# Evaluation of stress response in patients following cardiac surgery

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# EVALUATION OF STRESS RESPONSE IN PATIENTS FOLLOWING CARDIAC SURGERY

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# LIST OF ABBREVIATIONS

ACT – Activated clotting time
ACTH – Adrenocorticotropic hormone
ANOVA – Analysis of variance
AVP – Arginine vasopressin
BIS – Bispectral index
CABG – Coronary artery bypass graft
CB – Cannabinoid
CNS – Central nervous system
CPB – Cardiopulmonary bypass
CRH -– Corticotropin-releasing hormone
CRP – C-reactive protein
EC – Endocannabinoid
ECLIA – Electrochemiluminescence "sandwich" immunoassay
EuroSCORE – European system for cardiac operative risk evaluation
FSH – Follicle-stimulating hormone
GABA – Gamma-aminobutyric acid
GH – Growth hormone
GHRH – Growth hormone-releasing hormone
GLUT – Glucose transporter
GR – Glucocorticoid receptor
HIT – Heparin-induced thrombocytopenia
HPA – Hypothalamic-pituitary-adrenal
ICU – Intensive care unit
IGF – Insulin-like growth factors
IL – Interleukin
IQR – Interquartile range
LH – Luteinizing hormone
LVEF – Left ventricular ejection fraction
M.v. – Mechanical ventilation
MAP – Mean arterial pressure
MC2R – Melanocortin 2 receptors
MR – Mineralocorticoid receptor
NPY – Neuropeptide Y
OPCAB – Off-pump coronary artery bypass
PIF – Prolactin-inhibitory factor
POD – Postoperative day
PRL – Prolactin-releasing factors
PVC – Polyvinylchloride
PVH – Paraventricular hypothalamic nucleus
PVN – Paraventricular nucleus
RAAS – Renin-angiotensin-aldosterone system
SCN – Suprachiasmatic nucleus
SVRi – Systemic vascular resistance index
T3 – Triiodothyronine
T4 – Thyroxine
TNF-$\alpha$ – Tumor necrosis factor-alpha
TRH – Thyrotropin-releasing hormone
TSH – Thyroid-stimulating hormone
VIP – Vasoactive intestinal peptide

# 1. INTRODUCTION

### 1.1. Stress response to surgery

Alterations in hormones and metabolism ensuing after injury or trauma is called the stress response. Surgical stress leads to derangements of every homeostatic axis, including vast endocrinological, immunological and hematological impacts on the human body (Table 1) (1). The degree of response to surgery encompasses a wide range of factors, such as length of surgery, type and magnitude of surgery, patients age, intraoperative blood volume loss, postoperative pain and anesthetic management used (2).

It was first acknowledged by David Cuthbertson in 1920, who observed elevated urinary muscle breakdown metabolites in patients following surgery (3). The mechanism to adapt to various homeostatic challenges is evolutionary designed to increase animals' survival after injury, by mobilizing their stored energy reserves (1). Regarded as a defense mechanism, the exacerbated systemic responses after surgery can be implicated in increased morbidity and mortality (4). Therefore, focus needs to be directed at minimizing the systemic response to stress, to improve patient outcomes, reducing the period of hospital stay and lowering costs of patient care (5,6).

**Table 1.** Systemic responses to surgery. [Retrieved from Desborough JP] (1)

| |
|---|
| Sympathetic nervous system activation |
| Endocrine 'stress response' |
| pituitary hormone secretion |
| insulin resistance |
| Immunological and haematological changes |
| cytokine production |
| acute phase reaction |
| neutrophil leucocytosis |
| lymphocyte proliferation |

### 1.2. Endocrine Stress response

The endocrine response to surgery is characterized by increased levels of pituitary counter- regulatory hormones, namely cortisol, growth hormone, glucagon and catecholamines and activation of the sympathetic nervous system (7). A small number of other endocrine glands respond with a decline in secretion (Table 2).

**Table 2**. Principal hormonal response to surgery. [Retrieved from Desborough JP] (1)

| Endocrine gland | Hormones | Change in secretion |
|---|---|---|
| Anterior pituitary | ACTH | Increases |
| | Growth hormone | Increases |
| | TSH | May increase or decrease |
| | FSH and LH | May increase or decrease |
| Posterior pituitary | AVP | Increases |
| Adrenal cortex | Cortisol | Increases |
| | Aldosterone | Increases |
| Pancreas | Insulin | Often decreases |
| | Glucagon | Usually small increases |
| Thyroid | Thyroxine, tri-iodothyronine | Decrease |

ACTH, adrenocorticotrophic hormone (corticotrophin); AVP, arginine vasopressin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.

#### 1.2.1. Hypothalamic-Pituitary-Axis

##### 1.2.1.1. Physiology of the Hypothalamic-Pituitary-Adrenal (HPA) axis

The hypothalamic-pituitary-adrenal (HPA) axis is the humoral component of an integrated neuroendocrine system, which has crucial importance in regulating homeostasis throughout the body, by reacting to internal and external stressors to homeostasis. The axis is comprised of the hypothalamus, which provides regulation via secretion of corticotrophin-releasing hormone, the anterior pituitary, with release of adrenocorticotropic hormone (ACTH) and the final product of glucocorticoids from the adrenal gland (Figure 1) (8).

Stimulation of the HPA axis follows activation of the neurons in the paraventricular nucleus of the hypothalamus, resulting in synthesis and secretion of corticotropin-releasing hormone (CRH) into the hypothalamic-hypophyseal portal vessel. Corticotropin-releasing hormone, a hypothalamic releasing factor, in turn acts on G-protein coupled receptors (corticotropin releasing hormone R1 receptors) of corticotropes in the anterior pituitary

stimulating release of ACTH (9). Corticotropin-releasing hormone is needed for both basal and stress-induced ACTH secretion. An increase in ACTH is followed within minutes by a steep rise in adrenocortical secretion of cortisol through its binding on melanocortin 2 receptors (MC2R) in the zona fasciculata (10).

The paraventricular nucleus of the hypothalamus (PVN), the central regulator of the HPA axis, acquirers' circadian input from the suprachiasmatic nucleus (SCN) in addition to receiving afferent information from the limbic system and the brainstem. From the limbic system, cognitive and emotional stressors are identified and information about inflammation and homeostatic changes are converged from the brainstem (11). The circadian rhythm of glucocorticoid secretion results in secretory rates of CRH, ACTH and cortisol, being highest at awakening and reaching its nadir at around midnight. This oscillation is caused by a 24-hour cyclical alteration in the signaling pathways from the hypothalamus (12).

Cortisol has various catabolic effects on target organs. In terms of protein metabolism, it promotes proteolysis and decreased protein synthesis. Furthermore, it increases gluconeogenesis in the liver and decreases cell glucose utilization by decreasing translocation of the glucose transporters (GLUT) 4 to cell membranes. The effects of cortisol are also demonstrated by enhanced mobilization of fatty acids, leading to increased free fatty acids in plasma (13).

