EFFECTS OF GENERAL ANAESTHESIA AND INTRAVENOUS FLUID THERAPY ON RENAL BIOMARKERS IN CATS UNDERGOING OVARIOHYSTERECTOMY

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I would like to dedicate this thesis to my father,

Valsan Madhavan Vadakkath,

my source of positivity.

ABSTRACT

Traditional screening tests used to evaluate renal function have been demonstrated to be insensitive in detecting early kidney damage, such as subclinical acute kidney injury. It is probable that general anaesthesia and routinely performed surgical procedures could cause subclinical changes in the renal structure, predisposing the animal to subsequent functional impairment. However, these active changes might go undetected while screening using traditional renal markers, such as serum creatinine (SCr) and blood urea nitrogen (BUN). Novel urinary biomarkers, that indicate renal injury earlier than conventional markers, have been extensively studied in humans during the perioperative period. A feline model of mild acute kidney injury, potentially induced through general anaesthesia during routine surgeries, may prove useful in testing novel renal biomarkers and providing insight into the effects of anaesthesia on kidneys.

A randomised controlled trial was performed using 60 healthy cats presented to the Massey University Veterinary Teaching Hospital for routine ovariohysterectomy. Blood and urine samples were collected immediately before (0 h), and after (24 and 48 h) anaesthesia and spay surgery. Traditional renal marker levels (SCr, BUN) were measured from the serum samples. Commercial assays were used to detect the levels of novel markers, such as N-acetyl-β-D glucosaminidase (NAG) enzyme, Neutrophil Gelatinase-associated Lipocalin (NGAL), Retinol Binding Protein (RBP) and Kidney Injury Molecule-1 (KIM-1), in the urine samples. This study aimed to use these urinary markers to investigate the effects of general anaesthesia and intraoperative fluid therapy on feline renal structure.

Statistical tests such as t-test and ANOVA were conducted to establish differences in renal marker values between the time points and between treatment groups. Upon comparing the changes in renal marker concentrations, the study found no measurable evidence of structural or functional kidney damage in the cats. This is plausible since the vital parameters, such as arterial blood pressure and oxygenation levels, of the study cats were maintained within or near the borderline physiological range throughout the surgical procedure, resulting in the apparent absence of assessable kidney damage postoperatively. It is inferred that a more severe form of renal injury would be required to test the sensitivity of these novel renal markers in cats.

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Abbreviations

AA: Afferent arteriole
AKI: Acute kidney injury
AM: Acepromazine-Morphine-Alfaxalone
ANOVA: Analysis of variance
ARF: Acute renal failure
ASA: American Society of anesthesiologists
BP: Blood pressure
BUN: Blood urea nitrogen
C: Control
CIM-1: Cochlear injury molecule-1
CKD: Chronic kidney disease
Cl^-: Chloride ion
DAP: Diastolic arterial pressure
DCT: Distal convoluted tubule
DKT: Dexmedetomidine-Ketamine-Butorphanol
EA: Efferent arteriole
ECF: Extra-cellular fluid
ELISA: Enzyme-linked immunoassay
ET: Endotracheal tube
$ETCO_2$: End-tidal carbon dioxide
FiO_2: Fraction of Inspired Oxygen
GFB: Glomerular filtration barrier
GFR: Glomerular filtration rate
HAVCR-1: Hepatitis A virus-cell receptor-1
HMW: High molecular weight
HR: Heart rate
IM: Intra-muscular
IMW: Intermediate molecular weight
IRIS: International renal interest society
IV: Intravenous
JGA: Juxta-glomerular apparatus
JGC: Juxta-glomerular cells
KD: Kidney disease
KIM-1 OD: Kim-1 Optical density
KIM-1: Kidney injury molecule-1
l-MAP: Lowest MAP
l-DAP: Lowest DAP
l-SAP: Lowest SAP
l-SpO_2: Lowest SpO_2
l-Temp: Lowest temperature
LFA: Lateral flow assay
LMW: Low molecular weight
m- SpO_2: Mean SpO_2
MAP: Mean arterial pressure
m-DAP: Mean-DAP
m-MAP: Mean MAP
MMP-9: Matrix metalloproteinase-9
m-SAP: Mean SAP
m-Temp: Mean temperature
n: Number
Na^+: Sodium-ion
NAG: N-Acetyl β- D- Glucosaminidase
NGAL: Neutrophil Gelatinase-associated Lipocalin
OHE: Ovariohysterectomy
P.E: Physical examination
PCT: Proximal convoluted tubule
RBF: Renal blood flow
RBP: Retinol Binding Protein
RR: Respiratory rate
S3: Third segment of PCT
SA-HRP: Streptavidin-horse radish peroxidase
SAP: Systolic arterial pressure
SCr: Serum Creatinine
SDMA: Symmetric dimethyl-arginine
sP: Serum phosphorus
SpO_2: Oxygen saturation
T.P: Treatment protocol
T: Test
Temp: Temperature
TID: ter die sumendum
TIM-1: T-cell immunoglobulin-1
UCr: Urine creatinine
UNCR: Urine NGAL to creatinine ratio
uNGAL: Urinary NGAL
UP:C :- Urine protein creatinine ratio
UPC: Urine protein concentration
UPCR: Urine protein to creatinine ratio
uRBP: Urinary RBP
uRBP:Cr :- uRBP to UCr ratio
USG: Urine specific gravity
UTP: Urine total protein

