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# Acute restraint stress induces rapid and prolonged changes in erythrocyte and hippocampal redox status

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**Summary** The onset and consequential changes in reduction–oxidation (redox) status that take place in response to short-term stress have not been well defined. This study utilized erythrocytes and neural tissue from male Wistar rats to demonstrate the rapid redox alterations that occur following an acute restraining stress. Serial blood samples collected from catheterized animals were used to measure prolactin, corticosterone, glucose, general oxidative status, and glutathione/glutathione disulfide ratios. Restraint increased prolactin concentration by approximately 300% at 30 min and rapidly returned to baseline values by 120 min of stress. Baseline blood glucose and corticosterone increased during stress exposure by approximately 25% and 150% respectively. Over the experimental period, the erythrocytic oxidative status of restrained animals increased by approximately 10% per hour which persisted after stress exposure, while changes in the glutathione redox couple were not observed until 120 min following the onset of stress. Application of restraint stress increased hippocampal oxidative status by approximately 17% while no change was observed in the amygdala. It was concluded that while endocrine and metabolic markers of stress rapidly increase and habituate to stress exposure, redox status continues to change following stress in both peripheral and neural tissue. Studies with longer post-restraint times and the inclusion of several brain regions should further elucidate the consequential redox changes induced by acute restraint stress.


## 1. Introduction

Stress activates a number of hypothalamic pathways including the hypothalamic–pituitary–adrenal (HPA) axis, which mobilizes energy stores while simultaneously modulating parasympathetic activity (Chrousos and Gold, 1992; Ulrich-Lai and Herman, 2009). This is achieved in part via the release of several pituitary-derived hormones including prolactin and adrenocorticotropic hormone (ACTH) (Ren et al., 2010). Prolactin is a transiently stress responsive hormone representing a functionally distinct stress pathway from the HPA axis that is unaffected by glucocorticoid negative feedback (Weiser et al., 2011). Although the role of prolactin in the stress response remains unclear, it has demonstrated facilitatory effects on adrenal sensitivity to ACTH (Jaroenporn et al., 2007). Acute increases in

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glucocorticoids modulate mitochondrial function, in addition to catabolically increasing glycolysis, gluconeogenesis, glycogenolysis and lipolysis, ultimately increasing available circulating glucose (Teague et al., 2007). This glucocorticoid-induced increase in cellular metabolism produces enhanced reactive oxygen species (ROS) formation from the oxidative phosphorylation pathway of the inner mitochondrial electron transfer chain (Manoli et al., 2007; Du et al., 2009). Under normal conditions, ROS play important physiological roles for several cellular enzymes and transcription factors in addition to regulating cell cycle progression and stem cell differentiation (Pugazhenthi et al., 2003; Mohn et al., 2005; Valko et al., 2007; Wang et al., 2011). However, prolonged increases in ROS concentration cause cellular compartmental damage to proteins, nucleic acids, and lipid membranes in addition to regulating long term glucocorticoid negative feedback (Liu et al., 1994; Sato et al., 2010; Zhou et al., 2011).

This imbalance in reduction—oxidation (redox) status leads to oxidative stress, which is hazardous to cells containing high densities of phospholipids and polyunsaturated fatty acids, particularly in the presence of high relative concentrations of oxygen (Valko et al., 2007). This criterion is characteristic of erythrocytes, with numerous studies demonstrating increased markers of oxidation in conjunction with altered antioxidant activity following stress exposure (Sahin et al., 2004; de Souza et al., 2006; Sahin and Gümüslü, 2007). Using both acute and chronic stress treatment, Sahin and colleagues (2004) established that restraint stress altered catalase, superoxide dismutase, and glutathione peroxidase activities in addition to increasing lipid peroxidation and decreasing glutathione (GSH) content in erythrocytes. Tagliari and colleagues (2010) confirmed these findings in blood plasma and erythrocytes for lipid peroxidation and imbalanced catalase and superoxide dismutase activities in a chronic unpredictable stress model. Erythrocytes are particularly susceptible to oxidative damage due to the high membrane content of polyunsaturated fatty acids and high cellular concentrations of oxygen and hemoglobin (Kusmic et al., 2000). In an extracellular oxidative challenging environment, erythrocytes accumulate exogenous ROS through membrane diffusion and specialized anion channels causing methemoglobin production and iron release, which further exacerbates erythrocytic ROS production (Denicola et al., 1998; Kinoshita et al., 2007).