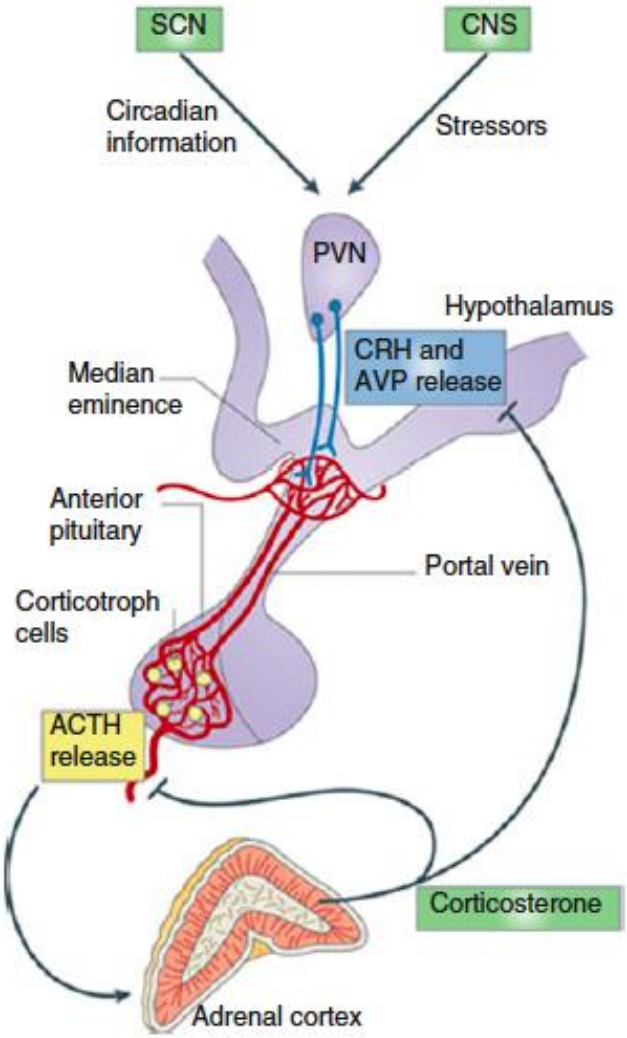


**Figure 1.** The hypothalamic-pituitary-adrenal (HPA) axis.

The hypothalamus receives circadian input from the suprachiasmatic nucleus (SCN) and stress-related inputs from the limbic system and brainstem.
[Retrieved from Lightman and Conway-Campbell] (14)

### 1.2.1.2. Regulation of the Hypothalamic-Pituitary-adrenal (HPA) axis

Within the central nervous system (CNS) glucocorticoid receptors control the feedback mechanism through binding of glucocorticoid to at least two intracellular receptor types; the mineralocorticoid receptor (MR) type 1 and the glucocorticoid receptor (GR) type 2 (9). The type 1 or MR receptor is saturated by basal levels of glucocorticoids, as it exhibits higher glucocorticoid binding affinity. It is responsible for regulating circadian and ultradian rhythms, therefore determining basal activity of the HPA axis. During peaks of circadian rhythm and during stress, higher levels of glucocorticoids activate lower affinity GRs (15–17).

As a consequence, GRs are responsible for mediating the stress response, resulting in increased mobilization of energy sources (18). Glucocorticoids initiate direct negative feedback mechanism of the HPA axis at the level of the hypothalamus. The binding of glucocorticoids to receptors causes rapid synthesis and release of endocannabinoids (ECs). Endocannabinoids bind to cannabinoid receptor type 1 (CB1) on presynaptic terminals, stopping release of neurotransmitter glutamate, leading to decreased CRH activation (19,20). In addition to the classical direct glucocorticoid negative feedback on CRH neurons, GABA, somatostatin, enkephalin and others also play a role in paraventricular hypothalamic nucleus (PVN) inhibition (8). The direct inhibitory feedbacks of cortisol are ineffective in situations of potent stress stimuli, resulting in prolonged and periodic exacerbations of cortisol secretion (12).

#### 1.2.1.3. Pathophysiology of the Hypothalamic-Pituitary-Adrenal (HPA) axis to surgical stress

Overall, the endocrine response to surgery leads to surplus of energy sources by increasing blood glucose via the action on protein, glycogen and lipid metabolism. Increased proteolysis results in profound weight loss and muscle wasting in patients after major surgery, which may lead to disastrous events in the malnourished patient (21). Decreased protein stores are associated with poor immunological function and lengthened recovery (22). Insulin resistance is particularly noted on postoperative day 1, with resistance evident for about 3 weeks, with the greatest impact on patients following major surgery (abdominal and cardiac surgery) (23,24).

Activation of the HPA axis in times of stress has a profound effect in regard to the body's immune system. It downregulates immune responses, stimulates cytokine production and interrupts inflammatory mediator synthesis (8). McIntosh *et al.* demonstrated that the pulsatile secretion of cortisol is maintained after surgical insult. A delay in secretion was observed on the 3rd day after surgery (25).

### 1.2.2. Sympathoadrenal response

Activation of the sympathetic autonomic nervous system leads to increased release of catecholamines (epinephrine and norepinephrine) from the adrenal medulla and norepinephrine from presynaptic nerve terminals (1). Stimulation of the adrenal medulla and direct sympathetic stimulation result in increased overall activity of the heart (increased rate and force) and constriction of systemic blood vessels, yielding increased arterial pressure. In addition, the sympathoadrenal response contributes to the metabolic effect of increased blood glucose by

hepatic glycogenolysis and gluconeogenesis, and by inhibition of insulin‘s mediated glucose uptake by muscle and adipose tissue (12). Increased serum glucose concentration is a major concern, mainly for cardiac surgery, as blood levels may increase up to 10 mmol $L^{-1}$ in non-diabetic patients (21). There is a higher prevalence of surgical wound complications, organ dysfunction, adverse cardiovascular events and mortality among patients with increased intra- and postoperative glycemic levels (26,27).

### 1.2.3. Response of the posterior pituitary

The hypothalamic neurosecretory cells, specifically the magnocellular neurons secrete arginine vasopressin (AVP). From cell bodies in the paraventricular hypothalamic nucleus (PVH), axons project to into the neurohypophysis. Synthesis and secretion are organized by dual systems of volume/pressure and osmotic regulation (8).

Tonic inhibition from high-pressure baroreceptors, situated in the carotid sinus and aortic arch and low-pressure receptors, located in the atria and pulmonary venous system exhibit tonic inhibition of the magnocellular neurons. A decrease in this tonic inhibition leads to increased circulating levels of AVP. It is also important to note, that the major hormonal regulation to control volume is the renin-angiotensin-aldosterone system (RAAS), that causes pronounced sodium excretion following increases of pressure and volume (28). Another superior regulatory system is described by means of osmoreceptor, compared to the less sensitive concept of vasopressin regulation, due to volume and pressure regulation (8). Osmoreceptors are located throughout the brain, sensing changes in plasma osmolality, being particularly sensitive to variations in sodium levels (29).