1 INTRODUCTION

Kidney dysfunction is a common finding in cats, especially in those aged over 12 years, with a significant number of cases being detected only when the animals have advanced stages of renal damage. Kidney disease (KD) is associated with a reduced quality of life, poor prognosis, and survivability once it becomes clinically apparent. In diagnosing renal damage, clinicians are sometimes limited by the absence of overt clinical signs delaying its detection until the damage becomes irreversible. The loss of function is masked by the compensatory action of the kidneys and nephrons (Lefebvre et al., 2015). Thus, the clinical signs of KD primarily manifest once the kidneys are incapable of compensating for the damaged renal mass.

Our current tests for kidney dysfunction rely on identifying elevated levels of specific markers in serum (e.g., serum creatinine, blood urea nitrogen). However, these traditional markers are not produced by the kidney, but rather filtered from the blood by the kidneys and excreted from the body. Thus, increased levels of these markers in the serum are correlated with a loss of kidney function as they accumulate in the blood due to improper filtration (Segev, 2018a). These markers are imperfect tools for diagnosing early renal damage, where we would expect to find evidence of structural damage occurring long before the loss of renal function.

Another limitation with the current panel of markers is that they are primarily produced extra-renally (Hokamp & Nabity, 2016). Therefore, their increase and decrease cannot be solely attributed to kidney damage. They can be influenced by factors not concerning the kidneys, such as diet, exercise, and pathology in other organ systems. This makes it difficult to interpret the significance of slight fluctuations in traditional renal marker values over a short period of time.

The inability to determine the effect of onset factors on renal function and structure, due to the insensitivity of our current diagnostic tools, is a major concern with renal studies. The causes mentioned above, including the delayed presentation with seemingly normal biochemical findings, make it nearly impossible to identify the inciting cause of kidney damage (Syme, 2019). Furthermore, it is currently believed

that the cause for KD is multifactorial, with the interplay of several inciters leading to the progression of kidney damage, as evidenced by a large proportion of clinical cases having no known cause for KD (Dunn et al., 1980; Jepson, 2016). While these shortcomings are receiving critical attention, more research is needed to understand and, thereafter optimize the use of these markers in studying the causation of KD.

There is a growing recognition of probable links causing the increasingly common occurrence of KD in the later years of a cat's life (Jepson, 2016). However, it is often difficult to determine an aetiological agent because mild kidney injury is subtle in its approach, with the cat showing no apparent clinical signs of KD. Furthermore, the traditional markers of renal dysfunction are inadequate tools for detecting mild kidney injury. It is necessary to conduct more studies to improve and develop the diagnostic panel in order to identify slight changes in kidney function and structure.

Identifying new biomarkers that can provide an immediate and accurate assessment ('snapshot') of the kidneys has become increasingly important. As humans and animals share many similarities, the novel markers used for the former are now being applied to animals to study their potential. There is a growing body of literature that recognizes the importance of developing these markers. There is evidence to suggest that these markers are proportional to renal structural damage rather than function and are, thus, more sensitive to renal damage (Vaidya, Ferguson, & Bonventre, 2008). Their rapid increase in response to injury will aid in quick diagnosis and reduce the incidence of higher grades of KD, by allowing for targeted therapy earlier in the course of the disease.

We have witnessed a dramatic shift in the use of general anaesthesia and elective surgical procedures in companion animals over the past century. It is well known that both of these factors can cause transient changes in the physiological state of animals (Grauer, 1996; Senior, 2017). There is still much uncertainty about whether these factors can affect renal structure, by creating an environment unfavourable for the renal tissue, especially in cats. Studies to identify the changes wrought by potential risk factors are made more challenging, with the increasing reliance on alternative study designs to animal testing, due to ethical concerns. Clinicians must take measures to minimize the risk of renal injury,

for example, by using anaesthetic agents that have been shown to have limited adverse effects on the kidneys. The increased use of general anaesthesia in recent years has raised the question of whether general anaesthesia is associated with the increased prevalence of renal dysfunction in cats as anaesthetics may affect renal perfusion through their cardio-depressant effects.

This study investigates the effects of general anaesthesia and intraoperative fluid administration on renal markers in cats and, thus, its effects on the kidney. We do so by determining the changes in serum and urine markers, after routine ovariohysterectomy, in those cats admitted to the teaching hospital. The use of novel and traditional markers will provide information on both structure and function of the kidneys after exposure. This will also help us compare the markers to validate the sensitivity of novel markers in cats, as reported in other studies. The following review covers the physiology and pathology of kidneys in cats, potential risk factors (specifically anaesthetic agents and fluids), and various renal markers used to detect renal dysfunction.

