Chapter 14
Salivary Bioscience and Pain

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Abstract Salivary biomarkers and analytes represent an important advancement in the field of pain by suggesting the possibility of identifying an objective measurement of individual differences in the experience of acute and chronic pain. In adults and children, the most widely studied salivary pain biomarkers are salivary alpha-amylase (sAA) and cortisol. Existing data suggest that, whereas in the acute pain context there are elevations in the production of salivary biomarkers, in chronic pain there is often a blunting effect. These findings support the overlap between acute pain and stress responses, as well as a chronic stress model where the experience of persistent pain is associated with sustained changes in stress biomarkers. However, individual factors and mechanisms contributing to this relationship remain unclear. Additionally, more research is needed to determine the specific and interactive factors and effects of individual salivary measures.

Keywords Cortisol · Salivary alpha-amylase · Acute pain · Chronic pain

14.1 History of Salivary Bioscience and Pain

As noted by the International Association for the Study of Pain (IASP), pain is a complex phenomenon involving "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP Task Force on Taxonomy, 2004). Because the experience of pain varies across individuals and is influenced by not just actual or potential tissue damage, but emotional, cognitive, familial, cultural, and environmental factors,

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assessment of a broad range of both subjective and objective markers of pain can inform effective interventions. Although self-report has historically been considered the gold standard for pain assessment and has largely been used to guide pain treatment decisions, critics of this approach argue that self-report oversimplifies the pain phenomenon and in fact, may contradict alternative measures of pain (Twycross, Voepel-Lewis, Vincent, Franck, & Von Baeyer, 2015). Accordingly, it is important to examine other individual or contextual factors associated with pain.

Salivary biomarkers prove one meaningful avenue to further understand the pain experience. Pain is conceptualized as a stressor that activates the autonomic nervous system (ANS) and subsequently the sympathetic nervous system (SNS), which triggers the changes that occur in physiological functioning during the fight-or-flight response. Several hormones are produced during this response and have been assessed as a part of the pain response, including cortisol and alpha-amylase. Cortisol production following a stressor is delayed and thus assessment must occur 15–20 min following the experience of pain; whereas alpha-amylase increases more quickly and increases are seen more proximal to the stressor. Pain biomarkers are effectively biological "signatures" that may represent specific phenotypic characteristics associated with either acute or chronic pain, specific chronic pain conditions, or someone at risk for transitioning from acute or recurrent to chronic pain. Identification of specific biomarkers is thought to be an important window into mechanisms of pain, which could allow for the development of novel therapies to address these mechanisms (Reckziegel et al., 2019). Additionally, salivary biomarkers, in particular salivary secretory immunoglobulin-A (s-IgA) and soluble tumor necrosis factor—alpha (tnf-alpha), demonstrate good reliability across time (Sobas et al., 2016). Salivary bioscience is critical to identifying biomarkers, as many different components of functioning, from gonadal hormones to salivary proteins, can be measured noninvasively.

14.2 Salivary Bioscience and Pain in Adult Populations

14.2.1 Clinical Pain Syndromes

14.2.1.1 Fibromyalgia

Fibromyalgia (FM) is a complex pain syndrome defined by chronic, widespread pain and tenderness in multiple body locations (Wolfe et al., 1990). Like other pain conditions, it is associated with deficits in central pain processing, although the exact mechanisms by which these deficits occur are not well understood (Eller-Smith, Nicol, & Christianson, 2018). A long-standing hypothesis is that alterations in the functioning of the hypothalamic–pituitary–adrenal axis (HPA axis) may be a significant contributor to deficiencies in pain processing; thus, salivary bioscience investigations have primarily focused on examining cortisol levels in this population. Because cortisol reactivity can be affected by so many possible factors (presence of

chronic pain, pain levels at the time of sampling), the data are hard to interpret. One early study examined cortisol in individuals with FM and found higher daily average cortisol levels than healthy controls, despite no differences in stress (Catley, Kaell, Kirschbaum, & Stone, 2000). Yet, hypocortisolism has also been demonstrated in women with FM (Riva, Mork, Westgaard, Rø, & Lundberg, 2010). For example, women with FM were shown to have significantly lower levels of cortisol compared to women with shoulder and neck pain and healthy women with no pain (Riva, Mork, Westgaard, & Lundberg, 2012). These studies either did not obtain current pain ratings or did not compare cortisol levels to pain ratings specifically, which makes it difficult to tease apart possible mechanisms contributing to differences in cortisol reactivity in FM.

A subsequent study did find a positive relationship between salivary cortisol levels and pain ratings at waking and one-hour post-waking, with cortisol levels explaining 38% and 14% of the variation in pain ratings, respectively (McLean et al., 2005). These data suggest that the relationship between cortisol and FM pain is dynamic and fluctuating throughout the day. A more recent study confirmed these findings in a sample of women with FM who provided both pain and stress ratings, as well as saliva samples six times per day for 14 consecutive days (Fischer et al., 2016). Results showed that stress predicted pain ratings, but not vice versa; additionally, cortisol levels were significantly positively related to pain levels only. However, additional research has found only slightly elevated cortisol levels in women with FM compared to healthy controls, despite no relationship of cortisol to laboratory-induced pain via pressure and heat pain thresholds (Wingenfeld, Nutzinger, Kauth, Hellhammer, & Lautenbacher, 2010). Yet, the opposite pattern was demonstrated in a separate study that found no differences in cortisol upon awakening or throughout the day, but significantly elevated cortisol in response to pressure pain threshold measurement (Geiss, Rohleder, & Anton, 2012). Social stressors, on the other hand, may result in attenuated cortisol responses in women with FM (Wingenfeld et al., 2008). These data highlight the potential utility of cortisol as a pain-specific biomarker in fibromyalgia, but also suggest the need for continued investigation of other factors that may alter the cortisol-pain relationship.