Vasopressin acts via V2 receptors in the renal collecting tubules, to enhance water and urea permeability to stabilize extracellular fluid osmolality in a range of 280 to 295 mOsm $kg^{-1}$ $H_2O$ (8). Ligand-receptor binding results in phosphorylation and activation of aquaporin II and incorporation of water channels into the luminal membrane to increase water reabsorption, in addition to increased synthesis of aquaporin II and permeability of the channel to water (30). Besides AVP’s primary effect on plasma osmolality, it responds to surgical stress by adding potency on CRH induced ACTH release in the pituitary (31). Arginine vasopressin is released from CRH nerve terminals, as approximately 50% of neurons co-express AVP and binds to V1b receptors subtype on corticotropes, synergizing CRH actions (32)

### 1.2.4. Growth hormone response

Growth hormone (GH) secretion is coordinated by two main hypothalamic peptides: growth hormone- releasing hormone (GHRH) and somatostatin (8). Growth hormone- releasing hormone is secreted by parvocellular neurosecretory cells in the arcuate or infundibular nucleus and stimulates the release of GH (33). It is assumed to be responsible for GH's pulse amplitude (34). In contrast, somatostatin inhibits GH secretion and drives GH pulse activity (35). Somatostatin neurons are located in the periventricular nucleus of the hypothalamus (33). Despite the above-mentioned primary GH regulators, GH release is stimulated by multiple mechanisms, numerous other hormones and substances (8).

Many effects of GH are mediated by somatomedins, the most important of which are insulin-like growth factors (IGF), notably IGF-1. Insulin-like growth factors are synthesized in the liver, muscle and other tissues following stimulation by GH. Besides its primary effect in growth stimulation and regulation, it substantially contributes to overall metabolism. It stimulates protein synthesis in most cells of the body, promotes increased mobilization of fatty acids from adipose tissue, with resultant higher body fluid concentrations of fatty acids, for energy production (12). In addition, it exerts an anti-insulin effect, diminishing glucose uptake by cells and utilization, thereby contributing to elevated plasma glucose levels. Growth hormones impact on metabolism is heightened in response to surgery, with the degree of elevation correlating with tissue injury (1).

At both the hypothalamus and the pituitary, growth hormones secretion is controlled via negative feedback from multiple substances. Firstly, GH in itself exerts direct negative feedback on its own secretion, as somatotrophs express GH receptors in the anterior pituitary, whereas GH's indirect feedback control is regulated by primarily increasing somatostatin secretion (36). The latter mechanism is explained by the expression of GH receptors on somatostatin neurons in the hypothalamus (8). Conversely, only a small number of GHRH neurons express GH receptors, which strongly supports the dominant role of activation of somatostatin neurons for feedback control of GH secretion (37). Secondly, IGF-1 has central inhibitory action on GH secretion, demonstrated by an increased concentration of somatostatin as well as a reduction on GHRH (38). Thirdly, ghrelin, a circulating hormone secreted by enteroendocrine cells of the gastrointestinal tract is thought to have both direct and indirect effects on increasing GH secretion. Ghrelin receptors are highly expressed on somatotrophs in the anterior pituitary and in the hypothalamus (8). Evidence suggests ghrelin's stimulation of GH receptors and ultimately GH secretion is not influenced by inhibitory effects of somatostatin (39). Moreover,

feedback effects of GHRH and somatostatin via a bidirectional interaction induce pulsatile GH secretion (40).

Besides hormonal regulatory mechanism, GH's release from the anterior pituitary is under the influence of neuronal control by efferent connections to the hypothalamus. Growth hormone-releasing hormone and somatostatin neurons express pre-and postsynaptic receptors for various neurotransmitters, emphasizing the role of norepinephrine and epinephrine for adrenergic stimulation during surgery as one example (8).

### 1.2.5. Prolactin response

Prolactin is a protein hormone similar in structure to that of growth hormone (1). Unlike other pituitary hormones, it is primarily under tonic inhibitory control from dopamine, as the main prolactin-inhibitory factor (PIF) in the hypothalamus (41,42). Dopamine inhibits prolactin secretion due to dopamine binding to D2 receptors expressed on lactotrophs. Despite the dominant inhibitory effect of dopamine on prolactin secretion, other mechanisms contribute to its regulation by either suppressing dopamine or by stimulating the release of prolactin-releasing factors (PRL) (8). The most important of the PRL's are thyrotropin-releasing hormone (TRH), oxytocin and thyrotropin-releasing hormone (VIP), but depending on the physiological situation, vasopressin, angiotensin 2, neuropeptide Y (NPY), galanin and neurotensin can also trigger prolactin secretion (41).

In the context of physiological stress AVP and peptide histidine isoleucine are co-released with CRH from parvicellular PVH neurons and may be specifically important for prolactin secretion (43). Stress stimuli have a powerful effect on prolactin secretion, but with its little metabolic activity, the significance of prolactin's increase is uncertain (1).

### 1.2.6. Responses of other pituitary hormones

As mentioned above, a marked increase of pituitary hormones after surgery has been noted in the context of stress responses following tissue injury. Plasma levels of other anterior pituitary hormones, namely thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are not altered significantly (1).

Of note is a decreasing concentration of thyroid hormones (triiodothyronine (T3) and thyroxine (T4) following stress stimuli (44). This change can be explained by interactions between the thyroid and HPA axes. It is hypothesized that glucocorticoids suppress the thyroid axis via direct and indirect actions in the brain, as the TRH gene encompasses a glucocorticoid response element, along with TRH neurons exhibiting glucocorticoid receptors (45,46).

### 1.3. Immunological and hematological changes

Tissue injury leads to increased circulating cytokines, including interleukins and interferons, with substantial role in mediating immunity and inflammation (47). Following cellular injury, neutrophils and macrophages release proinflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and several other interleukins, of which interleukin-1 (IL-1), and interleukin-6 (IL-6) are specifically notable in inducing the systemic response (48). Increased circulatory levels of these substances induces the production of acute phase proteins (C-reactive protein (CRP), ferritin, transferrin, albumin and fibrinogen) by the liver (21). Watt *et al*. have showed that the level of acute phase reactants correlates with the severity of the stress response (49). Metabolic cytokine production is proportional to degree of tissue damage and remain elevated for 48-72 h postoperatively (1). An increase in leukocyte infiltration to area of tissue injury as well an elevated circulating levels of dendritic cells can be observed too (50).

### 1.4. Stress response to cardiac surgery

The surgical stress response results from direct and indirect manipulation of cardiac surgery on tissue. Its direct effects are attributable to cellular injury (21). Greater disruption leads to augmented inflammatory mediators and cytokine release, that positively reinforces the resultant response to stress (51).

Indirect damage ensues from multiple components, such as perturbations in perfusion pressures, loss of blood and anesthetics affecting oxygen delivery to tissues. Resultant secondary cellular-and organ malfunction leads to the development of systemic inflammatory response syndrome (52). Chernow *et al*. demonstrated that hormonal responses reflect the degree of surgical stress. Oppositely to major cardiac surgery, in response to minimal surgical stress, hormonal responses are negligible (53). According to Kawahito *et al.,* the type of cardiac surgery procedures differs in regard to cytokine response, with valve replacement surgery resulting in higher circulating levels of IL-6 and monocyte chemotactic and activating factors, in comparison with coronary artery bypass graft (CABG) surgery (54). Furthermore, variations between surgery with or without cardiopulmonary bypass (CPB) for coronary artery grafting exist, as the former is associated with lower IL-8 levels, as compared to the use of CPB (55).