2 REVIEW

2.1 OVERVIEW OF RENAL PHYSIOLOGY

2.1.1 Renal Architecture

The ureotelic excretory system in mammals comprises the kidneys, ureters, urinary bladder and urethra. Normal kidney function is paramount for maintaining the blood volume, blood pressure (BP) and osmolarity for physiological homeostasis. The mammalian kidneys are paired organs found in the retroperitoneal space of the abdominal cavity (Aspinall, 2004), i.e., between the parietal layer of the peritoneum and the roof of the abdomen. The bean-shaped kidneys in cats are smooth and mobile (Sjaastad et al., 2016), with large capsular veins (Alpern et al., 2013, p. 596). Macroscopically, the kidneys are divided into the highly vascular outer cortex and the well-developed inner medulla, as shown in Figure 1. The medulla

consists of the renal pelvis (crest-type) and the renal sinus (Schmidt-Nielsen, 1987). The vascular, lymphatic and nervous branches enter the kidneys through the renal hilus, which opens into the renal sinus (Hall, 1983; Sjaastad et al., 2016).

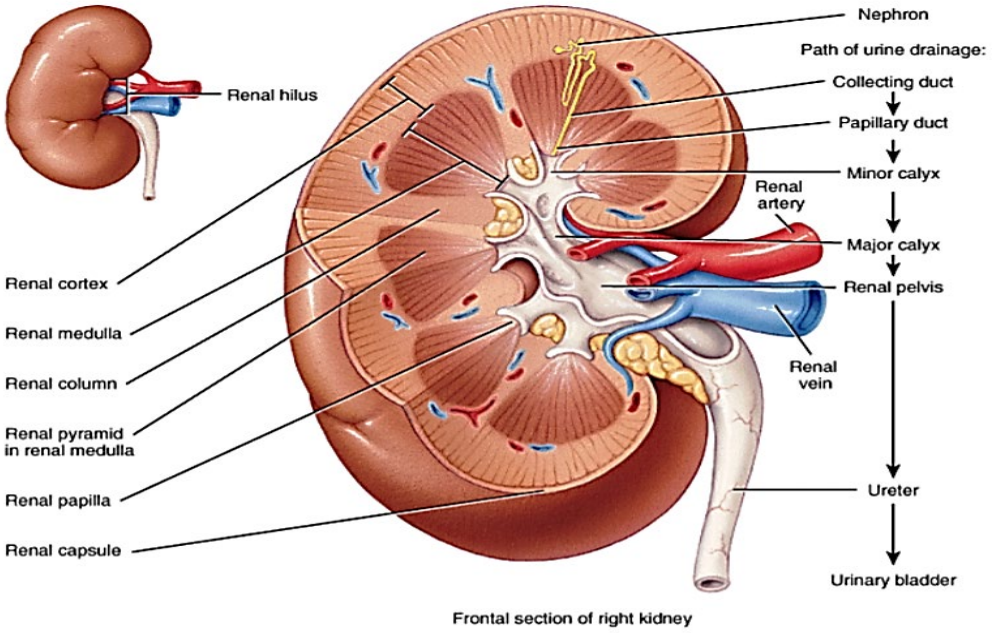


Figure 1 Kidney cross-section.

Diagrammatic representation of internal kidney structures within the renal cortex and medulla. The placement of a nephron (light yellow) has been depicted along with the path of urine drainage. Image reprinted from Maurya et al. (2018).

The nephron (*nephronum*) is the functional unit of the kidney. It consists of two main elements, i.e., the renal corpuscle (Malpighian corpuscle/*glomerulus corpusculi renalis*) and the tubular element (Aspinall, 2004), as seen in Figure 2 (A). The kidney contains around 1,90,000-2,00,000 juxtamedullary nephrons in cats (Dukes & Reece, 2004, p. 74). These nephrons are a type of mammalian nephron characterized by long tubular loops that dip into the medullary region of the kidney (Chmielewski, 2003; Dukes & Reece, 2004; Sjaastad et al., 2016). These nephrons are known for their urine concentrating role, above par due to the pressure and osmotic gradient across the cortex as well as the medulla (Chmielewski, 2003). While these nephrons are generally believed to have long tubular components, some of these nephrons in cats do not invade the deeper sections of the medulla. In fact, they resemble the cortical nephrons found in other species (Seldin & Giebisch, 1985, p. 272).