One possible pathway that the HPA axis becomes dysregulated in FM is through childhood maltreatment and neglect. A number of studies have demonstrated a relationship between childhood trauma and chronic pain (Davis, Luecken, & Zautra, 2005; Jones, Power, & Macfarlane, 2009), but the mechanisms by which childhood experiences exert their influences on physical health have not been elucidated. Disruption of HPA axis pathways during critical developmental periods may be one explanation; as a result, examining cortisol levels in individuals with childhood trauma and chronic pain may provide some answers. In support of this notion, cortisol has been shown to mediate the relationship between childhood neglect and current pain symptoms in women with FM (Yeung, Davis, & Ciaramitaro, 2016). Other investigations have shown significantly lower levels of cortisol in women with FM and a history of child abuse compared to women with FM and no history of abuse (McLean et al., 2005).

Other potential biomarkers, such as salivary alpha-amylase (sAA) have not been evaluated extensively. In women with FM, levels of sAA showed no relationship to self-reported daily pain (Fischer et al., 2016). There is some evidence that glucocorticoid sensitivity of inflammatory cytokine production is reduced in FM, and a pro-inflammatory cytokine, interleukin-6, is elevated following a pressure pain task and was significantly correlated with ratings of pressure pain and fatigue (Geiss et al., 2012).

Another potential utility of salivary bioscience in this population is to identify potential biomarkers that would facilitate diagnosis and disease prognosis. Differences in expressions of salivary proteins between those with FM and those without have been shown (Bazzichi et al., 2009). The authors attempted to further refine the identification of salivary proteins specific to FM by comparing them to individuals with rheumatoid arthritis, migraine, or healthy controls (Ciregia et al., 2019). Although expressions of specific salivary proteins were able to differentiate those with FM from the other groups in this specific study, additional research for salivary biomarkers is warranted.

14.2.1.2 Dental, Mouth, and Jaw Pain

Like in FM, salivary cortisol has been a widely used biomarker of pain in individuals with various forms of dental/mouth pain. Temporomandibular disorders (TMD) are chronic conditions of the mouth and jaw, with a common symptom of orofacial pain. Cortisol may be a useful biomarker for evaluating patients with TMD to develop new treatment approaches (Wadhwa & Kapila, 2008). Cross-sectional studies have found elevated levels of morning cortisol in TMD patients compared to controls (Chinthakanan et al., 2018; Vrbanović et al., 2018), although other studies have found no group differences and no relationship of self-reported pain to cortisol levels (Jo et al., 2016; Nilsson & Dahlstrm, 2010; Quartana et al., 2010). One study compared salivary cortisol levels in patients with chronic orofacial pain, acute orofacial pain, and healthy controls. This unique study design allows for comparison of duration of pain (chronic versus acute), which may help better determine whether alterations in salivary analyte levels are related to the ongoing experience of pain. Interestingly, no differences in cortisol levels were present between any of the groups, even though the chronic orofacial pain group reported significantly higher levels of pain and stress (Jasim, Louca, Christidis, & Ernberg, 2014). However, pain catastrophizing (which describes the tendency to describe pain in an exaggerated way) may help explain these findings. Pain catastrophizing has been linked to elevated salivary cortisol, *regardless of group* (i.e., TMD versus healthy control) in response to laboratory pain tasks (Quartana et al., 2010).

Patients with recurrent aphthous stomatitis (a relatively common condition where painful ulcers appear in the oral cavity) have shown hypocortisolism compared to controls, regardless of whether they were experiencing active lesions or if the lesions had healed (Rezaei, Aminian, & Raygani, 2017), and cortisol was also unrelated to pain severity. However, acute dental pain due to pulpal or periapical inflammation

has been associated with increased cortisol levels compared to controls (Haug & Marthinussen, 2018).

Additional salivary analytes such as dehydroepiandrosterone (DHEA; a steroid secreted by the adrenal glands) have not shown any group differences (Jo et al., 2016), although the research in these areas is still in its infancy. In a sample of individuals with tooth pain, sAA was related to the severity of self-reported pain (Ahmadi-Motamayel et al., 2013), and inflammatory cytokines have also been shown to be elevated in those with acute orofacial pain compared to controls (Haug & Marthinussen, 2018). Salivary total antioxidant capacity [TAC; a biomarker of the antioxidant potential of bodily fluids (Peluso & Raguzzini, 2016)] studies have not produced consistent findings. Two studies found lower levels of salivary TAC in patients with TMD (de Almeida & Amenábar, 2016; Rodríguez de Sotillo, Velly, Hadley, & Fricton, 2011), but two recent studies found significantly higher levels in patients with TMD compared to controls (de Almeida & Amenábar, 2016; Vrbanović et al., 2018). One possible explanation for this difference is that the participants in the study found that elevated TAC had documented chronic pain for at least 6 months; duration of pain was not reported in the other two studies. With regard to other salivary analytes, one recent study found salivary opiorphin (an endogenous opioid peptide) levels were significantly positively correlated to pretreatment tooth pain in participants who received root canals (Ozdogan et al., 2019), although a separate study found no significant relationship of s-IgA (an antibody located in the oral mucosa of the mouth) to self-reported pain during an archwire insertion (José da Silva Campos, César Souza Alves, Rezende Barbosa Raposo, Paula Ferreira, & Willer Farinazzo Vitral, 2010). These data highlight the importance of assessing dynamic changes occurring in salivary measures as a function of continued pain experience, which naturally introduces another potential variable and adds to the challenges in drawing significant conclusions.