For instance, CABG surgery is conducted for coronary revascularization for patients with obstruction of the left main coronary artery or for patients with three-vessel disease, which cannot effectively be treated by percutaneous coronary intervention (PCI). Isolated CABG surgery can be performed using cardiopulmonary bypass surgery, referred to as “on-pump” or executed on the “beating-heart”, described as “off-pump” surgery. Both procedures require a

median sternotomy, which is innately an invasive procedure (56). During CABG with CPB, pathological processes are amplified, which leads to the proposal of some clinicians, that off-pump coronary artery bypass (OPCAB) surgery is affiliated with risk reduction and complication, resulting in CABG operations being increasingly applied without CPB (57,58). Comparing outcomes between on- and off-pump surgeries in patients undergoing CABG surgery is beyond the scope of this research and therefore solely focuses on postoperative levels of cortisol between different surgical procedures, namely CABG, valvular surgery and combined CABG and valvular surgery, with and without the use of CPB.

### 1.4.1. Cardiopulmonary bypass

#### 1.4.1.1. History

The development of extracorporeal perfusion introduced by John H. Gibbson lead to immense advances in the field of cardiac surgery, making complex intracardiac procedures possible (59). The cardiopulmonary bypass machine was first used clinically in 1953 for repair of an atrial septal defect in an 18-year-old women (60). It resulted in conducting heart surgeries on a bloodless operating field, while preserving perfusion of heart and other organs (61).

#### 1.4.1.2. Technique

The CPB conduit consist of venous and arterial cannula, venous reservoir, pump, oxygenator and filter (Figure 2) (61). Anticoagulation with heparin (1.5 mg/kg) is required to reach an activated clotting time (ACT) of greater than 280 s, with normal levels being between 105 to 167 sec (62). The cannulas connect the patient to the CPB machine using polyvinylchloride (PVC) and wires preventing kinking of the material. Most commonly the proximal part of ascending thoracic aorta is used for arterial inflow, whereas the venous cannula is placed in the right atrial appendages. For venous return, either a single cannula or a dual system is used into the superior and inferior vena cava, respectively (61). Cardiopulmonary bypass is commenced, propelling blood from the heart into the venous reservoir. Mechanical ventilation is no longer required, as hemoglobin saturation is made possible via membrane oxygenators (59).

With the use of a heat exchanger, the blood is cooled between 28 to 32°C resulting in decreased tissue oxygen consumption, pump flow and tissue injury (63). For each 1°C decrease in temperature, cardiac output declines by 7 % (59). Currently, the use of hypothermia during CPB is under controversy, despite its presumed organ protective effects (64). It reversibly inhibits clotting factors and platelets adding to the already anticoagulative effects of heparin.

In addition, several studies have shown greater importance of the rate of rewarming and cerebral hyperthermia to prevent cerebral injury, rather than the actual absolute temperature used (65–67). It has been observed that normothermic cardiopulmonary bypass results in less cytokine and adhesion molecules than hypothermic CPB (68). However, it is uncertain if increased levels in the latter case solely reflects prolonged CPB time (69).

When the cardiac procedure is completed, the patient is rewarmed and weaned off CPB. During this process, ventricular fibrillation occurs frequently, requiring defibrillation. Protamine, in equivalent dose to heparin, is needed to reverse heparins anticoagulation to achieve hemostasis (61).

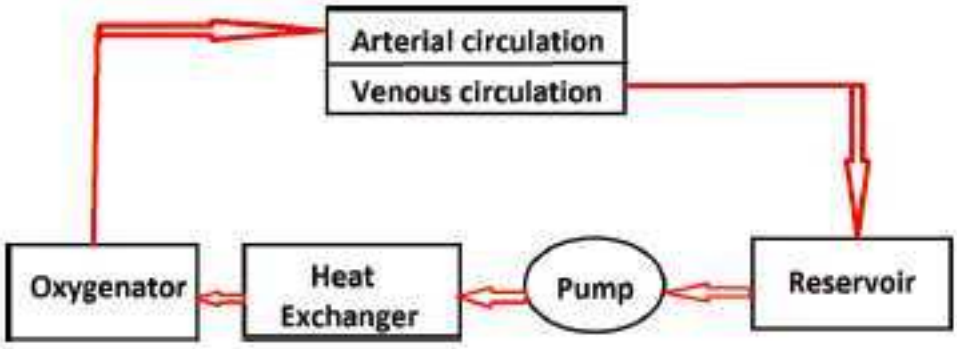


**Figure 2**. Cardiopulmonary bypass circuit. [Retrieved from Sarkar *et al*.] (59)
During CPB, venous blood is drained through gravity into a reservoir. The pump moves blood from the reservoir to the oxygenator through a heat exchanger, before returning it to the arterial circulation. Additional components include suckers (to remove blood from surgical field), vents (to decompress the heart), hemofilters (for ultrafiltration) and cardioplegia system.

### 1.4.1.3. Myocardial protection

The word cardioplegia is derived from the Greek word of cardio meaning "heart", and plegia "paralysis". A cardioplegic solution is one that results in electromechanical arrest of the heart, allowing the myocardium to be protected from cell death and for a motionless operating field for intracardiac repair (63). Potassium is the main electrolyte causing cardiac arrest, with circa 20 mmol $L^{-1}$ causing a reduction in myocardial membrane potential (70). Among institutions and surgeons, cardioplegic solution and infusion technique can vary substantially. Ongoing debate with respect to infusion method of antegrade versus retrograde, temperature, solution components (crystalloid or blood based) and etcetera are continuing (61). Most commonly a crystalloid solution in combination with autologous blood from the CPB system

is used (71). Infusion of cardioplegia solution into the aortic root and cross-clamping of the ascending aorta is the process of anterograde cardioplegia, while retrograde cardioplegia is delivered into the coronary sinus (63).

#### 1.4.1.4. Adverse effects

Despite ever occurring advances during surgery and CPB modification, it is not without its share of detrimental effects on various physiological processes, resulting in disordered hemostasis, enhanced inflammatory response, as well as end organ function (61).

Arterial cannulation poses a risk of bleeding, plaque dislodgement and dissection. Other mechanical complications can arise from any malfunction of the several devices making up the CPB machinery (59).

Systemic complications are related to the contact of blood with artificial surfaces in the bypass conduit, hypothermia and laminar blood flow (72). Nonpulsatile blood flow results in higher plasma concentration of renin, angiotensin II and aldosterone, potentiating the neurohumoral endocrine response (73). Inflammatory, coagulation, complement and fibrinolytic cascades are activated, leading to the formation of thrombin, which is responsible for converting fibrinogen to fibrin. It also causes qualitative and quantitative thrombocyte dysfunction (59).

Moreover, CPB interferes with the response of the immune system. The classic and the alternative complement pathways are activated and platelets and neutrophils stimulation releases acute inflammatory mediators and cytokine production, persisting after CPB procedure (73,74).

Rewarming adds to the release of inflammatory mediators and causes stress response, besides the surgical trauma itself that leads to complex inflammatory response after major surgery (75,76).

