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Beyond Cortisol! Physiological Indicators of Welfare for Dogs: Deficits, Misunderstandings and Opportunities

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ABSTRACT

This paper initiates new conversations about using physiological indicators to assess dog welfare. There are significant concerns about construct validity – whether measures used accurately reflect welfare's complexity. We propose evidence-based frameworks to advance canine welfare assessment through improved physiological measurement protocols, challenge over reliance on isolated cortisol measurements, and present actionable recommendations for research methodologies that better consider individual variation. Current evidence demonstrates that animal welfare assessment requires multiple physiological indicators reflecting distinct biological systems. In practice, indicators are frequently used in isolation with limited salient dog descriptors, failing to reflect the canine experience adequately. Using a systems approach, we explore various physiological systems and alternative indicators It is essential to consider factors like age, body weight, and sex when interpreting biomarkers, and researchers should report these. This discussion identifies indicators for both positive and negative experiences. We advocate for a practical, evidence-based approach to assessing indicators of canine welfare, including noninvasive collection methods. We acknowledge the complexity of evaluating experiential responses in dogs and the need for continued work to improve practices, ultimately advancing welfare assessment and improving dogs' lives.

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Animal welfare; assessment; canine; dogs; physiology; stress

Introduction

Animal wellbeing has become increasingly important as scientific understanding and modern community attitudes have evolved. Recent evidence confirms the sentience of many species living with people globally, including domestic dogs (*Canis familiaris*) (Browning & Birch, 2022; Browning & Veit, 2023). We find dogs fulfilling diverse roles globally, including: as human companions providing emotional support and social connection (Payne et al., 2015); as protection animals performing security, guarding, and military functions (Haverbeke et al., 2009); as assistance animals guiding the visually impaired, alerting to seizures, and supporting mobility needs (Audrestch et al., 2015); as detection specialists for narcotics, explosives, wildlife products, and disease (Lazarowski et al., 2020); as herders maintaining livestock management traditions (Early et al., 2019); as free-ranging animals forming ecological and cultural relationships with human communities (Serpell, 2017); and as entertainment or gambling focuses in racing, hunting, and competitive events (McEwan & Skandakumar, 2011). This understanding of sentience emphasizes that dogs value a "good life" of agency and positive mental experiences

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(Littlewood et al., 2023). With this comes a moral obligation for those involved in the regulation, care, and management of dogs (Cobb et al., 2021; Mackenzie et al., 2014). It is no longer enough to regulate that dogs' experiences are free from neglect, suffering, cruelty or harm; people should ensure that our canine companions and coworkers can lead a good life. Specifically, we should reduce or remove avoidable negative experiences (e.g., hunger, pain, exposure to extreme heat/cold, isolation, etc.) and strive to provide dogs with positive experiences (e.g., satiety, prompt veterinary care, comfort, social connection, etc.) (Lawrence et al., 2019; Mellor et al., 2020; Webster, 2016). Assurance of wellbeing will underpin the social license to operate in many interactions with dogs in working and companion roles (Hampton et al., 2020). Industries and practices that depend upon dogs will need to assure the wider community of this to be sustainable in the future (Cobb et al., 2021). While behavioral and physiological indicators give us important information about how dogs experience their lives (Polgár et al., 2019), current practices in canine welfare science don't align with the evidence base, specifically: 1) overreliance on single biomarkers, particularly cortisol; 2) failure to account for individual variation in physiological responses; 3) inadequate reporting of critical variables such as age, weight, breed, and sex; 4) inconsistent methodology in sample collection and analysis. This paper specifically reevaluates cortisol, heart rate variability, and immune markers as indicators of canine welfare, challenging the common practice of using single biomarkers (particularly cortisol) in isolation without considering individual characteristics or comprehensive physiological contexts. This discussion paper examines the use of physiological indicators in dog welfare assessment, evaluating current limitations, misconceptions, gaps and opportunities for future research.

Animal welfare assessment and assurance to safeguard dogs rely on many factors, including resources (including monetary budget, personnel, skills and equipment), transparency, traceability, and validated indicators of negative experiences (commonly referred to as stress, distress or suffering) and positive experiences (understood as wellbeing). These include observations of behavior and physiology. However, monetary budgetary constraints, evidence deficits, and misunderstandings within canine science have resulted in assessment practices that do not align with the evidence base or lack the evidence upon which to base good practice. In particular, there are significant concerns about construct validity – whether these physiological indicators actually measure what we claim they measure about dog welfare. There is a clear need (as identified relevant to animal welfare science more broadly by Mason, 2023) to explore the canine physiological indicators relating to affective states (negative and positive, see Mendl et al., 2022) that are used to assess welfare. This paper functions as a critical review, examining what evidence exists and evaluating the quality of this evidence, while identifying critical methodological gaps. This approach allows us to challenge established but problematic practices in canine welfare assessment while providing specific recommendations for improvement. This perspective builds on the existing canine science evidence base and cross-species physiological research to identify knowledge gaps and novel approaches in dog welfare assessment. We aim to advance the use of validated physiological indicators to meet the emerging need to center dog wellbeing in research, regulation, and human-animal interactions.

Aligning the meaning of "animal welfare" across multiple disciplines

Modern animal welfare science understands "animal welfare" as representing the full range of mental experiences in an animal's life, which is complex and cannot be directly measured (Mason & Mendl, 1993). This view recognizes that animals, including dogs, have essential affective (emotional), social, and cognitive needs beyond basic requirements (De Winkel et al., 2024; Flint et al., 2024). Providing animals with agency and opportunities for play, companionship, safety, and a diverse range of behavioral interactions creates positive experiences. These contribute to "a good life," one where the balance of experiences, when considered over time, is positive (see Figure 1). Individual variation across dimensions like personality, cognitive bias, prior experience, and social attachment can all influence how dogs may have different mental experiences (i.e., different welfare) in response to the same social or environmental conditions. Recent empirical work has identified specific behaviors

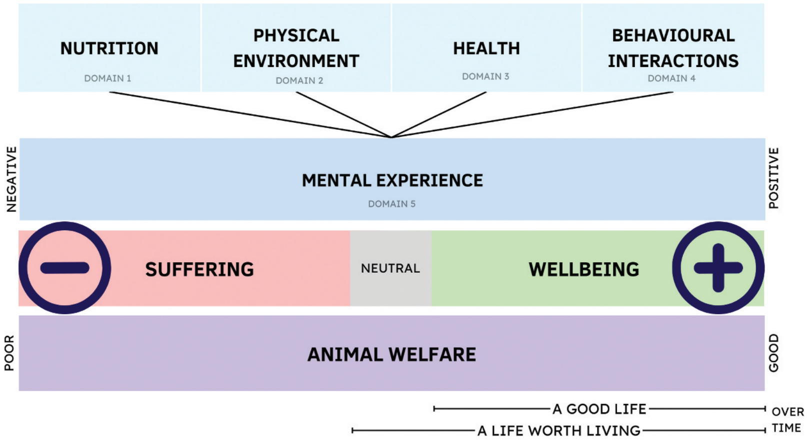

Figure 1. Conceptual alignment of an animal's welfare with constructs of suffering, wellbeing, a life worth living, a good life, animal mental experience and the five domains of animal welfare model.

associated with positive welfare states in dogs. Play behaviors, including play bows and soliciting chase, indicate that dogs feel comfortable within their environment (Miller et al., 2022). Affiliative behaviors toward humans, such as relaxed tail wagging and social licking, have been proposed as indicative of positive welfare (Miller et al., 2022). Dogs with environmental choice and control scored highest on welfare assessments, whilst those in unpredictable environments with limited choices scored lowest (Malkani et al., 2022). However, comprehensive empirical evidence for behavioral expressions of "a good life" in dogs remains limited; most to date research has focused on the absence of negative states rather than the presence of positive welfare indicators.

The Five Domains Model of Animal Welfare (Mellor et al., 2020) provides a framework widely used in animal welfare science and its translation to care provision and regulation (e.g., Beausoleil et al., 2023). It assessed indicators of good and bad effects that animals experience across the four physical and functional domains: 1. Nutrition, 2. Physical Environment, 3. Health and 4. Behavioural Interactions (with other animals, people, and the environment). When considered together at any point in time, these inform an animal's Mental Experience (domain 5), which represents their welfare. This framework has direct implications for physiological measurement strategies in welfare assessment. Each domain corresponds to distinct physiological systems that can be measured: nutritional state correlates with metabolic markers; environmental comfort with thermoregulatory indicators; physical health status with immune function parameters; and behavioral interactions with neuroendocrine indicators of social behavior.