14.2.1.3 Heterogeneous Chronic Pain

Cortisol has also been widely studied in heterogenous populations of chronic pain, with general trends toward evidence of hypocortisolism in patients with chronic pain. This population includes individuals with widespread musculoskeletal pain and FM. Cortisol has been found to follow a similar trajectory as reported pain intensity following a 4-week intensive pain management program (Evans, Douglas, Bruce, & Drummond, 2008). It also appears as though cortisol levels are negatively associated with degree of chronic pain, such that individuals with generalized chronic pain were three times as likely to have cortisol levels in the lowest third of a group of individuals that also included those "at risk" for chronic pain (as evidenced by high levels of somatization), and healthy controls. Similarly, the individuals in the "at risk" group were 1.8 times more likely than controls to have cortisol levels in the lowest third (Mcbeth et al., 2005). In a follow-up study by the same authors, those in the "at risk" group with new-onset chronic widespread pain showed significantly lower morning salivary cortisol and higher evening salivary

cortisol (indicative of a "blunting" cortisol response), suggesting that HPA axis dysfunction may be a predictor of who will develop chronic pain in those with psychological risk factors (McBeth et al., 2007). This evidence of at least morning hypocortisolism in individuals with chronic pain is a finding that has been supported by several other studies (Generaal et al., 2014; Turner-Cobb, Osborn, da Silva, Keogh, & Jessop, 2010).

Only one study to date has examined other salivary measures associated with chronic pain (in this case neuropathic pain) (Kallman, Ghafouri, & Bäckryd, 2018). The authors compared salivary concentrations of beta-endorphins and substance P in individuals with chronic neuropathic pain to healthy controls and found no significant differences in either salivary peptide.

14.2.1.4 Pelvic Pain

No significant differences have been found in salivary cortisol concentrations between women with menstrual pain and healthy women during menstruation, although this study did not assess the correlation of cortisol with pain ratings and also did not specify what time of day the samples were obtained (Park & Watanuki, 2005). Men with chronic prostatitis/chronic pelvic pain (CPP) appear to demonstrate elevated cortisol awakening responses compared to healthy controls (Anderson, Orenberg, Chan, Morey, & Flores, 2008), and diurnal salivary cortisol levels have been shown to be normal to low in women with CPP (Heim, Hanker, & Hellhammer, 1998) and women with endometriosis (Petrelluzzi, Garcia, Petta, Grassi-Kassisse, & Spadari-Bratfisch, 2008), despite women with endometriosis having higher levels of perceived stress (Petrelluzzi et al., 2008). This same pattern was found in another study of women with endometriosis compared to healthy women, although both groups in this study had similar levels of perceived stress (Quiñones, Urrutia, Torres-Reverón, Vincent, & Flores, 2015). Additionally, incapacitating pain was the strongest predictor of hypocortisolism.

In response to a corticotropin releasing factor (CRF; a neuropeptide that regulates activity in the HPA axis) stimulation test or dexamethasone suppression test (DST; a test that measures the responsiveness of the adrenal glands), women with CPP have decreased cortisol levels compared to healthy controls (Heim et al., 1998; Heim, Ehlert, Hanker, & Hellhammer, 1999). Yet, women with CPP showed identical cortisol response patterns as healthy women and women with fibromyalgia in response to a social stress task or an adrenocorticotropic hormone (ACTH; a hormone that also stimulates the adrenal glands to produce cortisol) 1–24 stimulation task (Wingenfeld et al., 2008). These data highlight the complexities of assessing cortisol reactivity.

Measuring prostaglandin levels is a novel application of salivary bioscience and is particularly relevant for women with dysmenorrhea, as elevated prostaglandin levels are believed to be a causal factor for menstrual pain. Only one study to date has examined salivary prostaglandin levels in women with menstrual migraines, each of whom received either a placebo pill or a pill containing sumatriptan

succinate and naproxen sodium (Durham et al., 2010). Results indicated elevated levels of prostaglandins following the migraine attack in women who received the pill placebo; women who received the active pill did not show these elevations. It is conceivable that salivary prostaglandin levels may be an indicator of treatment response.

One other area of physiological assessment in CPP is measuring levels of secretory immunoglobulin-A (s-IgA). S-IgA—an antibody that helps prevent adherence of potentially harmful microorganisms to healthy cells (Corthésy, 2010)—is a marker of immune function and may play a role in the experience of pain (Willemsen et al., 1998). A single study examined this measure in women with severe menstrual pain and found higher concentrations of s-IgA in women with menstrual pain compared to healthy controls during menses only; there were no group differences in non-menstrual phases of the cycle (Park & Watanuki, 2005).

14.2.1.5 Neck and Back Pain

In a study comparing the cortisol awakening responses of patients with acute low back pain to those with chronic low back pain, the authors found no group differences in cortisol profiles (Sudhaus et al., 2009). However, within the chronic low back pain group, those with elevations on psychometric measures such as avoidance of social activities, depression, and fatigue showed blunted or weakened cortisol responses compared to those who scored low on these measures. This relationship was not evident in the acute low back pain group. Lower cortisol has also been associated with increased health complaints, pain, and fatigue in patients with low back pain (Sveinsdottir, Eriksen, Ursin, Hansen, & Harris, 2016). Interestingly, one study examined gender-related salivary cortisol levels in both patients with chronic neck, back, or shoulder pain and healthy controls. Cortisol levels were significantly *higher* in the pain group, but for men only (Schell, Theorell, Hasson, Arnetz, & Saraste, 2008).

Salivary cortisol can also be a useful indicator of treatment response. A recent study manipulated treatment expectations for physical therapy (positive, neutral, and negative) in patients with neck pain (Malfliet, Lluch Girbés, Pecos-Martin, Gallego-Izquierdo, & Valera-Calero, 2018). Increased cortisol was observed in patients in the neutral and negative expectations groups, although not in the positive expectation group. Additionally, salivary cortisol was not related to pain intensity report. Another study found that significant increases in both morning and evening cortisol levels in patients with low back pain who were treated using Mindfulness-Based Stress Reduction (Ardito et al., 2017; Kabat-Zinn, 2006). Similarly, salivary cortisol may be a useful tool for identifying individuals who will transition from acute pain to chronic pain (Nees, Löffler, Usai, & Flor, 2019).