High concentration of unfractionated heparin during extracorporeal perfusion predisposes the patient to the development of heparin-induced thrombocytopenia (HIT) with occurrence rate of 1% to 5% (77). Heparin-induced thrombocytopenia is an immune mediated adverse drug reaction caused by the production of antibodies that activate platelets (78). Despite thrombocytopenia, bleeding is rare, whereas thrombosis is a feared situation as it is associated with a mortality rate of approximately 20 to 30% (79).

Cerebral, renal and mesenteric circulation are particular affected during CPB procedures, due to a combined effect of hypoperfusion, embolization and cytotoxic pathways of the immune response. They may present as neurocognitive deficits, respiratory failure and

injury to the renal parenchymal (80). Cerebral injury can manifest in a spectrum from postoperative cognitive dysfunction to cerebrovascular insult (59).

### 1.5. Stress response under anesthesia

Hormonal changes during sedation and general anesthesia are elements of the stress response, induced by stimuli from anxiety, pain, surgery, acids-base aberrations, hypoxia, hypothermia and overall circulatory instability (81).

Anesthesia influences the body response by afferent inhibition (local anesthesia), central modulation (general anesthesia) or by peripheral action within endocrinological systems (etomidate) (82). The choice of anesthetic technique may limit activation and influence the amplitude of the surgical stress response by use of regional anesthesia, deep general anesthesia, or pharmacological blockage of the sympathetic system (2).

Nociceptive stimuli from tissue injury is associated with a systemic neuroendocrine stress response, that is being proportional to the intensity of pain. The goal of anesthetic intervention must therefore not only provide ideal management during surgical intervention but also provide an anesthetic plan for the postoperative period, minimizing adverse effects of systemic responses to pain, contributing to improvements in morbidity and mortality (83).

#### 1.5.1. General anesthesia

Large doses of opioids inhibit the release of hormones involved in the endocrine stress response to surgery. McDonald *et al.* reported the suppression of the hypothalamic and pituitary hormone secretion with therapeutic doses of morphine (84). The effects of opioids have also been documented during cardiac surgery. Fentanyl is more effective than morphine in modifying neuroendocrine responses, although its efficacy is dose dependent (85). Large doses of fentanyl and sufentanil inhibit the release of pituitary hormones until the introduction of CPB. After initiation of CPB, the impact of stressors is so profound that hypothalamic responses cannot be absolutely inhibited by opioids (86). It remains unproven, if greatly attenuating the stress response with opioids have clinical benefits regarding outcome. Contrarily, side effects from large doses of opioids are readily apparent. High-dose opioids results in respiratory depression requiring ventilatory support for the patients in the postoperative period (83).

The short-acting intravenous agent etomidate is used for induction of general anesthesia. It reversiblly inhibits steroid 11-beta-hydroxlase encoded by the CYP11B1 gene, blocking the synthesis pathway for cortisol as well as aldosterone (87). One induction dose will suppress hormone production for 6-12 h, while infusion for 1-2 h will inhibit cortisol synthesis for up to

24 h (88,89). Across other studies, inhibitor effects of etomidate on cortisol production was not observed, owing to the fact, that inhibition of 11-beta-hydroxlase result in increased accumulation of 11-deoxycortisol, which cross-reacts with cortisol immunoassays, producing "falsely" elevated concentrations of cortisol (90).

Volatile anesthetics may be less effective than neuroleptic, spinal or epidural anesthesia techniques, in mitigating the stress response (82). Halothane and enflurane inhibit adrenal release of catecholamines (91).

### 1.5.2. Regional anesthesia

Regional anesthesia can reduce the stress response by modulating the pituitary-adrenocortical axis via sympathetic blockage in combination with afferent inhibition of central cord fibers (92). Neuraxial blockade of nociceptive stimuli have been shown to reduce the vast endocrinological, immunological and hematological responses to surgery. Compared to other anesthetic techniques, regional anesthesia has the most salutary effect on catabolism (83). To maximize its beneficial effect, neuraxial block should precede incision and continue 24-48 h postoperatively (93). In major thoracic interventions, thoracic epidural blockade with local anesthetic is a recommended method for enhancing speedy recovery, providing excellent analgesia, and promoting mobilization. In comparison with systemic opioids, thoracic epidural analgesia provides improved static and dynamic pain alleviation (83). Another study showed that regional, compared to general anesthesia, was associated with a 17% reduction in cortisol response over the first 24 h (94).

# 2. OBJECTIVES AND HYPOTHESES

## 2.1. Objectives

The aim of the current study was to evaluate the magnitude of cortisol response in patients undergoing elective cardiac surgery, with an additional focus on the impact of cardiopulmonary bypass on the cortisol response.

## 2.2. Hypotheses

1. Cardiac surgery will induce significant endogenous release of cortisol hormone.
2. Cardiac surgery with use of cardiopulmonary bypass will exhibit a more pronounced cortisol response compared to beating-heart surgery.

# 3. PATIENTS AND METHODS

### 3.1. Study Design and Participants

This prospective cohort study was performed at the University Hospital of Split in Croatia between March 2015 and June 2016. Ethical approval for this study (Ethical Committee N° 2181-147-01/06/J.B.-16-2) was provided by the Ethical Committee of the University Hospital of Split, Split, Croatia (Chairperson Prof J. Bagatin) on 10 March 2015. All patients provided written informed consent.

This study enrolled patients aged between 41 and 84 years who were scheduled for elective CABG, heart valve surgery, or a combined surgery (CABG and valve surgery) with or without CPB. Exclusion criteria were mental illness, adrenal gland disease; requiring steroid treatment for longer than 7 days in the past year; alcohol (> 20 g per day or > 150 g per week) or controlled substance abuse; and additional corticosteroid treatment throughout the study period.

### 3.2. Surgery and Anesthesia

For all patients, the procedure began at 08:00. Anesthesia was induced with fentanyl (5-7 μg $kg^{-1}$) (Janssen Pharmaceutica, Beerse, Belgium), midazolam (0.05-0.1 mg $kg^{-1}$) (F. Hoffman-La Roche Ltd., Basel, Switzerland), and vecuronium (0.1-0.2 mg $kg^{-1}$) (N.V. Organon, Oss, Netherlands). Anesthesia was subsequently maintained with fentanyl (10-20 μg $kg^{-1}$), midazolam (0.05-0.1 mg $kg^{-1}$), vecuronium (0.01-0.02 mg $kg^{-1}$), and 0.5-2.5% sevoflurane (Abbott Laboratories Ltd., Queenborough, U.K.) in a 50% O2/air mixture. The depth of anesthesia was titrated to achieve a bispectral index (Aspect Medical Systems Inc., Newton, MA, USA) between 40 and 55.