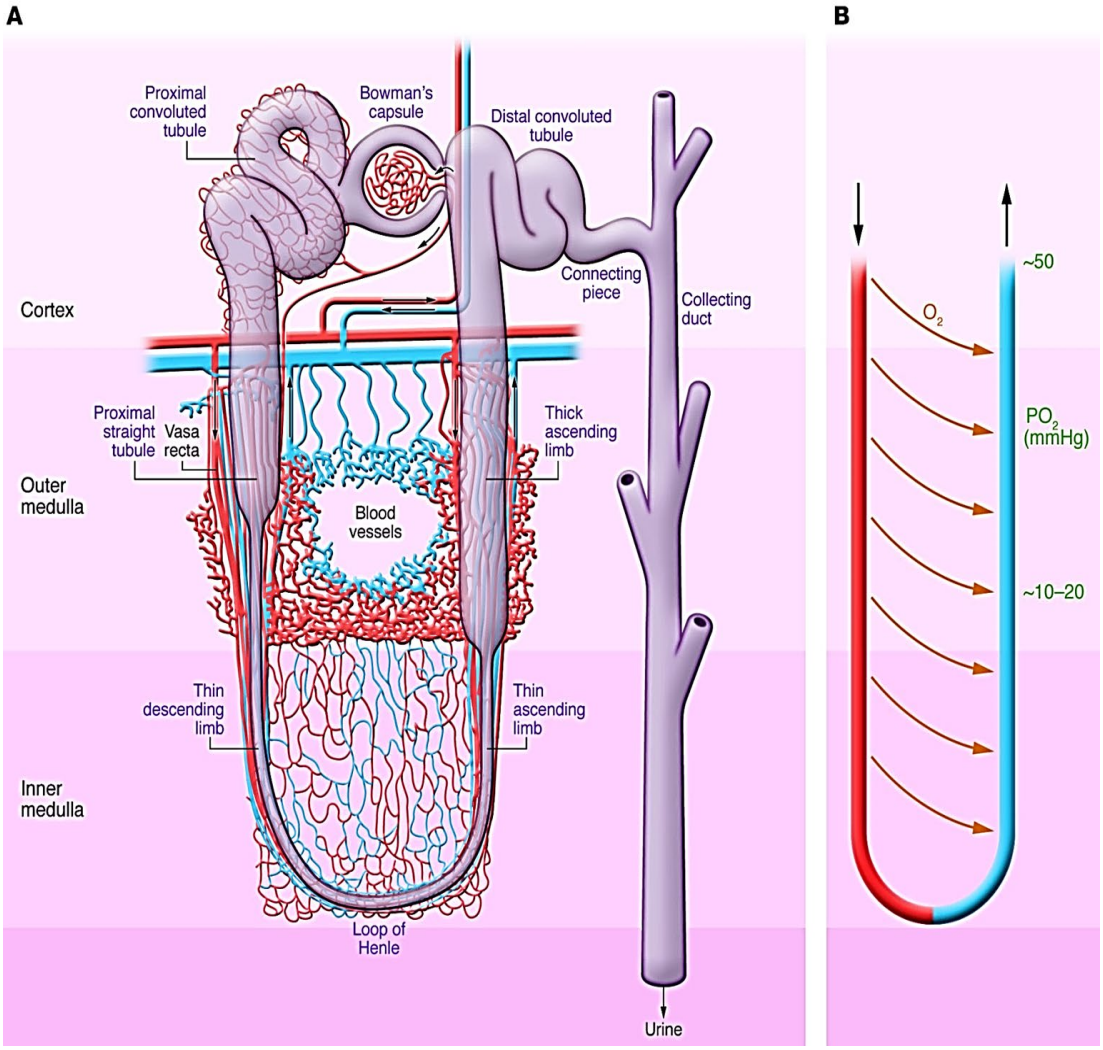


Figure 2 A) Parts of a nephron and B) oxygen gradient across the renal cortex, outer medulla, and inner medulla (Bonventre & Yang, 2011).

A) The nephron consists of the renal corpuscle (Bowman's capsule and glomerulus) and the tubular components: proximal convoluted tubule (convoluted and straight tubule), Henle's loop (descending, thin ascending, and thick ascending limb), distal convoluted tubule and collecting duct. The vasa recta are a network of arterioles which loop in and around Henle's loop and act as the main source of blood supply for the medullary structures. B) The countercurrent exchange of oxygen across the vasa recta network results in an oxygen gradient which maintains a near-hypoxic environment in the deeper layers of the kidney (medulla).

The glomerulus and Bowman's capsule, together, form the renal corpuscle. This structure serves as the primary site for fluid transfer, across the capillary bed and into the Bowman's space, a process regulated by the high-pressure environment found within the involved renal blood vessels (Dukes & Reece, 2004). The glomerulus is a mass of richly vascular,

interlinked capillary loop that dips into the Bowman's capsule (Chmielewski, 2003; Dukes & Reece, 2004). This vascular loop is an extension of the afferent arteriole (AA), which continues as the efferent arteriole (EA) as it exits the capsule. The AA carries the blood containing systemic metabolites and waste materials to the glomerulus before branching into vessels that are in close apposition with the Bowman's capsule. The vessels then continue as the EA, now carrying filtered blood. The EA divides to form an intricate network of peritubular capillaries (*vasa recta*) around the long tubule of the nephron. The vasa recta are a part of a low-pressure complex, essential for the re-absorptive and secretory function of the kidneys (Dukes & Reece, 2004; Finch, 2014; Sjaastad et al., 2016).