Laboratory studies of pain sensitivity have also revealed differences in salivary cortisol between patients with low back pain and healthy controls. Another study found significantly lower salivary cortisol (measured as "area under the curve," AUC) in chronic low back pain patients compared to patients with depression and

healthy controls, following experimental pain testing using heat stimuli (Muhtz et al., 2013). However, the opposite pattern has also been demonstrated—individuals with chronic low back pain show similar cortisol reactivity to healthy individuals following heat pain testing (Vachon-Presseau et al., 2013).

Like in other pain conditions, sAA has also been shown to be a possible biomarker for pain intensity in this population, with sAA levels positively correlating with reports of pain intensity using a visual analog scale (VAS) (Shirasaki et al., 2007). Similarly, substance P has provided some interesting results in its relationship to pain. There are data to indicate substance P levels are lower in patients with chronic pain compared to healthy controls (Parris, Kambam, Naukam, & Rama Sastry, 2006; Parris, Rama Sastry, Kambam, Naukam, & Johnson, 2006). However, no recent studies have continued this line of investigation with regard to this specific biomarker.

14.2.1.6 Headache/Migraine

Headache and migraine studies are unique in that they are recurrent pain conditions (typically not chronic), so that each individual experiences intervals of pain and no pain. Interestingly, only one study has examined salivary cortisol in chronic headache/migraine. Chronic migraine has been associated with increased diurnal cortisol levels compared to controls (Patacchioli et al., 2006). Two other studies have evaluated sAA as a potential biomarker for sympathetic arousal in this population. One study found that women with frequent, episodic tension-type headache had significantly higher sAA levels compared to healthy women, and sAA levels were significantly positively correlated with pain ratings in the headache group (Vahedi et al., 2018). A separate study went beyond evaluating headache/control group differences and compared sAA levels in four groups: individuals currently experiencing a migraine attack, those in post-attack, those who were in a pain-free interval between migraines, and healthy controls (Bugdayci, Yildiz, Altunrende, Yildiz, & Alkoy, 2010). Compared to the control group, sAA levels were significantly *lower* during a migraine attack and significantly *higher* in post-attack. The pain-free interval and control groups did not show any differences in sAA levels. Pain ratings were not correlated to sAA levels in any group.

A small group of additional salivary analytes have also been explored in this population. Salivary magnesium levels, which have been thought to contribute to the onset of headache, are lower in patients with migraine or tension-type headache compared to controls (Gallai et al., 1992). Nerve growth factor, Substance P, and calcitonin gene-related peptide (CGRP) levels have been shown to be higher in individuals with chronic migraine compared to healthy controls, with Substance P and CGRP highly positively correlated with reported pain intensity (Jang, Park, Kho, Chung, & Chung, 2011). Salivary measures of testosterone and dehydroepiandrosterone-sulfate have not shown group differences when comparing chronic migraine and healthy controls (Patacchioli et al., 2006). However, oxytocin and interleukin—1 beta did show elevations in migraineurs compared to controls,

with changes in those measures appearing to reflect degree of clinical improvement following vagal nerve stimulation (Boström et al., 2019).

14.2.1.7 Cancer Pain

To our knowledge, only one study has evaluated cancer-related pain and salivary biomarkers in adults. Thirty-eight patients with cancer were asked to rate their overall cancer pain intensity and provide a saliva sample for analysis. Levels of sAA were significantly positively correlated with pain intensity in this sample (Arai et al., 2009). Although it is difficult to draw conclusions about this population from this single study, the results support other findings of the positive relationship between sAA and indices of pain intensity.

14.2.1.8 Other Pain Syndromes

Other pain syndromes have not been studied widely, but some general patterns have revealed the possibility of using salivary biomarkers for diagnostic assessment. Alpha-amylase levels have been shown to successfully discriminate those with myocardial infarction from those with acute pain (and no myocardial infarction) (Shen et al., 2012) and women with and without carpal tunnel syndrome (Fernández-de-las-Peñas et al., 2014). Cortisol and sAA was also related to the severity of carpal tunnel syndrome. In patients with interstitial cystitis and chronic myogenous facial pain, there have been no differences found in cortisol levels compared to controls (Galli et al., 2008; Lutgendorf et al., 2002). However, cortisol levels do appear to be related to disease severity in a number of pain conditions, with hypocortisolism associated with greater symptom severity (Lutgendorf et al., 2002; Nierop et al., 2006), although in osteoarthritis (Carlesso, Sturgeon, & Zautra, 2016) and rheumatoid arthritis (Kim, Jeon, Koh, Park, & Suh, 2016), the opposite has been true.

Additional studies have found elevations in free radicals (peroxidase, superoxide dismutase) and uric acid and total antioxidant status in patients with Complex Regional Pain Syndrome—Type 1 (Eisenberg et al., 2008) and salivary exosomes in inflammatory bowel disease (Zheng et al., 2017).

14.2.2 Nonclinical Populations

Salivary bioscience has also been evaluated in nonclinical populations to determine normative levels of various analytes either in experimental or naturalistic settings.