Norepinephrine (0.05-1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) (Sanofi-Aventis S.p.A., Scoppito, Italy) was used to maintain mean arterial pressure (MAP) above 70 mmHg during beating-heart surgery, during the pre-CPB and post-CPB periods. During CPB, MAP was maintained within the range of 60-80 mmHg. Packed red cells were added when necessary to maintain hematocrit above 22% during CPB and above 26% during the pre-CPB and post-CPB periods. Access to the heart was achieved via a median sternotomy. Heparin (1.5 mg $kg^{-1}$) (25,000 IU, Fisiopharma S.R.L., Salerno, Italy) was administered prior to cannulation to achieve an activated clotting time (ACT) greater than 280 sec, and the effect of heparin reversed with an equivalent dose of protamine sulfate (Meda Pharma GmbH, Wangen-Brüttisellen, Switzerland) at the time of decannulation. During beating-heart surgery, the same principles of heparinization and neutralization were applied. Myocardial protection was induced with cardioplegia via antegrade removal and retrograde reinfusion of cold blood (4:1 blood-crystalloid ratio) (Bichsel AG,

Interlaken, Switzerland). CPB was performed using a non-pulsatile roller pump (Terumo Europe N.V., Eschborn, Germany) equipped with microporous membrane oxygenators containing integrated 40-µM arterial line filters (Medtronic Inc., Minneapolis, MN, USA) and heparin-coated circuits (Carmeda, Medtronic Inc., Minneapolis, MN, USA). During CPB, the alpha-stat technique was used along with maintenance of normothermia (35.5-36.5°C) or spontaneous hypothermia (as low as 32°C). A constant perfusion flow rate of 2.4 $L \cdot min^{-1} \cdot m^{2-1}$ was applied. In patients undergoing surgery without CPB, distal anastomoses were performed with the help of an Octopus tissue stabilizer (Medtronic Inc., Minneapolis, MN, USA). Proximal anastomoses were fashioned onto the aorta by means of a single side-clamp. During beating-heart surgery, cervical esophageal probes were employed together with a forced warm air blanket (3M Center, St. Paul, MN, USA) to maintain the core temperature near normothermia (36-37ºC).

After surgery, all patients were transferred to the intensive care unit (ICU) without extubation and were placed on mechanical ventilation (Evita XL, Dräger, Lűbeck, Germany). Extubation and ICU discharge were decided by the attending intensivist.

### 3.3. Laboratory Measurements

Cortisol levels in the patients were determined one day before surgery (at 08:00), as well as on the 1st (at 08:00, 16:00 and 24:00), 3rd (at 08:00) and 5th (at 08:00) postoperative days. For each sample, 5 mL of venous blood was collected into a biochemistry tube with gel to determine the concentration of cortisol.

Serum was prepared via centrifugation (Z 400, Hermle Labortechnik GmbH, Wehingen, Germany) at 3,500 rpm for 15 minutes. Serum cortisol levels were assessed using an electrochemiluminescence “sandwich” immunoassay (ECLIA) method (Cobas e601, Roche Diagnostics GmbH, Mannheim, Germany). The intra-assay and inter-assay coefficients of variation were less than 2.8% and 1.7%, respectively. The normal cortisol range between 7:00 and 10:00 am is 171-536 nmol $L^{-1}$, between 4:00 and 6:00 pm is 64-327 nmol $L^{-1}$, and at 12:00 am is 41-69 nmol $L^{-1}$ in the laboratory where the measurement was performed (95).

### 3.4. Statistical Analysis

Data analysis was performed using IBM SPSS Statistics, version 24.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as numbers and percentages. Continuous variables were expressed as the means and standard deviations or medians and interquartile range (IQR). The t-test was used to estimate significant differences in the

perioperative cortisol levels between cardiac surgery with and without CPB. Also, a general linear model mixed-design (between- and within-subjects) analysis of variance (ANOVA) was used to determine any differences in the cortisol levels between patients with and without CPB, in repeated experimental conditions. Levene's test for checking the variance equality of the cortisol levels across samples was not significant. Pearson correlation coefficient was used to test the correlation between age and postoperative cortisol levels. Statistical values were considered significant at 95% (two-sided $P < 0.05$).

# 4. RESULTS

Among the eligible patients, 125 patients provided written informed consent, passed exclusion criteria and were enrolled in the study. Three patients died in the early postoperative period, resulting in an overall in-hospital mortality rate of 2.4%. Two patients died of heart failure, and one patient died of sepsis and multiple organ failure on the 2nd and 3rd postoperative day, respectively. Two patients (1.6%) experienced a stroke during the perioperative period. Ultimately, we conducted analyses on 120 patients. The baseline demographic, clinical, surgical and postoperative characteristics of the patients are presented in Table 3.

**Table 3.** Demographic, clinical, surgical and postoperative characteristics.

| | Patients (n = 120) |
|---|---|
| Demographic characteristics | |
| Age, mean (SD), yr | 64.0 ± 9.4 |
| Male sex | 96 (80.0%) |
| Weight, mean (SD), kg | 85.5 ± 12.4 |
| Height, mean (SD), cm | 176.1 ± 8.1 |
| Elementary education | 22 (18.3%) |
| Secondary education | 77 (64.2%) |
| Higher education | 21 (17.5%) |
| Active smoking | 35 (29.2%) |
| Clinical characteristics | |
| General anesthesia | 28 (23.3%) |
| Hypertension | 86 (71.7%) |
| Insulin-dependent diabetes mellitus | 7 (5.8%) |
| Non-insulin-dependent diabetes mellitus | 31 (25.8%) |
| Hyperlipidemia | 89 (74.2%) |
| Carotid artery disease | 70 (58.4%) |
| Peripheral vascular disease | 12 (10.0%) |
| Atrial fibrillation | 18 (15.0%) |
| Myocardial infarction | 51 (42.5%) |
| LVEF, mean (SD), % [a] | 62.0 ± 10.9 |
| euroSCORE, median (IQR) | 2.0 (1.2-3.4) |
| Surgical characteristics | |
| CABG | 79 (65.8%) |
| Heart valve surgery | 26 (21.7%) |
| CABG plus valve surgery | 15 (12.5%) |
| Surgery with CPB | 56 (46.7%) |
| CPB duration, mean (SD), min | 101.0 ± 33.8 |
| Cross-clamp duration, mean (SD), min | 65.4 ± 25.6 |
| Lowest BIS, mean (SD) | 30.8 ± 7.0 |
| Lowest temperature, mean (SD), ºC | 34.2 ± 2.5 |
| Lowest MAP, mean (SD), mmHg | 56.1 ± 9.1 |
| Lowest hematocrit, mean (SD), % | 28.3 ± 6.3 |
| Highest glucose level, mean (SD), mmol $L^{-1}$ | 9.5 ± 3.2 |

| | |
|---|---|
| Insulin administered | 6 (5.0%) |
| Vasopressor administered | 70 (58.3%) |
| Inotropic agent administered | 60 (50.0%) |
| Blood transfusion, mean (SD), mL | 540.6 ± 579.4 |
| SVRi, mean (SD), dyne·sec/cm$^5$·m$^2$ | 1515.7 ± 516.5 |
| Surgery duration, mean (SD), min | 223.5 ± 62.1 |
| Postoperative characteristics | |
| Drainage in the first 12 hours, mean (SD), mL | 522.0 ± 570.3 |
| Duration of m.v. in the ICU, mean (SD), h | 19.4 ± 11.4 |
| Failed weaning from m.v. in the ICU | 2 (1.7%) |
| Time to extubation, mean (SD), h | 20.5 ± 11.9 |
| Length of ICU stay, mean (SD), h | 57.2 ± 32.2 |
| Length of hospital stay, mean (SD), d | 11.1 ± 2.9 |

Data are presented as numbers (%), unless otherwise indicated.
Levels of education according to Ministry of Science, Education and Sports of the Republic of Croatia range from elementary to secondary and higher education. General anesthesia – in the past five years. SVRi–value at the end of the surgical procedure.
[a] Results of echocardiography (Simpson's method).
LVEF, left ventricular ejection fraction; euroSCORE, European system for cardiac operative risk evaluation; IQR, interquartile range; CABG, coronary artery bypass graft surgery; CPB, cardiopulmonary bypass; BIS, bispectral index; MAP, mean arterial pressure; SVRi, systemic vascular resistance index; m.v., mechanical ventilation; ICU, intensive care unit.