In the brain, Lucca and colleagues (2009) systematically identified selective differences in oxidative parameters including protein carbonyls, lipid peroxidation, catalase, and superoxide dismutase activities in the cerebellum, prefrontal cortex, hippocampus, striatum, and cortex of chronically stressed rats. Liu and Zhou (2012) extended these findings to isolated mitochondria from the prefrontal cortex demonstrating direct increases in oxidative status and decreases in the glutathione redox ratio, superoxide dismutase activity, mitochondrial membrane potentials, and decreased hippocampal and striatal mitochondrial DNA content. Together with the findings of Wang and colleagues (2011) indicating that mitochondrial DNA oxidation elevated astrogliosis of hippocampal neural stem cells, the effects of stress-induced alterations in redox balance likely play key roles in neurogenesis. In the hippocampus, oxidative stress has been directly associated with administration of glucocorticoids *in vivo* which concurrently increased serum markers of oxidation (Sato et al., 2010). Furthermore, incubation of the synthetic glucocorticoid agonist dexamethasone selectively increased oxidation in organotypic hippocampal slice cultures in contrast to other steroid hormones such as progesterone and estradiol (You et al., 2009). Dexamethasone also up-regulates oxidant-producing enzymes such as monoamine oxidase-A, and inhibition of this enzyme reduced the dexamethasone-induced neuronal death, acting as a key antidepressant (Haynes et al., 2004; Duncan et al., 2012). These findings offer an explanation for the increased apoptosis and decreased proliferative capacity of the hippocampus following both acute and chronic stress exposure, as alterations in redox status regulate these processes (Menon et al., 2003; Heine et al., 2004; You et al., 2009). Although research has focused on chronic stress in the etiology of degenerative diseases, there is mounting evidence that acute stress initiates several long-term adaptations that exceed the time course of the stress response (Heine et al., 2004; Wiedenmayer, 2004). Importantly, exposure to acute stress sensitizes the HPA axis to potentiate subsequent responses, making it an appropriate model to investigate the possible origins of pathological states (Nakashima et al., 2002).

The lack of sensitive and minimally invasive biomarkers hinders the early diagnosis of degenerative diseases (Zhou et al., 2008). Recently, several clinical studies have reported indicators of altered oxidative metabolism in peripheral blood from Alzheimer's, amyotrophic lateral sclerosis, Huntington's, and Parkinson's patients (McGrath et al., 2001; Sohmiya et al., 2004; Klepac et al., 2007; Babu et al., 2008). These studies suggest that, in addition to diagnosis, these markers may be useful in monitoring disease progression and response to treatments. However, the time-course and sensitivity of the redox system to challenges under non-pathological conditions has not been well characterized. In this study, we have validated an acute restraining stress in rodents using prolactin, corticosterone, and glucose as stress markers in addition to utilizing both neural tissue and peripheral erythrocytes to demonstrate the subsequent redox alterations that occur.

## 2. Materials and methods

### 2.1. Experimental animals

Male Wistar rats (*Rattus norvegicus*) aged 6 weeks postnatal weighing 200—300 grams were housed individually under standard laboratory conditions (22 ± 2 °C; 55 ± 5% humidity) with a 12 h light—dark cycle (lights off at 12.00 h). Standard rat chow and water were available *ad libitum*. All experimental procedures were in accordance with regulations and policies outlined by The University of Queensland Animal Ethics Committee.

### 2.2. Jugular vein catheterization

Rats were anaesthetized with isoflurane delivered in medical oxygen (induction: 5% at 2 L/min; maintenance: 2.5—3.5% at 1 L/min). Both incision and exit sites were disinfected and a 0.5—1 cm incision was made in the ventrolateral aspect of the neck. Approximately 1 cm of the right external jugular

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