Positive experiences such as feeling comfortably satiated after eating, enjoying the sensation of lying in a soft bed, feeling strong while running fast and playing happily with their family all contribute to positive (sometimes referred to as good) animal welfare and wellbeing. However, feeling thirsty, uncomfortable from lying on wet concrete, having itchy skin from an untreated allergy and feeling lonely from social isolation are unpleasant. Such negative experiences contribute to poor animal welfare and suffering. In reality, most dogs live with a mix of these experiences, and so investigating their welfare requires us to consider additional aspects of their experiences such as duration, intensity, and significance to the individual. When considered over time, a life that has a balance of neutral or positive experiences (accepting that some negative experiences from time to time are inevitable) is considered a life worth living, and one that balances as positive is a good life (Lawrence et al., 2019).

Theoretical work by Broom (2023) reinforces the importance of understanding the balance between positive and negative welfare components in individual animals. Broom emphasizes that welfare assessment requires identifying both the extent of positive welfare indicators and the absence of negative welfare indicators, noting that "good welfare can often counterbalance poor welfare but does not do so in all circumstances." This perspective underscores the necessity for comprehensive physiological indicators that can capture both positive and negative affective states, rather than relying on single biomarkers that may overlook crucial aspects of an animal's welfare experience.

Adopting precise stress terminology in dog welfare research

Stress is a key biological concept, with the word widely applied across physiological, psychological, social, medical, and environmental fields over the last fifty years (Lu et al., 2021). During this period, our understanding of the stress concept has evolved significantly from its origins in Hans Selye's pioneering work defining stress as an adaptive general syndrome (Selye, 1936, 1946). Selye's original conceptualization emphasized that stress was fundamentally about the organism's ability to adapt to environmental challenges. This perspective remains relevant to contemporary welfare science, although our understanding of the concept is expected to continue developing. Referring to *stress* in animal welfare science can be confusing, as the word might be used to reference many relevant terms (stimulus, effect, affective state, etc.). Here, we follow the terminology of Lu et al. (2021), defining *stress* as "a state of homeostasis being challenged," and *stressors* as "factors with the potential to directly challenge homeostasis" (Figure 2).

These distinctions manifest through distinct physiological patterns. *Stressors* (the challenging factors) include environmental, social, or physical stimuli such as novel environments, social isolation, or painful procedures. *Stress* (the physiological state) manifests as measurable responses

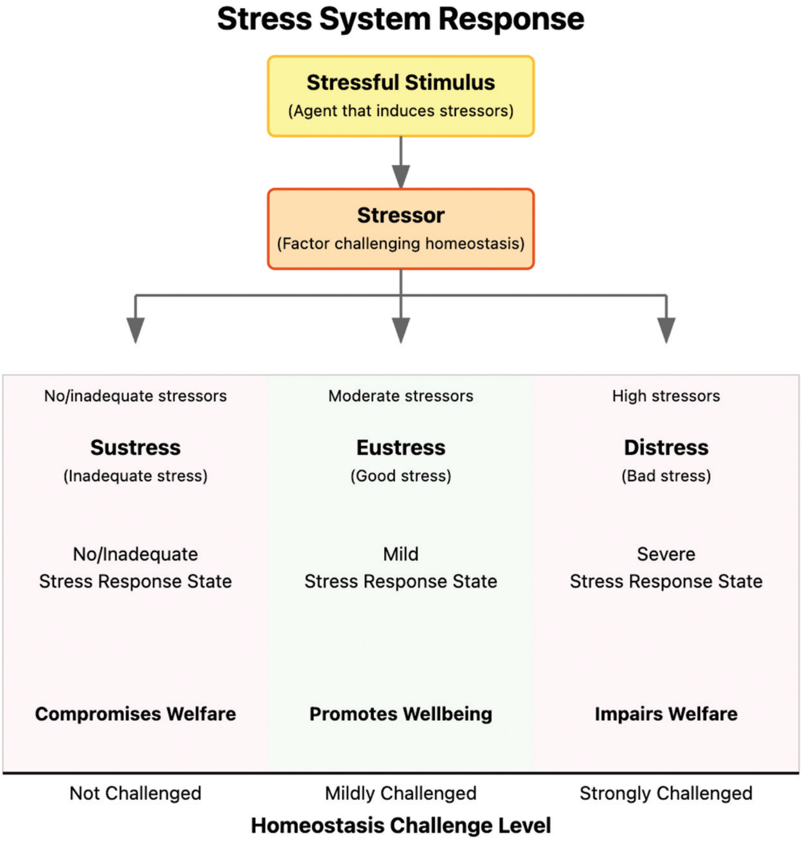

Figure 2. Conceptualisation of key physiological stress-related terms in the stress system response, following terminology from Lu et al. (2021).

(i.e., the stress system response) including activation of the HPA axis (elevated cortisol, ACTH), sympathetic nervous system activation (increased heart rate, catecholamine release), immune system changes (altered white blood cell distributions), and behavioral adaptations. The same stressor may elicit different stress responses depending on individual factors such as genetics, prior experience, and current physiological state. This highlights why measuring the stress response, rather than assuming the impact of a stressor, is important in welfare assessment. Homeostatic challenges vary in intensity and duration; a strong challenge may result from an intense and acute stressor, or when repeated (singular or multiple) low-to-moderate stressors are experienced persistently.

Using "stress" as a blanket term oversimplifies dogs' emotional and mental states, no longer aligning with the current scientific understanding of animal welfare. Although widely used, this term can potentially lead to misinterpretations when evaluating canine welfare. Instead, precise terminology should be used to improve the accuracy of our assessments, reflecting the specific negative and positive affective experiences of dogs. Mellor et al. (2020) categorize aversive experiences as either survival-critical negative affects, or situation-related negative affects. Survival-critical negative affects are the animal's sensory response to "imbalances or disruptions in the *internal* physical/functional state of animals" (Mellor et al., 2020). These are linked to inherent biological mechanisms that stimulate or relate to behaviors critical to the animal's survival, specific to the resolution of that experience. Survival-critical affects include breathlessness triggering increased respiration, hunger prompting food-seeking and eating, pain causing avoidance behaviors, and thirst driving water-seeking and drinking. These behaviors subside once the functional/physical problem has been met. When animals cannot effectively respond to aversive experiences (e.g., being tethered in the hot sun for a week), welfare impact is greater than from acute but brief experiences (e.g., hearing thunder twice in five minutes). In contrast, situation-related negative affects are derived from the animal's perception of its *external* circumstances. Examples include frustration, loneliness, fear, hypervigilance, and depression. These categories may overlap – a dog with a broken leg experiences both survival-critical affects (pain leading to avoiding movement of the injured limb, whimpering and guarding the injured area) and situation-related affects (fear of unfamiliar hospital environment and loneliness while away from social attachment figures). Addressing the pain, fear, and social isolation (both types of affects) is important for welfare improvement.

Objective animal-based evidence is needed to infer welfare-relevant affects. For example, a dog's awake inactivity could indicate comfortable relaxation or fear-induced withdrawal and immobility. Accurate assessment requires the consideration of multiple factors: species-typical behaviors (e.g., ear position, mouth tension, tail movement, sleep-activity budget), environmental conditions (e.g., temperature, loud noises, familiarity with surroundings) and physiological indicators (e.g., breathing patterns, heart rate variability). These combined sources of evidence help identify both negative and positive affective states, informing a welfare assessment (Fureix & Meagher, 2015). Identifying different stress states (*sustress*, *eustress* [considered "stimulation," per Broom, 2017], or *distress*; Figure 2), requires recognizing the multiple coordinated physiological systems in the individual animal (Figure 3). For example, sustress in dogs might occur in environments with inadequate stimulation, such as puppies born into intensive breeding facilities with minimal environmental complexity or dogs kept in social isolation with restricted movement from a young age (e.g., chained). These conditions can impair the development of coping competencies and adaptive responses, limiting opportunities for agency and environmental engagement that are crucial for positive welfare development (Littlewood et al., 2023; Webber et al., 2022).