14.2.2.1 Experimental Pain

Thermal Pain

The cold pressor test (CPT) involves participants putting their entire hand and forearm in a large container of cold water, typically around 12 °C. The CPT has been shown to be associated with increased cortisol responses and decreased tnf-alpha (a measure of inflammation) reactivity (Goodin, Smith, Quinn, King, & McGuire, 2012). However, positive social support can possibly attenuate cortisol reactivity to the CPT (Roberts, Klatzkin, & Mechlin, 2015). In response to a CPT, women were found to report higher levels of pain display more overt behavioral indicators of pain (i.e., jumping and cursing) compared to men; however, these differences were not accounted for by salivary testosterone levels (Archey, Goldey, Crockett, & Boyette-Davis, 2018). The CPT was also used to evaluate cortisol reactivity in healthy adults with different styles of coping with pain. People who reported being fearful of and avoiding pain were more likely to show an increase in cortisol responses at baseline and following the CPT, compared to people who reported using humor or distraction or persisting despite pain who tended to show lower cortisol baseline levels and decreased cortisol responses following the cold pressor (Sudhaus et al., 2015). Similarly, cortisol responses following a DST have been linked with pain sensitivity, with cortisol suppression associated with higher pain tolerance ratings during the CPT and impaired pain inhibition (Godfrey et al., 2017). The CAR is a significant predictor of postoperative pain (Lautenbacher et al., 2009). Studies have also investigated how the CAR may be related to cortisol and tnf-alpha reactivity following cold and hot water pain tasks. Increased CAR was associated with higher pain intensity and pain unpleasantness; however, CAR was not related to cortisol or tnf-alpha reactivity to the pain tasks (Goodin, Quinn, et al., 2012). However, the authors found the opposite results in a previous study where suppression of CAR was associated with increased pain intensity and unpleasantness during the CPT (Fabian et al., 2009). In support of these findings, a study by the same authors found that baseline cortisol and tnf-alpha levels (obtained prior to administering the CPT) were not related to the change in CPT pain responses following either a brief hypnosis session or no intervention, *even though hypnosis was associated with lower pain intensity and unpleasantness* (Goodin, Quinn, et al., 2012). Poor sleep has also been shown to be predictive of greater cortisol reactivity to the CPT and mediate the relationship between poor sleep quality and pain severity (Goodin, Smith, et al., 2012).

In a sample of healthy adults, sAA has been used to measure responses to heat pain stimuli. Even though sAA is a marker of sympathetic activity in response to stress, it can also be a potentially useful biomarker for pain reactivity in healthy populations. Levels of sAA have been shown to be related to self-reported pain intensity and unpleasantness of heat stimuli, but not pain tolerance (Wittwer, Krummenacher, La Marca, Ehlert, & Folkers, 2016). Sex differences in responses to thermal pain can also shed light on individual patterns of reactivity.

Other Models of Experimental Pain

Other studies have used different methods for delivering experimental pain. Although the exact relationship between cortisol and pain reactivity to electrical stimulation was not reported, one study found that pain ratings of electrical pain were higher when participants were under stress, and cortisol levels were also higher during stress (Choi, Chung, & Lee, 2012). Salivary melatonin also appears to be reactive to acute electrical pain stimuli; although the pattern of melatonin levels during recovery may show time-dependent both decreases and increases (Nelson, Farr, Ebadi, & Nelson, 2001).

14.2.2.2 Naturalistic Pain Settings

Evaluating physiological reactivity and nonexperimental stressors provides a unique method of assessing "real life" reactivity. One study evaluated women in labor by assessing salivary levels of chromogranin A (CgA), which is a marker of psychological stress, separate from physical stress. Results indicated that CgA levels were significantly higher when the sample was taken when the participant showed 10 cm of cervical dilation, as compared to 4–6 cm cervical dilation and immediately after birth (Iizuka, Masaoka, & Ohashi, 2018). In a similar population, there were no differences in salivary substance P between women in active labor, pregnant women not in labor, nonpregnant women without pain, nonpregnant women with acute pain due to postoperative hysterectomy (Dalby et al., 1997). However, sAA levels have not been shown to be related to pain during dental extraction (Lee & Bassiur, 2017). Salivary melatonin levels have been measured in relation to gastrointestinal symptoms in patients seeking psychiatric care (Söderquist et al., 2019). Melatonin levels after lunch were significantly related to report of gastrointestinal symptoms, particularly gastrointestinal pain and bloating. Other studies have shown elevated pro- and anti-inflammatory cytokine levels following an experimental cold pressor test as compared to an experimental warm water test, but no elevations were found following venipuncture (Cruz-Almeida et al., 2017). Additionally, blunted CARs have been shown during menses in women with premenstrual syndrome, as compared to other phases of the menstrual cycle. Menses was also associated with the highest pain intensity, although there were no significant correlations between cortisol measures and pain intensity (Ozgocer, Ucar, & Yildiz, 2017).

14.2.3 Methodological Considerations

With regard to adult pain, there have been mixed findings across both chronic pain diagnoses and healthy samples. One relatively consistent finding is that widespread pain conditions, such as FM, do appear to be associated with a blunting of salivary cortisol responses. Attempting to identify specific salivary biomarkers related to

adult pain presents a number of challenges. Varied methodologies, including the time of day the sample was obtained and the analytical method (e.g., using AUC or raw levels) have made it difficult to draw conclusions across studies, as well as across pain diagnoses (Sobas et al., 2016). Variations in sample collection methods (e.g., unstimulated whole saliva versus stimulated sublingual saliva, etc.) have also likely contributed to inconsistent findings across biomarkers and in different populations (Jasim, Carlsson, Hedenberg-Magnusson, Ghafouri, & Ernberg, 2018). Additionally, the number of potential configurations for comparing groups, such as chronic pain versus healthy, chronic pain versus healthy in response to acute pain, comparing specific pain conditions, etc., presents many challenges. Likely, inaccurate or nonspecific phenotyping of various pain conditions has contributed to the challenges of identifying specific salivary pain biomarkers.