The glomerular filtration barrier (GFB) is a complex trilaminar interface present between the glomerulus and Bowman's capsule. It comprises the endothelial cells (glomerular capillaries) held together by the glycocalyx, glomerular basement membrane and podocytes (epithelial cells of Bowman's capsule). These charged layers effectively prevent the loss of essential macromolecules, such as albumin, from the vascular lumen (Menon et al., 2012; Sjaastad et al., 2016). The pressured environment, within the vascular component of the corpuscle, ensures that the supplied blood is filtered through the GFB to produce urine.

The ultrafiltrate/primary urine filters into the Bowman's space and is carried through the tubular components of the nephron, i.e., the proximal convoluted tubule (PCT/ tubulus convolutes proximalis), the loop of Henle, distal convoluted tubule (DCT/ tubulus convolutes distalis), the collecting tube- joins those of the other nephrons to form an arcade- that finally continues as the cortical collecting duct (Seldin & Giebisch, 1985), which opens into the renal sinus. The juxtaglomerular nephrons are equipped with an additional limb (ascending) of the loop of Henle's (Chmielewski, 2003). The ultrafiltrate comprises ~ 27-31% of the supplied venous plasma in cats. It is subjected to secretory/excretory and re-absorptive physiological processes that enables systemic homeostasis (Finch, 2014; Sjaastad et al., 2016). Almost 99% of the filtrate is reabsorbed into the surrounding Extracellular Fluid (ECF), maintaining its osmolarity, a factor essential to ensuring the urine concentrating ability of nephrons.

2.1.2 Renal Vasculature

Each kidney has a rich source of blood supply, delivered by a direct branch of the abdominal aorta, i.e., the renal artery. The renal vascular system is a portal system, i.e., the capillary beds (glomerular and peritubular capillaries) are enveloped by arteries (Chmielewski, 2003). The renal arteries divide into smaller vessels: the segmental arteries, multiple interlobar arteries, interlobular arteries, arcuate arteries and cortical radiate arteries, until they finally continue as the AA (Glaser, 2017). The AA and EA form the distal limbs of the invaginating tuft of porous glomerular capillaries. The EA continues as the interlobular and interlobar veins and then exits the kidney as the renal vein (Aspinall, 2004) before emptying into the caudal vena cava.

The vasa recta, a division of the EA and peritubular capillaries, perfuses the tubular component of the nephron in the medullary region (Dukes & Reece, 2004), as shown in Figure 2. They play a significant role in osmoregulation through the counter-current mechanism- essential for urine concentration- and are responsible for carrying the venous blood from the deeper parts of the medulla (Chmielewski, 2003; Seldin & Giebisch, 1985).

The kidneys are supplied by 20% of the total cardiac output (Chmielewski, 2003); 90% of the renal blood supply is directed towards the cortex and about 7.5% to the renal medulla. This difference in blood supply across the regions, i.e., the renal cortex (high blood flow) and the outer-/inner- medulla (Eaton & Pooler, 2009, p. 13), is essential for the production and excretion of concentrated urine (Sjaastad et al., 2016, p. 558). Figure 3 illustrates the difference in renal BP across the different vessels, such as the difference between the AA and the peritubular capillaries, supplying the various structures in the human kidney, assumably like what would be seen across the renal vasculature in cats.

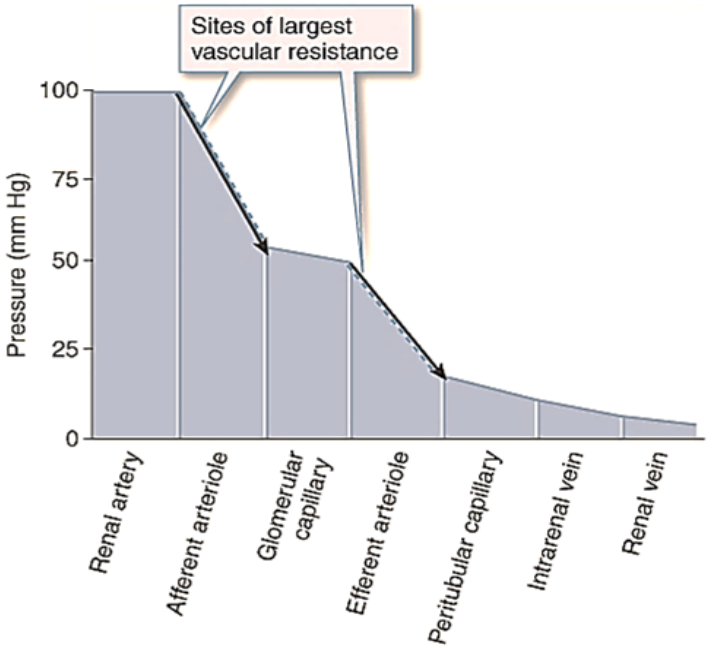


Figure 3 Difference in blood pressure across the renal vasculature in the human body.