The physiological systems maintain homeostasis through complex feedback loops, with disruption in one system triggering responses within that localized system and among the other systems, to bring the organism back to a stable state. The endocrine, nervous, and immune systems work in conjunction with the gastrointestinal, integumentary, cardiovascular and circulatory systems to restore and maintain stability. This interconnected quality highlights the risks of relying on single measures, like cortisol alone, provides insufficient evidence of stress or welfare. It appears there are

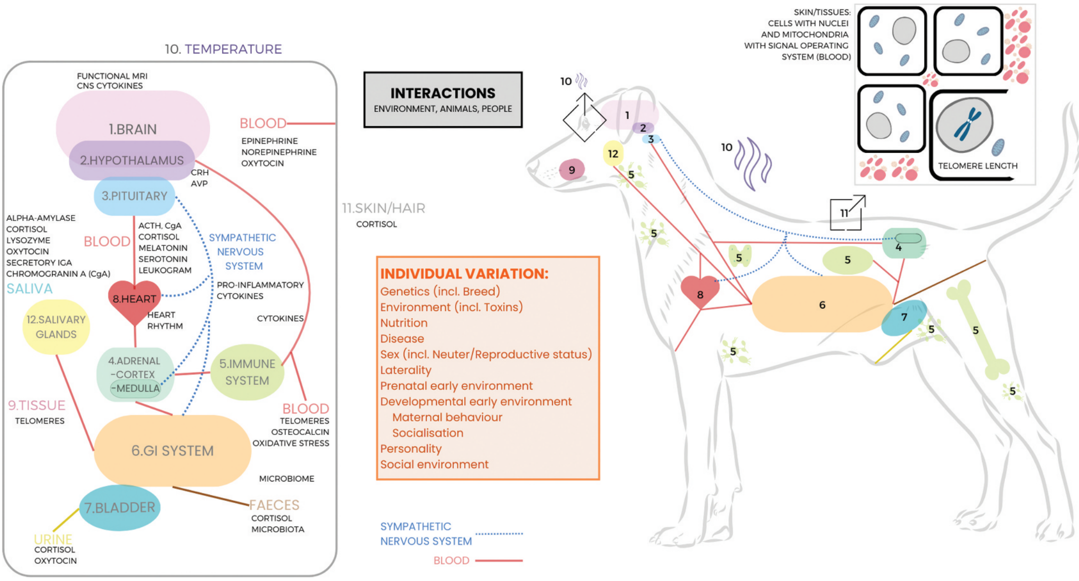

Figure 3. The systems and indicators of experiential physiological responses in dogs. This illustrates the complex network of integrated physiological systems involved in stress response, including the endocrine system, the autonomous nervous system (sympathetic and parasympathetic branches), the immune system, and metabolic pathways that allocate energy resources during challenge. These systems continuously interact through bidirectional feedback loops, demonstrating why isolated measurements of single biomarkers cannot adequately capture the complexity of stress responses or welfare states.

still fundamental deficits in our understanding of canine physiology in welfare science, and opportunities presented by new methodologies.

A systems approach to stress physiology and behavior

The nervous system determines whether a stress response is initiated based on individualized perception of stressors (Figure 3, “Individual variation”). This depends on many factors, including sensory capabilities, genetics, health, and prior experiences. A dog’s sensory perception of the world, including vision, hearing, olfaction, and touch, may determine what environmental conditions are perceived as stressors. Many of these features have genetic components (e.g., visual acuity, ear carriage, coat length). Even a dog’s level of pain perception (sensitivity) may vary by breed (Caddiell et al., 2023) and personality (Lush & Ijichi, 2018). The presence of disease can also affect an animal’s sensitivity to their environment. The limbic system processes these inputs, potentially activating the hypothalamus to mount a physiological stress response if homeostasis of the organism appears threatened. There is evidence that specific gene variants may play a role in this processing of a dog’s fear response (Zapata et al., 2016). The genome-wide association study by Zapata and colleagues (2016) identified specific loci associated with fear and aggression traits in dogs, with particular variants in genes associated with serotonergic neurotransmission, neural development, and fear memory formation. Physical environment (housing, temperature, humidity, nutrient availability, etc.) and social interactions (presence of conspecifics and other species, including humans) may also affect an individual’s stress response (McEwen, 2001). Prior experiences may affect whether they perceive a specific stimulus as aversive. Previous exposure to stimuli influences response, as seen in dogs with thunderstorm phobia responding to changes in barometric pressure or distant thunder sounds, while other dogs remain unaffected (Dreschel & Granger, 2005).

When a stimulus registers as a stressor, it initiates a systemic stress response (physiological processes occurring systematically in the whole body, independent of where stressors originated from, as per Lu et al., 2021). This fundamental response pattern was first identified by Selye (1936, 1946) as part of his general adaptation syndrome and represents highly conserved biological mechanisms across vertebrates. This response operates through two primary pathways, nervous and endocrine, respectively: the Sympathetic Nervous System (SNS) and the Hypothalamic-Pituitary -Adrenal (HPA) axis. As both systems have been well described (Chrousos, 1998), we include only a brief overview here to inform the discussion.

SNS activation begins when the hypothalamus signals through preganglionic neurons to the thoracolumbar gray column of the spinal cord, then to target organs via ganglionic axons, primarily using norepinephrine as a neurotransmitter (de Lahunta et al., 2021). This rapid, short-acting response is limited only by neural impulse speed and neurotransmitter (epinephrine and norepinephrine) actions at the widespread target organs. SNS responses could potentially be measured at many different locations and by their effects on other systems. The cardiovascular and circulatory systems respond with increased heart rate (frequency of cardiac contractions), pulse (palpable pressure wave through arteries), and changes in blood pressure and heart rate variability. Increased respiratory rate and pupil dilation occur. The immune system (comprising thymus, spleen, lymph nodes and bone marrow, refer Figure 3) responds with changes in white blood cell distribution and immunoglobulin levels. The enteric system responds with decreased motility of the stomach and intestines, and gastrointestinal sphincter contraction (McCorry, 2007). Salivary alpha-amylase increases have been noted in humans (Nater et al., 2006) and have also been measured in dogs following sympathetic stimulation (Contreras-Aguilar et al., 2017). The skeletal system may respond to acute stress via osteocalcin release (Berger et al., 2019).

The HPA axis, highly conserved among vertebrate species (Bouyoucos et al., 2021), activates in response to acute stressors through a cascade of hormones: the hypothalamus causes release of corticotropin releasing hormone (CRH) which travels to the anterior pituitary gland. This causes release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the circulatory system, triggering the release of corticosteroids (i.e., cortisol in canines) from the adrenal cortex into the circulating blood. The glucocorticoids have effects on multiple systems: immune, gastrointestinal, endocrine, and cardiovascular (McEwen, 2019). Communication occurs between the immune and endocrine systems and central nervous system via cytokines (Granger et al., 2003). There is evidence that the gut microbiota and permeability are also affected (Dinan & Cryan, 2012). This hormonal response (HPA activation) is relatively slower and longer acting than the SNS response, even after the stressor has subsided, and self-regulates through negative feedback, with glucocorticoids suppressing ACTH and CRH to restore homeostasis (Mârza et al., 2024). In chronic stress conditions, this negative feedback mechanism can become compromised through several mechanisms. These include the downregulation of glucocorticoid receptors in the hypothalamus and pituitary, reducing sensitivity to inhibitory signals (Mârza et al., 2024; Gadek-Michalska et al., 2013; Karin et al., 2020); the altered expression of mineralocorticoid receptors that normally help maintain basal HPA tone (Mârza et al., 2024; de Kloet et al., 1993; Gadek-Michalska et al., 2013); epigenetic modifications of key regulatory genes in the HPA axis (Mârza et al., 2024;); and inflammatory cytokine actions that can directly stimulate CRH production despite elevated cortisol levels (Mârza et al., 2024; Goddard et al., 2016).

The duration of homeostatic disruption significantly influences physiological responses. Acute responses occur with specific events (e.g., new environments, frightening sounds, brief transportation) and help survival through short-term challenges. Continued exposure to a stimulus results in two possible outcomes: habituation (the animal learns to no longer react to the stimulus as a stressor), or chronic stress (develops from repeated or continuous exposure to a stressor). Notably, chronic stressors (e.g., inconsistent training, resource competition) do not need to be intense to present a sustained negative experience for dogs and, subsequently, impact homeostasis over time. Prolonged exposure (a state of chronic stress) can lead to the continuous release of corticosteroids with deleterious effects on the organ and immune systems. This includes the possible fatigue and dampening of the physiological stress response and suppressing immune system response to antigens. The prenatal and early postnatal development of the HPA axis can be affected by the presence of maternal stress and circulating corticosteroids which have long-lasting effects on the endocrine, CNS and other systems (McEwen, 2019). No generalized endocrine profile exists for chronically stressed wild animals (Dickens & Romero, 2013), highlighting the complexity of measuring and interpreting chronic stress responses. We argue that there is also little evidence for a current endocrine profile of chronically stressed dogs.