14.3 Salivary Bioscience and Pain in Pediatric Populations

14.3.1 Neonatal Pain

A number of studies have examined salivary biomarkers in the context of procedural pain and have largely focused on salivary cortisol as a biomarker for pain as a result of early exposure to painful procedures in neonates. For example, the earliest study we identified documented increased salivary cortisol in a cross-sectional study of 2-, 4-, and 6-month-old infants in response to immunization and showed correlation in behavioral responses indicative of pain and cortisol levels (Lewis & Thomas, 1990). A significant concentration of the literature has also been on cortisol in preterm infants, with early work focused on the feasibility of salivary sampling for cortisol assay in infants in response to heel stick—a common painful procedure for neonates. This work demonstrated elevations in cortisol following the heel stick, although unlike the previous study, did not find correlations between cortisol levels and behavioral response to pain (Herrington, Olomu, & Geller, 2004). Additional work focused on older infants showed similar results, with 6-month old infants demonstrating increases in cortisol following routine immunizations, but no associations between cortisol and behavioral reactivity (Ramsay & Lewis, 2003). The inconsistency in findings related to cortisol and behavioral response in infants may reflect differences in observational measures of pain used in these studies. These discrepancies highlight the importance of relying on multimodal assessment of pain, particularly in infants.

Further evaluation of how exposure to distressing and painful procedures early in life may impact HPA axis functioning in preterm infants has resulted in some mixed findings, but examination of cortisol response in this population supports potential alterations in the stress response system. For example, early work showed that extremely low gestational age (ELGA) infants had altered cortisol response, including higher basal and more sustained cortisol levels, compared to very low gestational age (VLGA) infants, suggesting that increased exposure to painful procedures in

ELGA compared to VLGA infants may lead to alterations in the stress response system (Grunau, Weinberg, & Whitfield, 2004). Additional studies focused on preterm infants documented a blunted cortisol response compared to full-term or near-term infants, and this response was associated with the number of painful procedures the infant experienced (Provenzi et al., 2016) and sex, with male preterm infants showing more blunted response compared to female preterm infants (Grunau et al., 2010). Consistent with this later work, school-age boys who were born preterm who had higher neonatal procedural distress showed lower cortisol levels compared to full-term boys (Brummelte et al., 2015). Thus, early exposure to pain may lead to alterations in the HPA axis, as demonstrated by assessment of cortisol in preterm infants.

In addition to cortisol, salivary alpha-amylase, a salivary biomarker of the sympathetic nervous system component of the stress response, has also been examined in the context of painful procedures in infants (Davis & Granger, 2009). This work suggests that evidence of the sympathetic nervous system via sAA develops somewhere between 2 and 6 months of age and that infant sAA levels correlate with maternal levels (Davis & Granger, 2009). Although a number of investigators have examined sAA in neonates as a marker of stress, to date we were unable to locate any studies that included sAA as a biomarker of pain in infants. However, as will be presented later in the chapter, sAA in response to pain has been examined in school-aged children.

14.3.2 Children with Clinical Pain Syndromes

Chronic pain conditions are prevalent in children and although estimates vary widely, it is suggested that nearly 40% of children may experience chronic pain (Huguet & Miró, 2008). Recent research documents that depending upon the type of pain encountered, prevalence rates may even be over 80% for some pain conditions, such as headache (King et al., 2011). Chronic pain in children is associated with a wide array of negative sequelae, including difficulties with school attendance and academic performance, impaired sleep and social and role functioning (Haraldstad, Sørum, Eide, Natvig, & Helseth, 2011), and comorbid emotional disorders such as depression and anxiety (Kashikar-Zuck, Goldschneider, Powers, Vaught, & Hershey, 2001; Knook et al., 2011).

14.3.2.1 Functional Abdominal Pain

Functional abdominal pain (FAP) is common in children, with epidemiological research suggesting worldwide prevalence rates of 13.5% (Korterink, Diederen, Benninga, & Tabbers, 2015) and perhaps even as high as 53% (King et al., 2011). Although the body of literature on salivary bioscience and chronic pain in children is small, several studies have focused on FAP and such studies have examined cortisol

and sAA in children with FAP compared to healthy controls or have examined these biomarkers in children with FAP in response to a stressor. In examinations of children with FAP compared to healthy children, morning and total cortisol levels were found to be higher, although the authors noted that the cortisol results may have reflected comorbid depression in the FAP sample (Tornhage & Alfven, 2006; Törnhage & Alfvén, 2015). A study by Dorn and colleagues comparing cortisol response to a stressful procedure in children with FAP and those with anxiety disorders found no differences in baseline or maximum cortisol levels between groups (Dorn et al., 2003). Finally, children with FAP were found to have a blunted cortisol response to a stressful laboratory task compared to healthy children (Gulewitsch et al., 2017). Thus, based upon this small body of literature, it is difficult to draw conclusions of how FAP may impact response to pain as assessed by cortisol.

14.3.2.2 Juvenile Primary Fibromyalgia Syndrome

The only additional pediatric chronic pain article we located that included salivary biomarkers focused on children with juvenile primary fibromyalgia syndrome (JPFMS), which is an amplified pain syndrome associated with musculoskeletal pain in conjunction with fatigue and disruptions in sleep and emotional functioning (Anthony & Schanberg, 2001). In a comparison of salivary cortisol levels in children with JPFMS to a group of children with arthritis and a group of healthy controls in response to venipuncture, no significant differences were found between groups (Conte, Walco, & Kimura, 2003). However, the authors noted the procedure may not have induced a significant stress response in children and that the baseline saliva sampling may have been too temporally proximate to the procedure. Additionally, circadian rhythm and baseline cortisol levels were not taken into account.

14.3.3 Pain Associated with Medical Disease

14.3.3.1 Cancer

Salivary biomarkers of pain have been examined in children with cancer, a pediatric population that is subjected to repeated painful medical procedures and experiences chronic and recurrent pain associated with cancer and its treatment (Miller, Jacob, & Hockenberry, 2011). Two studies have examined sensitivity to pain and physiological pain responses using salivary cortisol, connecting psychosocial and physiological aspects of pain in children diagnosed with cancer (Chen, Craske, Katz, Schwartz, & Zeltzer, 2000; Firoozi & Rostami, 2012). Both studies showed that children who had lower levels of pain tolerance demonstrated increases in cortisol following a medical procedure, including a lumbar puncture (Chen et al., 2000) and administration of chemotherapy (Firoozi & Rostami, 2012). In examining a range of

self-report, observational, and physiological indices of pain during a lumbar puncture, including salivary cortisol, low associations between assessment modalities were found (Walco, Conte, Labay, Engel, & Zeltzer, 2005). For example, cortisol increased significantly over the course of the painful procedure, even when observational and/or self-report assessments did not show elevations in pain. Thus, it is important to note that these various modalities of pain assessment should not be used in isolation or to the exclusion of other modalities.