Perioperative levels of cortisol in the patients are presented in Table 4 and Figure 3. Postoperative cortisol levels were markedly beyond normal range in all measurements. We did not identify statistically significant differences in cortisol levels, regardless of the use of CPB, except on the third postoperative morning ($P = 0.003$). Although this difference is statistically significant when analyzed with a bivariate t-test, the repeated-measures general linear model ANOVA with a factor CPB showed that there was no significant impact of the cortisol level ($F = 2.02$, $P = 0.103$, Figure 3); thus, the result of the t-test is considered a false positive.

**Table 4.** Cortisol levels in the patients.

| | | Patients | *P* value |
|---|---|---|---|
| Baseline 08:00 | Cortisol overall | 438.4 ± 223.8 | 0.387 |
| | Cortisol on-pump | 419.4 ± 192.4 | |
| | Cortisol off-pump | 455.1 ± 248.4 | |
| POD 1 08:00 | Cortisol overall | 1124.2 ± 539.2 | 0.103 |
| | Cortisol on-pump | 1210.0 ± 633.0 | |
| | Cortisol off-pump | 1049.0 ± 432.5 | |
| POD 1 16:00 | Cortisol overall | 985.6 ± 490.6 | 0.782 |
| | Cortisol on-pump | 972.4 ± 472.0 | |
| | Cortisol off-pump | 997.4 ± 510.1 | |
| POD 1 24:00 | Cortisol overall | 914.7 ± 472.4 | 0.681 |
| | Cortisol on-pump | 933.8 ± 483.5 | |
| | Cortisol off-pump | 897.8 ± 465.6 | |
| POD 3 08:00 | Cortisol overall | 1046.2 ± 389.7 | 0.003 |
| | Cortisol on-pump | 1155.8 ± 431.3 | |
| | Cortisol off-pump | 948.7 ± 321.8 | |
| POD 5 08:00 | Cortisol overall | 819.2 ± 236.2 | 0.958 |
| | Cortisol on-pump | 817.9 ± 239.9 | |
| | Cortisol off-pump | 820.2 ± 234.8 | |

Data are shown as the means (cortisol-nmol $L^{-1}$) with SD.
The cortisol levels were determined one day before surgery (at 08:00), on POD 1 (at 08:00, 16:00, and 24:00), POD 3 (at 08:00), and POD 5 (at 08:00).
POD, postoperative day.

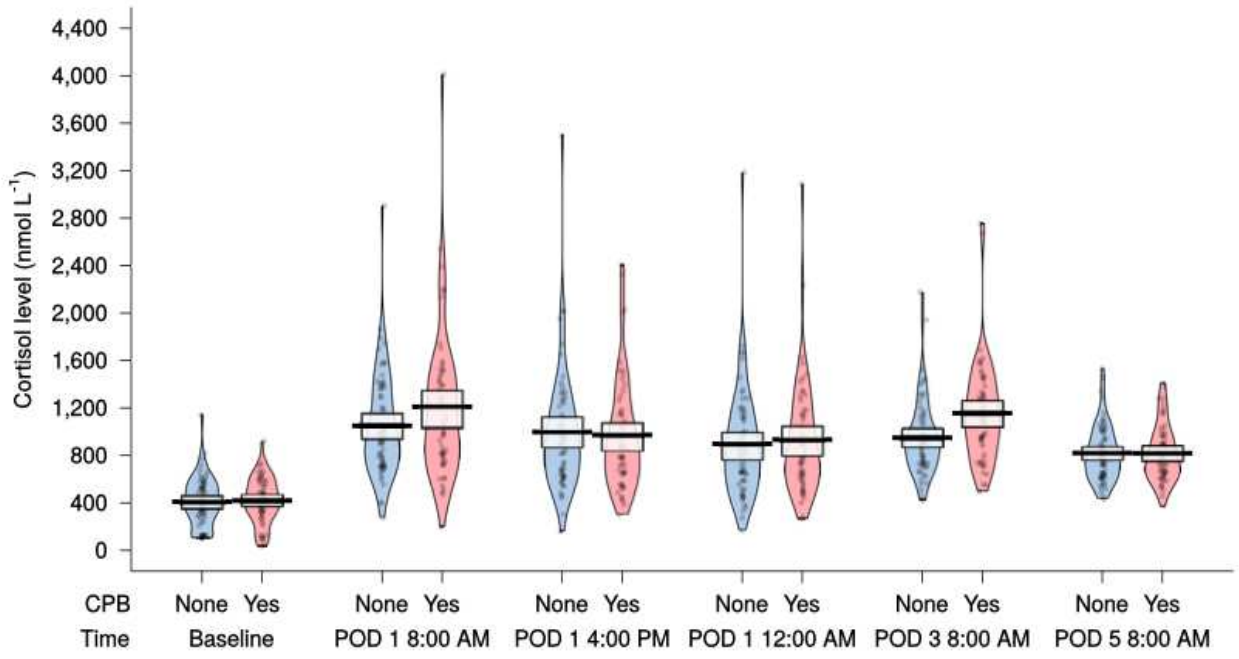


**Figure 3.** Repeated measures of the cortisol levels according to the surgical technique.
General linear model ANOVA showed no statistically significant difference in the cortisol levels between cardiac surgery with *(red)* and without CPB (*blue)* at six perioperative time points (F = 2.02, $P = 0.103$).
CPB, cardiopulmonary bypass; POD, postoperative day.

We also demonstrated a significant correlation between old age and cortisol levels on the 1st (at 12:00 am; Pearson correlation coefficient = 0.208, $P = 0.023$) and 5th (Pearson correlation coefficient = 0.183, $P = 0.045$) postoperative days. On the 3rd postoperative morning, the correlation was almost statistically significant (Pearson correlation coefficient = 0.171, $P = 0.063$).

# 5. DISCUSSION

The current prospective cohort study including 125 cardiac surgery patients has revealed that cardiac surgery stimulates a prolonged and extremely pronounced cortisol response and severely disrupting the circadian rhythm regardless of the applied surgical technique.