Behavioral changes provide visible evidence of physiological responses to challenges. The SNS "fight or flight" response manifests in observable canine behaviors including activity level changes, facial, and postural adjustments (e.g., eyes, mouth, ears). In addition, vocalizations and subtle indicators, like lip licking, panting, yawning, shake-offs, etc. can occur (Beerda et al., 1997; Beerda et al., 1999; Grigg et al., 2021).

Beyond cortisol

While physiological stress responses are well-documented across species (Hochachka & Somero, 2002), dog-specific evidence remains limited, often extrapolated from human and rodent studies. Recent decades have seen an increased focus on dog welfare (e.g., Beerda et al., 1999; Edwards et al., 2019; Protopopova, 2016), but research has primarily measured HPA and SNS activation through cortisol and heart rate. This narrow focus potentially overlooks the complex interactions between the different physiological systems (Oken et al., 2015). Canine science often misinterprets stress responses by oversimplifying their relationship with glucocorticoid levels (MacDougall-Shackleton et al., 2019). Treating cortisol levels in dogs as a simple indicator of stress response state or welfare (low = good, high = bad) can lead to incorrect conclusions that can be harmful to dogs. As noted by Broom (2017), cortisol has roles in both positive and negative situations, including learning, and has been observed to increase during activities such as mating, and food acquisition, making it "erroneous to consider it to be always or generally harmful to the individual." Moreover, Broom emphasizes that "the absence of an increase in cortisol concentration does not indicate that there is no problem for the individual." This highlights the critical importance of individual and contextual interpretation when using cortisol measurements in canine welfare assessment. For example, Hennessy et al. (2001) found that shelter dogs' cortisol levels dropped over several days of confinement despite continuing behavioral signs of distress, potentially leading to false conclusions about adaptation if cortisol alone was measured. Most recently, Ferrans et al. (2025) provided direct evidence supporting these concerns, demonstrating poor correlations between salivary and serum cortisol in healthy puppies and adult pet dogs, with arousing events successfully increasing serum but not salivary cortisol concentrations. Researchers may conclude an experience (e.g., novel environment, human interaction) is not aversive to dogs when, in fact, the experimental protocol has not adequately evaluated how dogs have responded. Multiple researchers have emphasized that glucocorticoids alone cannot adequately characterize vertebrate stress response (e.g., Breuner et al., 2013; Romero & Beattie, 2022). The increasing tendency to label cortisol (or other biomarkers) as "stress hormones" is inaccurate, further oversimplifying its complex role (Dickens & Romero, 2013; MacDougall-Shackleton et al., 2019).

Glucocorticoids serve multiple physiological functions beyond stress response (Broom, 2017; Vera et al., 2017; Gormally & Romero, 2020), including coordinating metabolic rates and daily activities, and regulating sleep patterns (McEwen, 2019). Cortisol serves age-specific functions across the lifespan of an individual: neonatal amygdala development, adolescent growth promotion, and potentially accelerating aging when found in excess (McEwen, 2019). Stressor-induced cortisol levels do not create homeostatic challenge (i.e., stress), they help minimize stress responses (MacDougall-Shackleton et al., 2019), and normally fluctuate given a certain environment or circadian cues (McEwen, 2019). There are some common misconceptions regarding cortisol concentration that should be clarified. Namely, that acute high cortisol concentrations equal an animal in distress when it really implies that the animal is appropriately coping with the stressor (Romero & Beattie, 2022).

In turn, that low acute cortisol concentrations depict a healthy animal, when this could mean an animal that has stopped appropriately responding to its environment due to persistent stressors causing HPA axis fatigue and burnout (Romero & Beattie, 2022). Consistent elevation in cortisol doesn't necessarily indicate compromised welfare, since cortisol alone is not a good predictor of stress response state (Romero & Beattie, 2022).

When investigating physiological stress responses, study design must consider and report individual dog characteristics (genetics, personality, physical traits like weight), as well as the duration of stimulus exposure (e.g., acute, chronic, or unknown). The process of measurement itself can lead to spurious and invalid results. Collection method invasiveness ranges from noninvasive (e.g., collecting a post-excretion fecal sample), to mildly invasive (e.g., collection of hair or saliva), to more invasive (e.g., blood draw), to highly invasive (e.g., organ biopsy). Ethical guidelines for biomarker collection in stress-sensitive canine populations should include: application of the harm-benefit principle, ensuring scientific value justifies any distress caused (Coghlan, 2025); implementation of the 3Rs (replacement, reduction, refinement) through pilot studies, power calculations, and least invasive sampling methods; obtaining informed consent from owners/guardians with clear explanation of procedures; incorporating habituation protocols to minimize handling stress; and ensuring personnel are trained in low-stress handling techniques (Scalia et al., 2017) and recognizing facial expressions and behavioral indicators of pain or discomfort during sample collection procedures (Mota-Rojas et al., 2021). Dogs' individual histories, any early-life trauma, training, or body sensitivity will affect their response to sampling methods. A key consideration includes the time delay between handling and physiological changes. Further replication is indicated to confirm if practices, such as blood samples taken within four minutes by an experienced person (as stated in Hennessy et al., 1997), avoid reflecting the physiological changes induced by handling. This is especially important given that handling-induced distress responses have been documented to significantly alter multiple biomarkers in dogs (Cobb et al., 2016; Grigg et al., 2022). Study design using pre- and posttest responses, including a return to baseline within a single animal may alleviate some of these challenges (Lensen et al., 2019).

Given domestic dogs' exceptional morphological and physiological diversity Bryce et al. (2021), dog age and body weight variability (i.e., reference ranges) for each stress biomarker should be established and reported. Ideally, these should be considered prior to establishing the variability of these parameters in response to acute and chronic stressors. Additional considerations should include sex and reproductive status of the dog at the time that the biomarker was taken (refer Cobb et al., 2016). A clear understanding of the biomarkers' properties, circadian cycles, allostasis (refer Lu et al., 2021), and interpretation is also necessary. In the following section of this discussion, we explore indicators of the physiological stress response commonly used in canine science, as well as the opportunities for future biomarker development and interpretation.

Physiological indicators of the stress response in dogs – A summary of the evidence

Cortisol

Circulating cortisol can be measured in multiple biological samples: blood, saliva, urine, feces, and hair (Beerda et al., 1996; Kooriyama & Ogata, 2021; Schatz & Palme, 2001; van der Laan et al., 2022). Each sample type reflects different timeframes, from immediate circulating levels in blood to longer-term (weeks or months) in hair. The distribution of cortisol in these different modalities may also be affected by the presence of corticosteroid-binding globulin. Cortisol measurement in all sample types has been widely used as an indicator of stress response in canine research, though it is often misinterpreted due to a lack of contextual reporting and understanding (Broom, 2017). In a meta-analysis of data from 61 peer-reviewed studies using salivary cortisol, Cobb and colleagues (2016) found a significant effect on cortisol levels for sex and neuter status, age, environment, and sample collection media. No significant effects of dog breed, body weight, dog type, or coat color were noted. Substantial variability between and among individuals complicates interpretation, makes it difficult to compare findings from different studies, and limits population-level conclusions from cortisol measurements alone. More recent work in dogs determined that dogs with known adverse early life backgrounds had variable salivary cortisol responses compared to other dogs in some situations (Buttner & Strasser, 2022; Buttner et al., 2023). These findings reiterate the significance of testing environment and attachment to people present on observed stress responses. Collection methods, subsequent sample processing, and materials also play a role in salivary cortisol measurement (Chmelíková et al., 2020; Dreschel & Granger, 2009). Pre-analytical procedures significantly impact results, including collection materials, transport conditions, storage parameters, and sample handling. Analytical procedures themselves introduce further variation: different assay methods (RIA, EIA, LC or GC) yield different absolute values; protocols with or without extraction measure different cortisol fractions; and laboratory-specific validation parameters affect precision and accuracy (El-Farhan et al., 2017; Gatti et al., 2009). As with any biomarker, the potential impact from the sample collection experience must be considered and controlled for in the study design.

Heart rate and heart rate variability

Heart rate (HR) and heart rate variability (HRV) serve as proxies for SNS response in dogs (Amaya et al., 2020). These measures reflect the balance between sympathetic and parasympathetic influences on cardiac function. Sympathetic activity (such as excitement or anxiety) increases HR through beta-adrenergic receptor stimulation in the sinoatrial node, while parasympathetic activity decreases it through muscarinic acetylcholine receptor activation via vagal nerve stimulation (Bidoli et al., 2022; Höglund et al., 2012). The root mean square of successive differences of inter-beat intervals (RMSSD) provides a time-domain measure of HRV, primarily reflecting parasympathetic modulation of heart rate through vagal tone (Amaya et al., 2020). During stress responses, distinct patterns emerge in these measures. Sympathetic dominance typically manifests as increased HR with decreased HRV, indicating reduced parasympathetic modulation. In contrast, vagal withdrawal may occur independently or precede sympathetic activation, appearing as moderate HR increases with proportionally larger decreases in HRV parameters.