As depicted in the graph, the blood pressure across the renal vasculature drops, especially in the afferent and efferent arterioles. The higher glomerular pressure ensures proper filtration in the renal corpuscle, while the lower pressure in the peritubular capillaries maintains the osmotic gradient with the tubule system. Permission for reprinting was granted by The McGraw-Hill Companies Inc.

The parts of the nephron found in the medulla are suited to function in a low-pressure environment as the tubular fluid is of a different composition than blood (containing charged, heavy molecules). The vasa recta supply the outer medulla with blood containing low oxygen reserves (Kanagasundaram, 2015), playing a role in urine concentration. Essentially, this unique environment of varied blood supply across the cortex and the medulla places regions such as the PCT and the loop of Henle (highly metabolically active) at an increased risk of injury (Kirita et al., 2020)- especially if there is a discrepancy in renal blood flow or RBF (De Loor et al., 2013), such as a decrease leading to anoxia in the medulla (Evans et al., 2020), as it is already maintained at a precariously hypoxic environment to enable the process of urine concentration (Brezis & Rosen, 1995), as seen in Figure 2 (B).

2.1.3 Nervous Innervation of the Kidneys

The sympathetic/adrenergic efferent nervous system works in tandem with the vital systems in the body to maintain homeostasis. The postganglionic sympathetic neurons innervate the renal tissue, including specialized cells, i.e., the juxtaglomerular granular cells or JGC (Dukes & Reece, 2004; Seldin & Giebisch, 1985). Sympathetic stimulation, in stressful conditions, reduces urine production by mediating the vasoconstriction of the AA and the release of norepinephrine from the adrenal medulla. This reduction in urine production occurs due to the resulting decrease in the RBF (Glaser, 2017). The sympathetic nervous system also plays a role in the autoregulation of renal BP (Sjaastad et al., 2016).

2.2 RENAL FUNCTION

The kidneys perform several vital functions in the body (Aspinall, 2004, pp. 134-144; Hall, 1983; Sjaastad et al., 2016), as shown in Figure 4. Namely, 1) excretory function, which involves the removal of non-gaseous metabolites such as nitrogenous wastes, excess water, detoxified products and inorganic ions, 2) production of proteinaceous substances such as erythropoietin (RBC production), renin (BP regulation) and calcitriol (calcium homeostasis), and 3) Ionic balance maintenance through secretory/re-absorptive processes carried out in the tubular portions of the nephron. Tubular functions include removal/absorption of salt, proteins, glucose, and electrolytes (Potassium, sodium).

Other functions include: 4) osmoregulation which helps maintain the composition, and volume of ECF among other body fluids. This is carried out by a complex interplay of factors such as renin, angiotensin, aldosterone, antidiuretic hormone, baroreceptors, and osmoreceptors. The kidneys also 5) maintain the systemic acid-base balance, and 6) act as a site for gluconeogenesis**.**

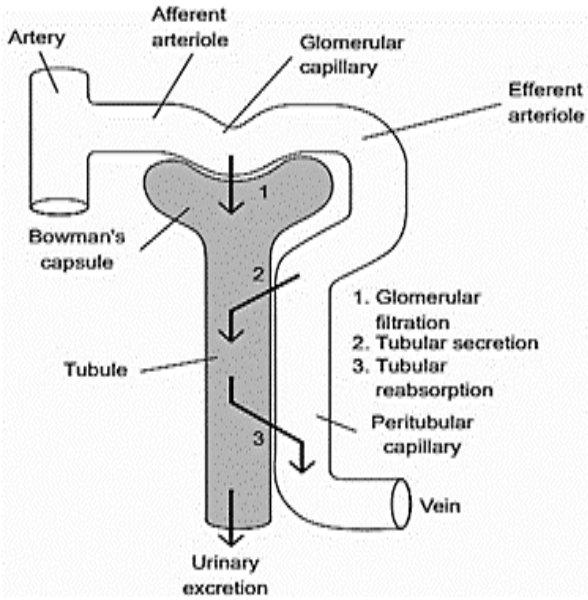


Figure 4 Functions of the nephron.

Diagrammatic representation of the direction of nephronal processes: (1) glomerular filtration, (2) tubular secretion and (3) tubular reabsorption. This image has been reprinted, with permission, from Vander's renal physiology.

The kidneys are capable of autoregulating renal BP, irrespective of the changes to arterial BP (to a limit), which is initiated by the secretions of the Juxtaglomerular apparatus (JGA). The JGA consists of (1) macula densa, a group of specialized chemosensory epithelial cells found at the point of contact between the DCT and the glomerular AA. The macula densa can detect changes in the tubular fluid electrolyte levels (sodium and chloride ion) and tubular fluid volume. (2) juxtaglomerular granular cells (JGC): barosensory myocytes in the AA wall, which are stimulated by the nervous system to produce Renin, an enzyme that helps autoregulate renal BP and maintain renal function when homeostasis is affected (Sjaastad et al., 2016). The extra-glomerular mesangial/Lacis/Goormaghtigh cells, found between the macula densa and the glomerular capillaries (Dukes & Reece, 2004; Seldin & Giebisch, 1985, p. 297), also play a role in maintaining the glomerular filtration rate (GFR; described below), as well as the osmolarity of the filtrate (indirectly), through the contraction and relaxation of the specialized myocytes (Glaser, 2017).