Jenkins and colleagues examined sAA in response to an experimental pain task in children undergoing treatment for cancer (Jenkins et al., 2018). Experimental pain tasks allow for safe induction of pain that allows for methodological standardization in order to better isolate aspects of pain response, including tolerance and sensitivity. In this study, children ages 8 and up were randomized to one of three emotional regulation conditions (reassurance, reappraisal, or distraction) and participated in the CPT, which involves submerging the nondominant hand in 7 °C water until the child can no longer withstand the pain. Results demonstrated that certain emotion regulation strategies seemed to reduce the stress response to pain as reflected by lower levels of sAA during the CPT. Specifically, children in the distraction condition demonstrated greater sAA reactivity (levels that continued to rise following the CPT) compared to children in the other two conditions (Jenkins et al., 2018).

14.3.4 Pain Associated with Neurodevelopmental Disorders

Assessment of pain in children with neurodevelopmental disorders can be challenging as evidence suggests such children are at high risk for experiencing pain and many children with neurodevelopmental disorders are unable to verbally report pain (Oberlander, O'Donnell, & Montgomery, 1999). Accordingly, strategies that focus on nonverbal modes of pain assessment, such as observational and physiological measures are important. The Paediatric Pain Profile (PPP) is one such measure that was developed for use in children with severe neurological disability (Hunt et al., 2007). In efforts to validate the PPP, salivary cortisol was analyzed in relation to observational pain ratings; however the authors did not find significant correlations between cortisol levels and pain severity (Hunt et al., 2007). This may be due to methodological issues, such as the timing of the sampling, which was not necessarily conducted in the context of painful events, and may also be a result of the finding that salivary cortisol concentrations in this population were lower when compared to data on healthy controls.

14.3.4.1 Epilepsy

Evidence suggests that children with epilepsy may be at higher risk for the experience of pain syndromes given the high rates of depression found in people with epilepsy, the high comorbidity of chronic pain and depression, and higher rates of

pain in two conditions related to epilepsy—traumatic brain injury (TBI) and migraine headaches (Finocchi, Villani, & Casucci, 2010; Kanner et al., 2012). We identified one investigation of pain response via sAA in response to a venous blood draw in children with epilepsy compared with healthy controls (Ferrara et al., 2013). Results showed that children with epilepsy experienced greater sAA reactivity compared to healthy controls, despite no differences in self-reported pain scores in the epilepsy group over the course of the procedure. Conversely, the self-reported pain severity of the healthy control group increased significantly over the course of the procedure. The authors could not rule out possible interactions between anti-seizure medications and alpha-amylase; nonetheless, this study represents an important potential avenue for assessment of pain in neurologically impaired children.

14.3.4.2 Attention-Deficit/Hyperactivity Disorder

Typical cortisol levels follow a circadian rhythm, with baseline levels at their lowest in the morning followed by a peak that gradually decreases throughout the afternoon and evening. Children with attention-deficit/hyperactivity disorder (ADHD) have been found to have atypical cortisol patterns, including decreased awakening cortisol levels and blunted cortisol levels in response to stressors (Kariyawasam, Zaw, & Handley, 2002; Randazzo, Dockray, & Susman, 2008). Thus, McCarthy and colleagues examined salivary cortisol levels in response to an intravenous (IV) catheter insertion in children with and without ADHD (McCarthy et al., 2011). In this study, children with ADHD demonstrated higher (although not significant) baseline cortisol levels at home prior to the painful procedure but significantly lower cortisol levels before and after the IV insertion compared to children without ADHD. This study provides some evidence that physiological response to stressful and painful procedures may be altered in children with neurodevelopmental disorders such as ADHD.

14.3.4.3 Cerebral Palsy

Pain in individuals with cerebral palsy (CP), the most common cause of severe neurological impairment in childhood, is frequently present as a result of spasticity, contractures, and bony deformities (Surveillance of Cerebral Palsy in Europe, 2000). In children with CP, pain is frequently as issue due to early developmental interventions that involve physical manipulation (Zhao, Chen, Du, Li, & Li, 2015). Two studies identified have focused on salivary cortisol response to pain in children with CP. To document the feasibility of saliva collection and cortisol as a possible marker to discriminate pain versus no pain in children with CP, Symons and colleagues examined cortisol response to a medical procedure in children with CP with and without pain (Symons et al., 2015). Findings showed that cortisol levels were higher in children with pain compared to those without. Zhao and colleagues examined cortisol response to neurodevelopmental intervention programs and found

significant increases in cortisol in response to the majority of interventions conducted with children ages 1 to 4 years (Zhao et al., 2015). Thus, although this body of literature is scant, there is some evidence that cortisol may be a useful salivary biomarker of pain in children with CP.

14.3.5 Pain in Children Without Medical or Developmental Disorders

A few studies located have focused on salivary biomarkers of pain in healthy children or children without any specified disease or condition. One of these represented a descriptive study to provide normative data on cortisol response to IV placement in children and included a large sample ($N = 384$) of children ages 4 to 10 years (McCarthy et al., 2009). Baseline samples were collected from children at home prior to the procedure and then again on the day of the procedure prior to and following their IV placement. Results showed that baseline cortisol levels reflected the expected pattern, peaking in early morning and then gradually decreasing throughout the day. On the day of the painful procedure, cortisol levels were higher than baseline and increased significantly at IV insertion, suggesting that cortisol may be a valid biomarker of pain in healthy children (McCarthy et al., 2009).