Research shows stressors typically increase HR and decrease in RMSSD in dogs (Katayama et al., 2016; Zupan et al., 2016). When approached by a threatening stranger, dogs showed increased HR regardless of the owner's presence, while another study showed HRV decreased in dogs only when their owner was absent (Gácsi et al., 2013). Similar HR changes occur during anxiety and fear responses in the Ainsworth's Strange Situation (Lu et al., 2021; Palestrini et al., 2005). Environmental studies have shown mixed results, with shelter dogs displaying unchanged HRV but increased rest behavior during music exposure (Amaya et al., 2020). In animal-assisted education settings, dogs deemed "problematic" (behaviorally challenging) showed a significant increase in HR compared with their "innocuous" (not behaviorally challenging) counterparts (Bidoli et al., 2022). While HR and HRV can indicate stress states, the relationship between behavioral and HRV indicators remains debated (see Maros et al. (2008) and Bergamasco et al. (2010), for example). These findings confirm the physiological basis for heart rate measurements as welfare indicators in dogs. Clinical applications for HR and HRV in dogs are reviewed in Kartashova et al. (2021).

Understanding HR and HRV responses requires consideration of age and body weight effects. Hezzell and colleagues (2013) found HR to be negatively correlated with body weight in healthy dogs, though age and HR demonstrate a more complicated relationship with changes across the dog's lifespan. Some breeds, namely, Border Collie, Golden Retriever, Labrador Retriever and Springer Spaniel, demonstrated significantly lower HR; whereas, Cavalier King Charles Spaniels, Staffordshire Bull Terriers and Yorkshire Terriers had significantly higher HR (Hezzell et al., 2013). Most of the research on dogs of different body weights and ages does not seem to report on, nor correct for, these variables.

Oxytocin

Oxytocin, a peptide hormone produced in the hypothalamus and released by the posterior pituitary gland (Onaka, 2004), regulates social behavior, attachment, anxiety, fear and stress response (Kikusui et al., 2019). It moderates stress responses by inhibiting ACTH secretion, thereby decreasing cortisol production (Li et al., 2019; Suh et al., 1986). However, the interplay between oxytocin and the HPA axis is more complex than simple inhibition, as demonstrated in a meta-analytic review by Brown et al. (2016), which revealed that the relationship between peripheral oxytocin and cortisol in humans is dynamic, context-dependent, and influenced by factors such as social context. Oxytocin is a hormone of interest in human-animal relationships (Beetz et al., 2012; Carter & Porges, 2016; MacLean & Hare, 2015)., Both species demonstrate an increase in oxytocin (evident in urine, blood and saliva) following positive social interactions (MacLean, Gesquiere, Gee, et al., 2017; Nagasawa et al., 2009, 2015). Oxytocin increases in urine of dogs following eating or exercise, suggesting this hormone may serve as an indicator of positive emotions (Mitsui et al., 2011).

When administered as intranasal spray, oxytocin enhanced social play (Romero et al., 2015), improved dogs' understanding of human social cues (Oliva et al., 2015), specifically by increasing their accuracy rather than their number of attempts at an object choice task (Oliva et al., 2019), and strengthened both intraspecific and interspecific bonds between dogs and humans (Romero et al., 2014). Intranasal spray of oxytocin also decreases HR and increases HRV in dogs, demonstrating the inter-connectedness between oxytocin and the SNS (Kis et al., 2014). Research on oxytocin production in dogs has not thoroughly examined effects of body weight or reproductive status, though limited evidence suggests age and sex influences (Mengoli et al., 2021). Breed differences exist in response to exogenous oxytocin (Kovács et al., 2016). This hormone can be measured in urine, blood, and saliva in domestic dogs (MacLean et al., 2018), though with substantial methodological considerations and limitations. These analytical challenges include: the influence of extraction procedures on measured concentrations (Bienboire-Frosini et al., 2017); immunoassay cross-reactivity with oxytocin metabolites affecting measurement validity (MacLean et al., 2019); the detection of biologically active oxytocin fragments that may have distinct effects (Uvnäs-Moberg et al., 2019); and variability in sample processing methods yielding inconsistent results across studies (Carter, 2022; Gnanadesikan et al., 2022). While oxytocin is measured peripherally, the action is at the brain level. Current research is focused on the transport of oxytocin across the blood-brain barrier (Higashida et al., 2024) and the potential of neurophysin I, the oxytocin carrier molecule, as an analytically robust surrogate biomarker for oxytocin that may overcome some measurement challenges (MacLean et al., 2024). The presence of these receptors likely affects the individual response to peripheral and exogenous oxytocin levels.

Temperature

Infrared thermography (IR) offers a noninvasive method to measure temperature changes as an indicator of stress responses in dogs. This technique detects surface temperature and micro-circulation variations, providing a visual representation of body temperature (Speakman & Ward, 1998) and insights into both acute and chronic stress states in animals (Stewart et al., 2005, 2007). Recent reviews have specifically addressed the application of infrared thermography and heart rate variability for evaluating autonomic activity in domestic animals, including dogs (Ghezzi et al., 2024). The neurophysiological basis for stress-induced temperature changes involves complex thermoregulatory pathways, with the hypothalamus serving as the central control center that coordinates temperature regulation. During stress responses, the activation of the preoptic anterior hypothalamus initiates sympathetic responses that affect peripheral vasoconstriction and thermogenesis. Catecholamine release and HPA axis activation redirect blood flow to skeletal muscles and vital organs while increasing heat production and loss, altering surface temperature distribution patterns detectable via thermography (Bouwknecht et al., 2007; Ghezzi et al., 2024). A positive correlation between core body temperature and stress response state is well-established (i.e., Bouwknecht et al., 2007). Eye temperature determined by IR strongly correlates with autonomic nervous system (ANS) activity (Travain et al., 2015).

Most vertebrates regulate internal body temperature (Tb) through behavioral and physiological mechanisms linked to metabolism (Zhao et al., 2022). Internal body temperature can increase due to a state of stress, thus termed "thermal stress." Body size significantly influences this regulation with larger animals having lower mass-specific metabolic rates than smaller ones (McNab, 1997). This relationship reflects adaptations to heat dissipation based on the animal's surface area:volume ratio (SA:V). Smaller animals, having larger SA:V, exhibit higher heat production rates and mass-specific metabolic rates (Schmidt-Nielsen & Knut, 1984). While large endothermic animals primarily generate heat through cellular metabolism, with a small contribution from thermogenesis, smaller ones lose heat at faster rates than larger ones. Thus, smaller endotherms should be able to orient their physiology toward heat production rather than the generation of adenosine triphosphate (ATP), the primary carrier of energy in cells, used for storing and transferring energy, when needed (Abreu-Vieira et al., 2015). The 44-fold variation in domestic dog body sizes creates significant differences in thermal regulation across breeds (Jimenez, 2016). Heat limitation particularly affects smaller breeds due to their inherently high metabolic heat production (Jimenez, 2016). This will also limit the thermal stress response of dogs. Body mass shows positive correlation with internal temperature and heat dissipation rates in dogs across seasons, while negatively correlating with mass-specific metabolism (Jimenez et al., 2023). Network analyses confirm body mass as a central factor in dogs' thermal regulation. While breed significantly effects thermoregulation, age, and sex do not show notable influence (Jimenez et al., 2023).

IR studies reveal increased eye temperature in domestic dogs during stressful situations like veterinary visits, with peak core temperatures occurring during clinical examination when dogs exhibit frozen behavior (Travain et al., 2015). This indicates that temperature changes reflect stress response rather than physical activity (Travain et al., 2015). Comprehensive coverage of IR principles and applications in dog welfare science is available in Travain and Valsecchi (2021). For a review on the usefulness of IR in a clinical setting, please see Casas-Alvarado et al. (2020) and Mota-Rojas et al. (2022).

Salivary alpha-amylase

Salivary alpha-amylase (sAA) serves as an SNS indicator for psychosocial stress in humans, responding to social defeat, confrontations, and trauma (Lu et al., 2021; Nater et al., 2006; Granger, et al. 2007). However, its role in dogs remains uncertain. While α-amylase mRNA transcripts appear absent in the canine parotid glands, they exist in other tissue, including pancreas, liver, intestines, and fallopian tube (Mocharla et al., 1990). Though detectable at low concentrations in canine saliva (Contreras-Aguilar et al., 2017), sAA's stress-related changes lack investigation regarding age, weight, sex, or reproductive status effects (Kooriyama & Ogata, 2021).