Cortisol, which is the end-product of the HPA axis, acts as a key regulator of inflammation and metabolic activity and a primary agent of the neuroendocrine stress response. In the unstressed subject, cortisol secretion levels follow a typical circadian rhythm, sharply increasing within 1 hour after waking and steadily declining thereafter, reaching a nadir in the late evening hours (96). In contrast, surgery stimulates the endocrine response, and cortisol levels may increase greater than 1,500 nmol $L^{-1}$, with phase shifting of the physiological rhythm depending on the degree of surgical stress (1,53). Nonetheless, our study revealed that cardiac surgery stimulates a prolonged and severe cortisol response with complete disrupted circadian rhythm. Notably, we noted a thirteen-fold increase in cortisol level on the 1st (at 24:00) postoperative day, compared with the normal range.

Furthermore, we did not observe significant differences in postoperative cortisol levels regardless of the use of CPB. Compared with the controlled systemic flow conditions of CPB, cardiac manipulation during beating-heart surgery to expose target coronary arteries can lead to significant hemodynamic impairment, with transient reductions in cardiac output despite relative preservation of the mean arterial pressure. Thus, in terms of stress hormone response, the benefits conferred by avoiding CPB might be negated by the cumulative hemodynamic stress of beating-heart surgery (97).

Finally, a significant association between old age and cortisol levels on the 1st and 5th postoperative day was demonstrated in this study, which can be explained by the gradual loss of corticosteroid receptors in the hypothalamus during the process of aging. These results support the recent findings of reduction of the negative feedback mechanism, with an inappropriate secretion of corticosteroids, in the elderly population (98). Moreover, additional contributing factors to older participants having higher perioperative cortisol responses may be; multiple comorbidities, lower preoperative health patient status as well as a higher risk of postoperative complications.

A strength of our study is that, we used anesthetic gases, which only mildly suppress the cortisol response to surgery, with avoidance of total intravenous anesthesia with propofol, which significantly blunt the cortisol response (4). Also, we did not use etomidate, an anesthetic agent that may reversibly inhibit 11-hydroxylase enzyme and subsequently decrease cortisol secretion from the adrenal gland (99).

The current study has several limitations. We measured only cortisol hormone, while other markers of stress such as catecholamines, growth hormone, prolactin, vasopressin, thyroid hormones, etc. may have yielded more useful insights in the mechanism of stress response prompted by cardiac surgery. Next, we did not investigate the possible association between heightened cortisol levels and morbidity as well as mortality in enrolled subjects. Finally, differences in baseline demographics, and clinical and surgical characteristics of patients, represent another possible limitation of this study.

# 6. CONCLUSION

In conclusion, this prospective cohort study with a longitudinal assessment of perioperative cortisol levels showed a significant cortisol response with an altered circadian pattern in cardiac surgical patients. However, it seems that use of CPB does not enhance the cortisol response compared to beating-heart surgery. Finally, we revealed a positive correlation between old age and postoperative cortisol levels. Because of the certain limitations of our study, we believe that this topic merits further research.

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# 8. SUMMARY

**Objectives:** The aim of the current study was to investigate the magnitude of stress response in patients undergoing cardiac surgery, with an additional focus on the possible variations of stress response between cardiac surgery with and without CPB.

**Patients and methods:** This study enrolled 125 patients, aged between 41 and 84 years, who were scheduled for elective CABG, heart valve surgery or combined surgery, with or without CPB. Excluded from the study were patients with mental illness; adrenal gland disease; requiring steroid treatment for longer than 7 days in the past year; alcohol (> 20 g per day or > 150 g per week) or controlled substance abuse; and additionally, corticosteroid treatment throughout the study period. Patient serum cortisol levels were determined 1 day before surgery (at 08:00) and on the 1$^{st}$ (at 08:00, 16:00 and 24:00), 3$^{rd}$ (at 08:00), and 5$^{th}$ (at 08:00) postoperative days.

**Results:** Postoperative cortisol levels were markedly beyond normal range in all measurements. However, a significant difference in the postoperative cortisol levels between cardiac surgery with and without CPB was not observed. Significant positive correlation was found between old age and cortisol levels on the 1$^{st}$ (at 24:00; Pearson correlation coefficient = 0.208, $P$ = 0.023) and 5$^{th}$ (Pearson correlation coefficient = 0.183, $P$ = 0.045) postoperative days.

**Conclusion:** Cardiac surgery induced a significant cortisol response with completely disrupted circadian rhythm in patients. The use of CPB during cardiac surgery had no effect on the level of cortisol response. Old age was associated with a more severe degree of the surgical stress response.

# 9. CROATIAN SUMMARY

**Ciljevi:** Cilj istraživanja je bio ispitati stupanj stresnog odgovora kod bolesnika nakon kardiokirurškog zahvata, s dodatnim osvrtom na moguće razlike u stupnju stresnog odgovora ovisno u uporabi izvantjelesnog krvotoka tijekom zahvata.

**Ispitanici i postupci:** Istraživanje je uključilo 125 bolesnika, dobi između 41 i 84 godine, predviđenih za elektivni zahvat aortokoronarnog premoštenja, operacije srčanih zalistaka i kombinirane operacije s ili bez uporabe izvantjelesnog krvotoka. Bolesnici nisu bili uključeni u istraživanje ukoliko su bolovali od psihijatrijske bolesti; bolesti nadbubrežne žlijezde, alkoholne (> 20 g dnevno ili > 150 g tjedno) ili druge ovisnosti; te ukoliko su bili pod kortikosteroidnom terapijom dulje od sedam dana u posljednjih godinu dana. Također bolesnici su se isključili iz istraživanja ukoliko su liječeni kortikosteroidima tijekom trajanja studije. Vrijednost kortizola kod svih bolesnika određena je jutro pred operaciju (u 8:00 sati), prvi poslijeoperacijski dan u tri vremenske točke (u 8:00, 16:00 i 24:00 sata), treće i peto poslijeoperacijsko jutro (u 8:00 sati).

**Rezultati:** Poslijeoperacijske vrijednosti kortizola u svim mjerenjima značajno su povišene u usporedbi s referentnim vrijednostima. Nije uočena značajna razlika poslijeoperacijskih vrijednosti kortizola između kardiokirurškog zahvata s ili bez uporabe izvantjelesnog krvotoka. Utvrđena je značajno pozitivna korelacija između starije dobi i vrijednosti kortizola prvi poslijeoperacijski dan (u 24:00 sata, Pearson koeficijent korelacije = 0.208, $P = 0.023$) i peto poslijeoperacijsko jutro (Pearson koeficijent korelacije = 0.183, $P = 0.045$).

**Zaključak:** Kardiokirurški zahvat je uzrokovao značajan stresan odgovor organizma i u potpunosti poremetio cirkadijalni ritam kortizola kod bolesnika. Uporaba izvantjelesnog krvotoka nije pokazala značajan utjecaj na poslijeoperacijske vrijednosti kortizola. Starija dob je bila značajno povezana s izraženijim stupnjem stresnog odgovora izazvanog kardiokirurškim zahvatom.

# 10. CURRICULUM VITAE

**Personal information:**

| | |
|---|---|
| Name: | Lara Sophia Vötgen |
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| 08/2010 – 06/2013 | Geschwister-Scholl-Schule Melsungen, Germany |
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**Work Experience:**

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| | |
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