Allen and colleagues examined cortisol response to experimentally induced pain in healthy children and specifically focused on sex differences, given sex differences found in adults (Allen, Lu, Tsao, Worthman, & Zeltzer, 2009). Participants included 235 healthy children ages 8–18 years who completed three laboratory pain tasks, including pressure, heat, and CPT. Salivary cortisol samples were taken at baseline, after completion of all tasks, and 20 min post-completion. Cortisol levels did not increase over the course of the study, in fact, the highest cortisol level in both boys and girls was the baseline sample, suggesting that as children became familiar with study procedures, stress levels decreased. Boys' baseline cortisol levels were higher, although not statistically, compared to girls'; thus, it remains unclear whether sex differences in cortisol response to pain emerges in childhood (Allen et al., 2009).

Finally, Payne and colleagues examined sAA and cortisol in response to experimentally induced pain in healthy children with and without social anxiety (Payne, Hibel, Granger, Tsao, & Zeltzer, 2014). Their sample included 231 children ages 8 to 18 who completed three laboratory pain tasks, including cold, pressure, and heat. Saliva samples were obtained at baseline, after completion of all tasks, and 20 min post-completion. Overall, sAA did not change across time and cortisol decreased from time one to time two and then remained stable (Payne et al., 2014). Children with social anxiety had higher levels of sAA, but not cortisol at each assessment point. Thus, although cortisol reactivity has been demonstrated in a study of healthy children undergoing a painful medical procedure, neither cortisol or sAA increased in experimentally induced pain, suggesting that laboratory pain models may not be ideal for salivary biomarkers of pain in healthy children.

14.3.6 Methodological Considerations

Saliva sampling is often a preferred approach to assessment of biomarkers, particularly in children, because it is simple, noninvasive, and painless. In the context of multimodal pain assessment, salivary biomarkers are useful to provide a more complete picture of the pain experience in children and are particularly helpful in nonverbal populations, such as infants and cognitively impaired children. However, it is important to keep in mind that although biomarkers such as cortisol and alpha-amylase may increase in the context of pain, they reflect the stress response more broadly and thus should not be used as a sole indicator of pain. There is also no standardization in interpreting changes in these biomarkers in a clinically meaningful manner. That is, although self-report scores of 0–10 are often accepted as the gold standard for assessment of pain severity and treatment decisions, with numeric ratings reflecting categories of pain severity (e.g., ≥ 3 = mild pain, 4–6 = moderate pain, etc.) (Boonstra et al., 2016) there are no such standardized tools that include clinically meaningful interpretations of changes in physiological parameters of pain.

In terms of sampling, although saliva is objectively noninvasive, as noted by Granger and colleagues, there are some populations in which saliva sampling may pose particular challenges (Granger et al., 2007). For example, collecting saliva from preterm and newborn infants can be difficult due to the inability to collect a sufficient volume of saliva. This is particularly concerning due to evidence that insufficient sample volume, particularly when absorbent collection devices are used, can influence the accuracy of assay results (Harmon, Hibel, Rumyantseva, & Granger, 2007). In addition, it is not well established when neonates establish diurnal cortisol patterns and data regarding normative development of basal cortisol levels in infants is lacking (Tryphonopoulos, Letourneau, & Azar, 2014). In older infants, additional challenges can hamper collection, including sleep-wake cycles, residual food and liquids in the mouth, and the simple unwillingness of an infant with stranger anxiety to allow a researcher to collect saliva (Granger et al., 2007). Nonetheless, there are alternative sample collection strategies (e.g., microsponge, paper) that are proving promising in aiding in sample collection in infants (Granger et al., 2007; Tryphonopoulos et al., 2014; Voegtline & Granger, 2014). Jessop and Turner-Cobb (2008) published a review of factors that can impact the accuracy of cortisol assessment in children and noted that age, sex, body mass index, conditions of sampling, units of measurement, assay conditions, and compliance are all important factors that can lead to variations in the precision and accuracy of salivary cortisol assessment (Jessop & Turner-Cobb, 2008). These authors suggest that rather than focusing on the establishment of normative basal cortisol levels, it is important to understand how these factors can impact the diurnal cortisol pattern so we can begin to understand atypical cortisol responses and their sequelae (Jessop & Turner-Cobb, 2008).

14.4 Future Directions

More accurate phenotyping of participants is the next critical step in identifying candidate salivary biomarkers for pain. The mere presence of a pain diagnosis as the primary inclusion criterion results in tremendous variability within the group, which is likely obscuring individual differences that could identify subtypes. Assessing the duration of pain, the contribution of additional body pain sites (that may not necessarily be chronic pain), and the influence of gonadal hormones and the menstrual cycle, are just some of the important variables to consider when attempting to identify different pain signatures associated with salivary biomarkers. Related to this, perhaps moving away from comparing pain diagnoses and instead focusing on those with central sensitization will result in a more accurate and generalizable phenotype (Woolf, 2011). Newly identified salivary biomarkers of pain will hopefully shed additional light on how pain can affect various bodily functions. Using markers of uric acid levels to assess painful conditions that are not typically thought of as chronic pain, such as gout, may provide additional answers about the role of pain in chronic medical conditions. Salivary oxytocin, which is beginning to be used intranasally as an anesthetic, (Paloyelis et al., 2016) and salivary opiorphin also show promise as pain biomarkers (Rock, Kataoka, & Lai, 2013). Future work in the area of salivary bioscience and pain should also focus on standardizing methodological approaches. For example, many studies have used the method of analyzing the AUC for salivary cortisol across time (e.g., Muhtz et al., 2013), but this is not entirely consistent. Using a standardized analytical approach will facilitate the generalizability of results.

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