Melatonin

Melatonin synchronizes dogs' internal biological clock with the day-night cycles, with its concentration (in blood) showing natural fluctuations (Chojnowska et al., 2021; Zan et al., 2013). Beyond circadian regulation, melatonin influences sleep-wake cycles and responds to environmental cues including daylight and temperature (Saarela & Reiter, 1994). It also serves as an antioxidant in normal biological processes, like oxidative stress, where it helps to maintain balance between free radicals and antioxidants to prevent cellular damage and disease (Konturek et al., 2007). Salivary melatonin levels don't reliably reflect pineal gland production, making blood the preferred mode of measurement (Kennaway, 2020). In dogs, blood melatonin follows circadian patterns like humans, peaking at night and reaching lowest levels at dawn (Zan et al., 2013), with seasonal variation showing higher levels during thermally stressful (winter) months (Dunlap et al., 2007). While melatonin shows promise for understanding and improving mental states related to anxiety, aging, and sleep quality in dogs, research has yet to explore relationships between melatonin and factors such as age, body weight, sex, and reproductive status.

Chromogranin A

Chromogranin A (CgA) and its epitopes, catestatin and vasostatin, can be measured in both blood and saliva, remain stable across age, gender, breed, and time of day in healthy dogs (Srithunyarat et al., 2017). While blood levels show no significant stress-related changes, salivary catestatin increases significantly when dogs encounter stressful situations like pain, unfamiliar environments or sampling methods (Srithunyarat et al., 2018). CgA and its epitopes could be valuable tools for evaluating the effectiveness of stress-reduction interventions. However, comprehensive research on age and body weight effects remains limited (Kooriyama & Ogata, 2021).

Arginine Vasopressin

Arginine Vasopressin (AVP) levels correlate with specific behavioral patterns in dogs: higher plasma AVP has been associated with increased aggression toward other dogs (MacLean, Gesquiere, Gruen, et al., 2017), while salivary AVP increased in dogs lacking human interaction during human-animal interaction social studies (MacLean, Gesquiere, Gee, et al., 2017). Though AVP responds to acute stress (Kooriyama and Ogata (2021) and could provide insights into both stress states and aggressive predispositions, research has not yet examined effects of age, body weight, sex or reproductive status.

Serotonin

Serotonin influences multiple affective states and stress-related behaviors, including aggression, impulsivity, and reaction to pain (Berger et al., 2009). This neurotransmitter is commonly measured in blood, with peripheral serotonin representing a distinct pool from central serotonin due to the blood-brain barrier and playing an important role in regulating metabolic homeostasis (El-Merahbi et al., 2015). Lower blood serotonin levels in dogs correlate with reduced human sociability and increased aggression in dogs (Alberghina et al., 2017). While studies have investigated age and sex effects (Alberghina et al., 2017, 2019) and preliminarily explored body weight relationships (Bochiș et al. (2022), sample sizes remain small and reproductive status effects unexplored (Ogi & Gazzano, 2023).

Prolactin

Prolactin represents another promising biomarker for canine welfare assessment. This pituitary hormone has been suggested as an indicator of psychological stress, anxiety, and chronic stress in humans (Lennartsson & Jonsdottir, 2011; Turner et al., 2002) and in dogs (Gutiérrez et al., 2019; Mengoli et al., 2021; Pageat et al., 2007). Prolactin concentrations increase in response to acute stressors and may remain elevated during periods of chronic stress, potentially serving as a marker for sustained psychological distress. Research in dogs has demonstrated associations between elevated serum prolactin and anxiety-related behaviors (e.g., Pageat et al., 2007), with responses to treatment using selegiline or fluoxetine.

Microbiome/microbiota relationships

Research across species demonstrate important multidirectional interactions between brain, gut microbiota, and stress response, including relationships with anxiety behaviors, early life stress effects on the adult microbiota, and microbial influence on HPA axis development (Dinan & Cryan, 2012). Limited canine studies show variable results (Cannas et al., 2021; Perry et al., 2017;

Venable et al., 2016), though one larger working dog study found microbiome markers predicted several relevant working dog characteristics, including motivation, sociability, and gastrointestinal issues, but not stress responses (Craddock et al., 2022). While microbiota markers hold promise as indicators of acute or long-term stress responses, specific recommendations do not currently exist.

Immune response

Secretory immunoglobulin A

Immunoglobulin A (IgA) is a group of antibodies found in the mucous membranes and its level in saliva has been shown to respond to changes in mood in humans and both chronic and acute stress response states in dogs (Kooriyama & Ogata, 2021). Studies have found that sIgA levels in dogs tend to decrease following both acute and long-term stress, suggesting it could be used to measure stress responses. However, sIgA exhibits diurnal variation and may respond differently in puppies versus adult dogs, so further research is needed to fully validate its use as a reliable indicator of canine mental states and welfare across different contexts, time of day, and age groups (Kikkawa et al., 2003; Skandakumar et al., 1995; Svobodová et al., 2014). Salivary IgA measurements require careful interpretation, considering factors such as saliva flow rate, which is an important consideration for all measurements in saliva, including salivary cortisol and salivary oxytocin, as flow rate variations can significantly affect biomarker concentration independent of actual production changes. Additionally, dental disease, age (especially during tooth eruption), sex, reproductive status, and potential sample contamination (e.g., fecal, blood, etc.) should all be assessed and reported.

White blood cell distribution

Leukocytosis (increased white blood cell numbers in blood) reflects sympathetic nervous system and/or HPA axis activation. The veterinary clinical pathology evidence refers to the response of white blood cells to epinephrine release as a “physiologic leukogram” (McCourt & Rizzi, 2022). This rapid, transient response involves neutrophil demargination from tissue to circulation, lymphocyte mobilization from the thoracic duct and blockage of lymphocytes entering the lymph nodes, along with potential increases in monocytes and eosinophils (Stockham et al., 2003). The resulting broad increase in white blood cell counts in response to epinephrine, provides a measurable indicator of acute stress response. Increased red blood cell numbers (erythrocytosis) can also occur following epinephrine release due to splenic contraction (Stockham et al., 2003). In contrast, a “stress leukogram” reflects white blood cell count changes from endogenous or exogenous glucocorticoids (McCourt & Rizzi, 2022). Like the response to epinephrine, corticosteroid release triggers neutrophil movement from tissues to circulation and bone marrow release, (causing neutrophilia, and possible monocytosis), corticosteroid response differs by redistributing lymphocytes to bone marrow and lymphatic tissue, reducing blood lymphocyte count (Stockham et al., 2003). Eosinopenia can also occur through tissue repartitioning and decreased bone marrow release (McCourt & Rizzi, 2022). These changes – neutrophilia, monocytosis, lymphopenia, and eosinopenia – emerge within hours of a challenging experience and may persist for days, depending on corticosteroid exposure duration (McCourt & Rizzi, 2022). Neutrophil/Lymphocyte ratios serve as HPA axis stress response indicators across species (Davis et al., 2008), including studies of transportation-related stress in dogs (Herbel et al., 2020). However, interpretation requires careful consideration of sampling-induced stress effects from restraint and needle puncture, and the difficulty of distinguishing stress-related leukocyte changes from those caused by inflammation and infection.

State of the evidence

While research to date confirms various physiological indicators relate to stress response in dogs (refer Table 1), most studies inadequately account for crucial variables like body weight, age, sex and reproductive status that influence the underlying physiological processes. Common indicators such as cortisol show significant individual variability, limiting their reliability as standalone measures. Advancing canine

Table 1. Summary of physiological measures.