2.2.1 Glomerular Filtration Rate (GFR)

The glomerular filtration rate (GFR) is the rate at which the ultrafiltrate is formed (Dukes & Reece, 2004; Eaton & Pooler, 2009). The net filtration pressure across the glomerular apparatus is essential for maintaining the GFR. The filtration pressure is maintained by the difference in hydrostatic pressure and plasma-oncotic pressure between the sites involved, i.e., the glomerular capillaries and the Bowman's space (Sjaastad et al., 2016, p. 560). The arterial BP influences the hydrostatic pressure in the glomerular capillaries. GFR is, thus, influenced by arterial BP (Eaton & Pooler, 2009) and the resistance offered by the renal arterioles (Finch, 2014; Sjaastad et al., 2016). The measurement of GFR helps monitor renal filtration and excretion, thereby providing information on renal function in a clinical setting (Von Hendy-Willson & Pressler, 2011).

2.2.2 Autoregulation of Renal Blood Flow (RBF)

The GFR is regulated by intrinsic reno-protective mechanisms (Kanagasundaram, 2015) that stabilize RBF, irrespective of systemic BP under steady conditions to a certain level (Cupples & Braam, 2007). Any slight variation in homeostasis, such as a drop in MAP, which could potentially affect renal function by causing a change in renal perfusion, stimulates the autoregulatory mechanism in the kidneys. This mechanism, functional only when the renal BP is within certain bounds, i.e., ~ 75 mmHg to 160 mmHg in dogs (Cupples & Braam, 2007), drives the excretion or absorption of hormones, salts and water to help maintain normal kidney function and, thus, GFR (Eaton & Pooler, 2009), as shown in Figure 5. Furthermore, a MAP of 60-70 mmHg is required to ensure organ perfusion (Mazzaferro & Wagner, 2001).

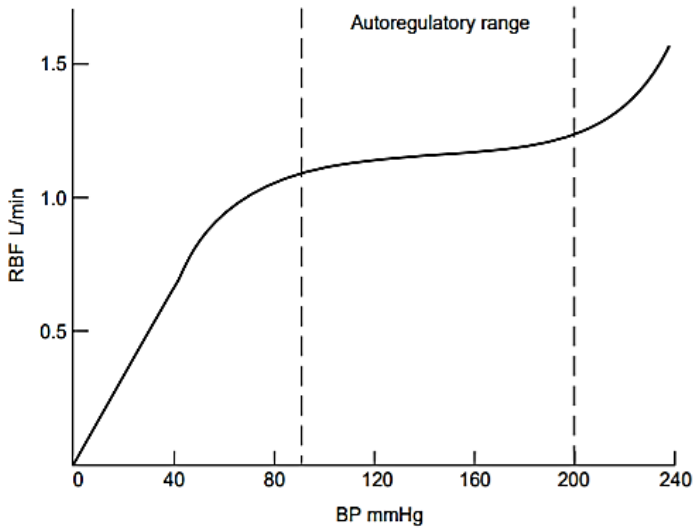


Figure 5 Effect of arterial blood pressure (BP) on renal blood flow (RBF) and, consequentially, on GFR.

Graph depicting the correlation between mean arterial blood pressure and renal blood flow. Changes in the BP within the autoregulatory range observes minimal variation in RBF due to the intrinsic renal autoregulatory mechanisms. However, if the BP falls below the lower threshold of this range, the renal autoregulatory mechanism would fail leading to decreased RBF and an increased risk of renal damage. This image has been reprinted, with permission, from Vander's renal physiology.

The process of renal autoregulation is mainly mediated by two mechanisms (Dukes & Reece, 2004; Sjaastad et al., 2016): 1) myogenic manipulation (rapid), and 2) tubuloglomerular feedback (slow). The former is stimulated by arterial wall tension, releasing eicosanoids (Kanagasundaram, 2015) and inducing a change in the AA's myogenic membrane potential (Cupples & Braam, 2007). This enables the AA to vasoconstrict/-dilate, as required, to regulate renal BP and, thus, prevent any negative impact on renal function. The latter mechanism relies on the JGA to maintain a normal environment in the kidney, undeterred by any changes in the systemic BP to a certain extent. The macula densa (JGA) secretes mediators (renin) that cause vasoconstriction/vasodilation of the AA when blood flow is affected, thereby modulating RBF to bring GFR to baseline.

