COCORO meter, NIPRO, Osaka, Japan) with disposable test strip- assessment if reaction time of a hydrolyzing reaction **Saliva type:** Unclear **Salivary Collection method:** test strip **OUTCOMES** No change in salivary alpha-amylase in the different time points in the control group. In the YKS group, salivary alpha-amylase scores directly before operation were significantly lower than those on the day before the surgery and one day postoperatively **Correlation with pain ratings:** Not analysed **Sex effects:** Not applicable
44	Wittwer (2016)	**AIM** To investigate the effects of acute heat pain on salivary alpha amylase activity

	Alpha-amylase	**METHOD** **Country:** Switzerland **Participants** **Recruitment:** Not stated **Age:** Mean 26 **Sex:** 13 F, 14 M **Total number of participants:** 27 **Dropouts:** 4 **Reason for drop out:** Unavailable pain intensity data (2), took analgesics (2) **Revised sample size:** 23 **Analgesia intake:** None **Chronic conditions:** Healthy (psychiatric conditions and chronic pain not specifically reported) **Restrictions:** Caffeine, alcohol, food, precautions to avoid blood contamination **Study design:** Salivary AA measured before and after induced noxious heat stimulus **Other measurements:** Pain intensity, mood and anxiety **Intervention:** Acute heat pain induced using Medoc TSA-II thermode **Comparison:** Change from baseline **Salivary assay:** Alpha amylase enzyme activity using reagents **Saliva type:** Unstimulated saliva **Collection method:** Swab **OUTCOME** Significant rise in sAA after heat pain **Correlation with pain ratings:** Positive correlation between sAA activity and pain intensity **Sex effects:** No correlation **NOTES** No significant correlation found between the trait anxiety score and the pre-test sAA level After the pain assessment, irrespective of gender, participants felt significantly calmer and their mood was better
51	Yamaguchi (2006) Alpha-amylase	**AIM** To validate the use of salivary amylase activity, as an indicator of pain in people with severe disability who required the daily replacement of gastric and/or bronchial tubes **METHOD** **Country:** Japan

		Participants **Recruitment:** From a hospital setting, no further details **Age:** Mean 20.5 **Sex:** 3 F, 7 M **Total number of participants:** 10 **Dropouts:** 0 **Reason for drop out:** Not applicable **Revised sample size:** 10 **Analgesia intake:** Not reported **Chronic conditions:** Severe motor and cognitive disabilities (psychiatric conditions and chronic pain not specifically reported) **Restrictions:** None reported **Study design:** Salivary AA measured before and after a noxious medical procedure stimulus **Other measurements:** Pain intensity, heart rate **Intervention:** Acute pain after gastric or bronchial tube exchange **Salivary assay:** Enzymatic reagent method **Saliva type:** Stimulated **Collection method:** Monitor device with test strip under the tongue **OUTCOMES** Significant rise in sAA **Correlation with pain ratings:** Significant positive correlation found **Sex effects:** Not analysed
45	Youssef (2018) Alpha-amylase	**AIM** To investigate differences in cardiovascular biomarkers between different cold stimulus responder types **METHODS** **Country:** Canada **Participants** **Recruitment:** By social media, e-mail, and informational flyers **Age:** Mean 24.7 **Sex:** 16 F, 16 M **Total number of participants:** 32 **Dropouts:** 2 (both male) **Reason for drop out:** Test not completed due to pain (2 M) **Revised sample size:** 30 **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions and chronic pain not specifically reported) **Restrictions:** Caffeine, exercise, alcohol, food

		Study design: Salivary AA measured before and after an induced noxious cold stimulus **Other measurements:** Measures of cardiovascular sympathetic tone (blood pressure, heart rate, cardiac output, stroke volume, left ventricular ejection timee, pre-ejection period), pain intensity **Intervention:** Cold pain induced by CPT **Comparison:** Change from baseline **Salivary assays:** sAA kinetic reaction immunoassay **Saliva type:** Stimulated saliva **Collection method:** Swab **Restrictions:** All of food, alcohol, smoking, caffeine **OUTCOMES** Significant rise in sAA after cold pain **Correlation with pain ratings:** Not analysed **Sex effects:** Not analysed
46	Zimmer (2003) Cortisol	**AIM** To examine sex differences in subjective pain and cortisol response to a noxious stimulus **METHOD** **Country:** Germany **Participants** **Recruitment:** University students (no other details) **Age:** 22.32 **Gender:** 42 F, 42 M **Total number of participants:** 84 **Dropouts:** 8 **Reasons for drop out:** Multivariate outliers (2 F, 3 M), strong autonomic reactions to noxious stimulus (3 F) **Revised sample size:** 76 (37 F, 39 M) **Analgesia intake:** Not reported **Chronic conditions:** Healthy (psychiatric conditions and chronic pain not specifically reported) **Restrictions:** Smoking **Study design:** Salivary cortisol measured before and after an induced noxious cold stimulus **Other measurements:** Pain intensity & unpleasantness, anxiety, distress, blood pressure, heart rate **Comparison:** Change from baseline. Comparison of change between the sexes **Intervention:** Cold pain (plunge test) **Salivary assay:** ELISA

		Saliva type: Unstimulated **Salivary collection method:** Swab **OUTCOMES** Salivary cortisol increased in both men and women, greater increase in men **Correlation with pain ratings:** Significant positive correlation **Sex effects:** Significantly greater increase in salivary cortisol from baseline in men at 20 minutes after plunge test **NOTES** **Inclusion in the cortisol- cold pain quantitative analysis:** Yes **BIAS** **Bias Type:** Moderate in confounding, selection of participants, departures from intended exposure, missing data and selection of reported result **Author's judgement:** Moderate ROB **Support for judgement:** No control but steps taken to minimise participant stress. Recruitment limited to university students. Interactions between participants and experimenters not fully described. Missing data not included in analysis. No published pre-specified protocol.