Measure	Mode of Measurement	Acute or Chronic	References
Heart rhythm (HR, HRV)	Pulse, Stethoscope, ECG, HR monitor	Acute	Gácsi et al. (2013); Katayama et al. (2016); Zupan et al. (2016); Amaya et al. (2020)
Oxytocin	Blood, saliva, urine	Acute	Nagasawa et al. (2009); Nagasawa et al. (2015); MacLean, Gesquiere, Gee, et al. (2017)
Cortisol	Plasma, saliva, urine, faeces, hair	Acute	Beerda et al. (1996); Cobb et al. (2016); Chmelíková et al. (2020); Schatz and Palme (2001); van der Laan et al. (2022)
Body temperature	IR thermography, thermometer (rectal, ear, microchip)	Acute, Chronic	Travain et al. (2015); Travain and Valsecchi (2021); Ghezzi et al. (2024)
Salivary Alpha-amylase (sAA)	Saliva	Acute	Kooriyama and Ogata (2021)
Melatonin	Blood	Chronic	Dunlap et al. (2007); Zan et al. (2013); Kennaway (2020)
Chromogranin A (CgA)	Blood, saliva	Acute	Srithunyarat et al. (2018); Kooriyama and Ogata (2021)
Arginine vasopressin (AVP)	Blood, saliva	Acute	MacLean, Gesquiere, Gruen, et al. (2017); Kooriyama and Ogata (2021)
Serotonin	Blood	Acute, Chronic	Alberghina et al. (2017); Alberghina et al., 2019
Prolactin	Blood	Acute, Chronic	Pageat et al. (2007); Gutiérrez et al. (2019); Mengoli et al. (2021)
Microbiome	Faeces	Acute, Chronic	Craddock et al. (2022)
Secretory IgA	Saliva	Acute, Chronic	Skandakumar et al. (1995); Kikkawa et al. (2003); Svobodová et al. (2014); Chojnowska et al. (2021); Kooriyama and Ogata (2021)
White blood cell amounts and ratios	Blood	Acute, Chronic	McCourt and Rizzi (2022); Stockham et al. (2003)
Lysozyme	Saliva	Acute in humans	Chojnowska et al. (2021)
Catecholamines (epinephrine and norepinephrine)	Blood	Acute	Not measured yet in dogs
Pro-inflammatory cytokines	Blood	Acute and Chronic	Jiménez and Strasser (2024)
Telomeres	Blood, mucosal tissue (cheek swab)	Chronic	Not measured yet in dogs
Oxidative stress measures	Blood	Acute, Chronic	Jiménez and Strasser (2024)
Osteocalcin	Blood	Acute	Not measured yet in dogs
CNS measures	MRI, EEG		Not yet measured in relation to welfare

welfare science requires establishing standardized protocols and reference ranges that consider individual and demographic variation for these indicators. Several promising new biomarkers from other fields of research may also merit investigation in dogs to expand our measurement toolkit.

Opportunities for future research

Several physiological indicators show promise for canine welfare assessment but remain unexplored. A brief description of these markers and what is known about them thus far in the canine or in other species follows.

Lysozyme

Lysozyme, a protein produced by immune cells (monocytes and macrophages), and present in saliva, provides innate defense against pathogens (Chojnowska et al., 2021). While human studies link it to acute stress response, its potential as a canine welfare indicator has not been explored.

Catecholamines: Epinephrine and norepinephrine

Catecholamines are key biomarkers for ANS activity. Epinephrine and norepinephrine are mainly synthesized by the central noradrenergic neurons and are released mainly from the adrenal medulla into the bloodstream to mount the sympathetic-dominated "fight-or-flight" response (Segerstrom & Miller, 2004). Many surrogate markers of ANS activity (e.g., HR, respiratory rate, blood pressure, etc.) have been proposed. Despite their importance, research on these compounds in dogs remains limited, partly due to sampling challenges. As with other markers, the collection of blood for sampling could cause spurious results.

Pro-inflammatory cytokines

Pro-inflammatory cytokines offer another promising avenue, particularly given the immune system's age-related changes in dogs. Aging affects T-cell immunity and immunoglobulin levels (Day, 2010), with studies showing decreased mitogen stimulation and changes in white blood cell populations (Greeley et al., 1996; Strasser et al., 1993). However, current research lacks comparisons between breeds and consideration of size-specific aging effects.

While chronic inflammation underlies many age-related diseases in humans, including cardiovascular disease, arthritis, and cancer (Chung et al., 2009; Davizon-Castillo et al., 2019) involving multiple cytokines and molecular pathways (Franceschi & Campisi, 2014), only limited data exists for dogs. Disease progression seems to be a causative factor linked to the inflammatory process (Chung et al., 2006). Humans demonstrate an increase in interleukin (IL)-6 and tumor necrosis factor α (TNF-α) levels with increased age and a decrease in insulin growth factor (IGF)-1 levels with increasing age (Xia et al., 2016). Proinflammatory cytokines IL-1β, TNF-α, and IL-6 in humans act both in paracrine and autocrine manners by developing insulin resistance, and they can interfere with insulin signaling, lipid and protein synthesis, potentially inducing metabolic disorders, including diabetes (Lontchi-Yimagou et al., 2013; Sung et al., 2018). One study found that increases in age demonstrated increased IL-6 concentrations in dog serum, but no changes with age in IL-1β, TNF-α (Jiménez, 2023). Another study found that shelter dogs, when compared with client-owned dogs, showed increased IL-1β levels, potentially leaving the shelter-dog populations at higher risk for zoonotic diseases (Jiménez & Strasser, 2024). Human studies link chronic stress experiences to increased cytokine levels (Gouin et al., 2012; Lennartsson et al., 2016), suggesting potential applications in canine welfare assessment.

Telomere length

Telomere attrition shows promise as a potential biomarker for assessing cumulative stress and welfare in dogs. Telomeres are protective caps at the end of chromosomes that are linked to genomic stability. As cells divide with increasing organismal age, or in times of increased oxidative stress, these caps get shorter (Richter & von Zglinicki, 2007). The mechanistic relationship between telomere dynamics and neuroendocrine function operates through several pathways: 1) Chronic activation of the HPA axis increases oxidative stress through elevated glucocorticoids, which directly accelerates telomere shortening; 2) Stress-induced catecholamine release increases cellular metabolic activity and mitochondrial reactive oxygen species production that damages telomeric DNA; and 3) Inflammatory cytokines produced during chronic stress responses activate cellular senescence pathways that affect telomerase activity (reviewed in Houben et al., 2008; Mathur et al., 2016).

Chronic stress experience has been associated with decreases in telomere length in humans and other animals. This work has been reviewed by Houben et al. (2008) and Mathur et al. (2016), with research suggesting that telomere length can provide a molecular measure of an animal's lifetime experiences, with negative experiences accelerating telomere shortening and positive interventions potentially mitigating or even reversing this attrition. There are no studies looking at stress and telomere length in domestic dogs, but others have established a link between telomere dynamics and the lifespan of different breeds of dogs without consideration for sex or reproductive status (Fick et al., 2012). Measuring telomere length could offer a valuable tool for evaluating the long-term impacts of various husbandry practices, environmental conditions, and life events on canine mental states and overall welfare between different populations of dogs.

Oxidative stress

Mitochondria structure and function play a central role in organismal homeostasis (Calhoon et al., 2014; Havird et al., 2019; Hood et al., 2018), acting as both targets and mediators of stress responses (Picard and McEwen, 2018). During "fight or flight" responses, they meet increased ATP demands (Manoli et al., 2007), but approximately 5% of oxygen processed becomes a reactive oxygen species (ROS) (Hulbert et al., 2007). While low ROS levels serve as important signaling molecules for gene regulation, cell signaling, and cell processes (apoptosis) (Dowling & Simmons, 2009; Monaghan et al., 2009; Sohal & Orr, 2012), high levels can damage lipids, proteins, and even DNA (Finkel & Holbrook, 2000; Monaghan et al., 2009).

Oxidative stress reflects the balance between pro-oxidants from aerobic metabolism and protective antioxidants (Ayala et al., 2014; Halliwell & Chirico, 1993). Oxidative damage accrues when ROS production overwhelms the antioxidant system (Dowling & Simmons, 2009; Monaghan et al., 2009; Sohal & Orr, 2012). Oxidative damage can happen to many biologically relevant molecules, such as proteins, DNA and lipids (Hulbert et al., 2007). Lipids are among the molecules most affected, and two of the most prevalent pro-oxidants that can initiate damage to lipid membranes are hydroxyl radicals (OH•) and hydroperoxyl radicals (OOH•) (Ayala et al., 2014). The process of lipid peroxidation (LPO) continues unabated until the propagation of damage is halted by an antioxidant molecule (Ayala et al., 2014; Halliwell & Chirico, 1993). Enzymatic antioxidants, such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), function by catalyzing the oxidation of less biologically insulting molecules. Other antioxidant molecules, such as vitamin E and C, act as chain-breaking antioxidants; they scavenge for ROS, remove them once they are formed, and further halt the propagation of peroxidation (Halliwell & Chirico, 1993). The following consideration is of utmost importance: The relationship between metabolism and ROS production is not straightforward, though many assume that an increase in oxygen consumption (i.e., increase in metabolic rate) should yield an increase in ROS production (Hou et al., 2021). Oxygen consumption can either be coupled with ATP production or heat depending on whether the ATP synthase or mitochondrial uncoupling proteins are driving respiration (Divakaruni & Brand, 2011; Hou et al., 2021), thus, it should never be assumed that this process is linear.

To accurately measure oxidative stress within an organism, the "damage" side and the antioxidant side should be measured together. That is, at least one measure of oxidative damage should be considered with at least one measurement of antioxidant capacity. Additionally, accurate estimations of whole-animal oxidative stress should be done in separated blood, as blood is a metabolic reservoir for these types of molecules (Jimenez & Downs, 2020) or in metabolically active tissues. Saliva and urine are not good sources for measuring oxidative stress in animals, and do not demonstrate a whole-animal perspective for the process. The connection between glucocorticoid secretion and OS increases has been documented in dogs (Ferreira et al., 2014); preliminary work has suggested OS could be used with dogs responding to different environments (e.g., shelters per Passantino et al., 2014). Age, body weight, and breed considerations to oxidative stress have been measured in domestic dogs (Jimenez & Downs, 2020; Jiménez & Strasser, 2024), sex and reproductive status have also been considered using primary fibroblast cells (Jimenez et al., 2018, 2020).

Osteocalcin

Osteocalcin, an osteoblast-produced protein abundant in the bone matrix, influences glucose metabolism and fat mass in metabolic homeostasis (Lieben et al., 2009). Berger et al. (2019) review the role of osteocalcin in mediation of the acute stress response. Exposure to a variety of physical and emotional stressors in rodents and humans leads to a rapid increase in circulating bioactive osteocalcin. There is evidence that this release leads to a suppression of the parasympathetic nervous system, allowing the acute stress response to proceed (Berger et al., 2019). Researchers have reported different patterns of change in serum osteocalcin and salivary cortisol in humans exposed to low- and high-intensity physical threat scenarios (Vít et al., 2023). Given its presence across vertebrates, osteocalcin warrants investigation as a canine stress biomarker, though factors like age, activity, nutrition, body condition, and other factors will likely play a role in its release and subsequent interpretation.

How could we better represent animal experiences?

With the increase in the use of artificial intelligence and newer technologies, there are great opportunities to combine and model the approach to stress and animal welfare measurement.

Emerging technologies

Recent technologies enable enhanced monitoring of canine behavior and physiology through wearables (e.g., actigraphy for sleep-activity patterns, accelerometers for movement), ingestible sensors (e.g., microsensors for temperature, heart rate, respiratory rate), portable devices (e.g., electroencephalogram), and smart (sometimes responsive) environments. Facial expression analysis through AnimalFACS (Facial Action Coding System) represents another promising approach for welfare assessment, allowing objective quantification of facial movements associated with emotional states in dogs (Mota-Rojas et al., 2025). However, applying these technologies requires careful consideration of the potential impacts on dogs' experiences (Webber et al., 2022). For example, devices emitting ultrasound above human hearing range (20 kHz) but within canine (45 kHz) could induce anxiety or behavioral changes if dogs cannot avoid the sound (Grigg et al., 2021). Such unintended effects might compromise the very observations these technologies aim to capture. While these emerging tools offer exciting opportunities for welfare assessment and canine welfare science, limited dog-specific validation currently restricts their scientific value. Future advances may come from adaptation of precision livestock farming technologies to canine applications (e.g., Neethirajan, 2024).

Existing technologies also continue to expand research capabilities in canine welfare science. Noninvasive resting-state functional magnetic resonance imaging (RS-fMRI) allows investigation of brain-behavior connectivity in awake, trained dogs (Berns et al., 2012). The ability to use these tools to predict the future success of working dogs based on trainability influences the welfare of those dogs going through the training process (Deshpande et al., 2024). Wider availability and decreasing costs relating to the use of this technology may open even more opportunities for its application in the study of canine welfare.

Artificial intelligence (AI) offers new opportunities for analyzing canine welfare indicators. Recent studies demonstrate AI's potential for predicting working dog success through personality assessment (Amirhosseini et al., 2024) and olfactory detection capabilities based on behavioral traits and environmental conditions (Eyre et al., 2023). However, accurate prediction of working dog suitability remains challenging, likely due to epigenetics (Bray et al., 2021). Dogs continue to change as individual genetic potential, personality, environment and learning experiences interact while dogs mature throughout their early years of life. Big data projects are becoming more common in canine science (e.g., ManyDogs; VetCompass; Generation Pup; The Dog Aging Project; DogslifeUK; etc.) and we expect these data sets will offer new insights into epigenetics and behavior informatics for which AI will undoubtedly prove to be a valuable tool to advance canine welfare.

We hope to see such validation and wider incorporation of these technologies within research as canine experience indicators become routine inclusions within human-animal interaction research. For too long, the focus within the field of anthrozoology and even canine science, has been to maximize the benefit to people from dogs (MacLean et al., 2021). Funding bodies increasingly require canine-specific welfare measures, though comprehensive assessment demands substantial resources, including funding, equipment, personnel, time, and subject dogs.

Behavioral factors in assessing canine welfare

Although this discussion paper emphasizes physiological indicators, behavioral factors are also important when assessing canine welfare. We know that many behavioral factors can influence how a dog copes in given situations. These include canine personality (Posluns et al., 2017), attachment (Payne et al., 2015), temperamental traits like shy-boldness (Starling et al., 2013), and cognitive bias (Barnard et al., 2018). Inclusion of observed behavior (e.g., McGreevy et al., 2012), qualitative behavior analysis (e.g., Flint et al., 2024), and/or other survey-based profiling (e.g., Ley et al., 2009) will serve to provide helpful information from which to contextualize and interpret physiological data.

Research design and analysis

No single experimental design suits all assessments of canine physiological indicators at this time. Study design depends on the focus – whether examining arousal, emotional valence, or acute versus chronic distress (Dickens & Romero, 2013; Gormally & Romero, 2020). Modern research increasingly incorporates dog agency, allowing subjects to opt-in or opt-out of participation (Horowitz, 2021; Littlewood et al., 2023), reflecting emerging concepts of canine consent and emotional needs (Jones, 2024). Environmental presentation order can also influence canine responses (Paul et al., 2023), adding another layer of complexity. Research design must balance multiple interacting factors: research questions, statistical approaches, project budget constraints, timelines, sample size and availability of participating dogs, and sampling invasiveness. These considerations inform our recommendations while acknowledging the need for continued discussion and methodological advancement in canine welfare science.

Key recommendations:

(1) Avoid using salivary cortisol alone to infer canine affect and welfare.
(2) Design research that records multiple indicators from different physiological systems to capture the dog experience. Ideally, these will rely on a) minimally invasive sampling that is b) most relevant to the system being evaluated in c) the context of that system's role in responding to the experience being evaluated. This approach supports interpretations and provides data for further research about the relationships between these markers and their relative physiological systems interactions.
(3) Include validated behavioral measures and individual characteristics (such as dogs' sex, age, breed, weight, personality, owner presence, etc.) in study design and reporting, acknowledging that physiological systems' interactions may yield varying interpretations. This comprehensive approach enables more meaningful cross-study comparisons and advances field-wide understanding.
(4) Give precise information about the context of the stressor/stressful procedure when reporting results, including duration, intensity, novelty, controllability, and predictability factors.

Animal welfare implications

This discussion advances canine welfare assessment by highlighting traditional biomarker limitations and advocating for a comprehensive systems approach, paving the way for more accurate and comprehensive assessments of canine welfare. Emphasizing the importance of individual factors and appropriate research design enables a better understanding of positive and negative affective states in dogs, which is crucial for promoting their wellbeing. While significant work remains to be done in exploring physiological indicators, we encourage further developments in this field and welcome debate. The recommendations provided can lead to improved practices, enhanced scientific understanding, and better outcomes for the welfare of dogs.

Conclusion

Canine welfare science requires multiple physiological indicators because welfare's complexity cannot be captured by any single measure. Converging evidence from various indicators (like cortisol, heart rate, immune markers and behavior) validates that we are evaluating welfare rather than some other physiological process (like age) or external factor. We advocate for practical, evidence-based approaches using noninvasive methods while acknowledging the challenges in evaluating physiological indicators across different situations. Continued refinement of practices and terminology will enhance our understanding of dog welfare and inform assessment standards. This comprehensive approach promises to advance both scientific knowledge and practical care, ultimately improving the lives of dogs.

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Author contributions

Conceptualization: AGJ, MLC; Investigation: AGJ, NAD, MLC; Methodology: AGJ, NAD, MLC; Project administration: AGJ, MLC; Visualization: MLC; Writing (original draft): AGJ, NAD, MLC; Writing (review and editing): AGJ, NAD, MLC.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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