Renin-Angiotensin-Aldosterone System (RAAS)

The RAAS system is involved in BP regulation (Coleman & Elliott) and is stimulated by the tubuloglomerular feedback mechanism. A loss of arterial wall stretch incites the JGA, i.e., through reduced blood volume or lowered BP. The change in BP (reduced RBF) subsequently causes a drop in the GFR, leading to changes in the tubular fluid salt concentration (Jepson, 2016). These variations in the delivery of sodium (Na^+) ions are detected by the macula densa cells (Sjaastad et al., 2016).

Renin, secreted by the JGA, is responsible for converting angiotensinogen to angiotensin I in the kidneys. Angiotensin I is converted to Angiotensin II in the presence of Angiotensin-Converting Enzyme (ACE), which is mainly present in the endothelial cell membrane of the lungs (Sjaastad et al., 2016). Angiotensin II induces vasoconstriction of the renal AA, thereby increasing the hydrostatic pressure of the glomerular capillaries, leading to glomerular hyperfiltration. This increase in renal BP helps maintain homeostasis for a short duration; however, extended periods of these episodes requiring autoregulation will have detrimental effects, such as fibrosis/sclerosis of tissue (Finch, 2014).

2.3 FELINE RENAL DISEASE

Prevalence

Renal disease is a prevalent affliction in geriatric animals and is a significant cause of mortality in cats. It has been hypothesized that animals become susceptible to KD through exposure to subclinical injuries (Bland et al., 2014; Vaidya, Ferguson, & Bonventre, 2008). These injuries may cause maladaptive changes that disturb the delicate balance between the reparative and degenerative processes of renal parenchyma; initiating a "self-perpetuating vicious cycle" of kidney damage.

While progress is being made in improving therapeutic protocols for KD, the mortality rate for cats affected with KD has not declined over the years (Segev, 2018a). The leading cause for the failure to effectively treat the condition is the inability to detect KD in its

preliminary stages of damage. Therefore, there is a need to educate owners on the signs of KD and improve our diagnostic tests to detect it in its initial phases to improve the responsiveness to treatment.

Classification

Renal disease can be broadly classified into acute kidney injury (AKI) and chronic kidney disease (CKD). Other categories, such as Acute-on-Chronic (Segev, 2018a) and chronic-on-acute kidney disease, have been identified, wherein either condition occurs in individuals with pre-existing KD. However, clinical cases are currently identified as either AKI or CKD as this helps determine the severity of KD, determine the prognosis and allow for subsequent treatment planning.

Both AKI and CKD were initially believed to be separate disorders with different pathophysiology. However, over recent years, this belief has transmuted as the pathophysiology of the two conditions is, in fact, quite similar (Segev, 2018a, 2018b). Both conditions involve the impairment of nephrons, and there can be a bidirectional communication between the two disorders, i.e., AKI being an initiating cause for CKD (Schmiedt et al., 2016) and vice versa (Cowgill et al., 2016). Segev (2018a) suggested that active renal injury could be used to differentiate KD, i.e., an increase in active renal injury indicators hints at AKI, while its presence in lower concentrations may hint at a slower progressive KD.

Grading/Staging

Kidney Disease, i.e., both AKI and CKD, is generally staged or graded using International Renal Interest Society (IRIS) guidelines that uses conventional markers, such as serum creatinine (SCr) and symmetric dimethyl-arginine/SDMA (Segev, 2018b), to gauge the severity of the condition. The two conditions, i.e., AKI and CKD, differ in the rate of progression, ultrasonographic findings and histology. Clinical cases are generally identified as AKI if the renal parameters change significantly over a short period, i.e., hours or days (Schollum, 2012). They are identified as CKD if the parameters gradually develop and persist for over two to three months (Jepson, 2017).

As mentioned before, it has been suggested that mild, transient/ongoing AKI could be a precursor for CKD (Chen et al., 2019; Cowgill, 2016; Cowgill et al., 2016; Katayama et al., 2020; Schmiedt et al., 2016; Vaidya, Ferguson, & Bonventre, 2008). While the study by Schmiedt et al. (2016) found experimentally induced AKI to have long-lasting malfunctioned reparative effects on feline kidneys, a strong correlation between mild AKI and CKD has yet to be proven (Bland et al., 2017; Cowgill et al., 2016). This uncertainty is, in part, because subclinical cases of renal injury frequently remain undetected and, thus, have not been longitudinally studied as clinical cases.

Clinical signs of KD can be non-specific. These include polyuria, polydipsia, lethargy, vomition, cachexia, inappetence, dehydration, halitosis, melena, diarrhoea and uremic stomatitis. Other apparent complications observed in clinical cases include peritoneal/pleural oedema/effusion, hyphema, retinal detachment and acute blindness (Eatroff; Segev, 2018b; Syme, 2019). It has been suggested that owners sometimes fail to notice subtle signs of illness especially in cats, a species that can mask the disease. Owners are also prone to attribute some of the signs to the normal ageing process.

Kidney diseases such as AKI and early CKD can be challenging to diagnose in cats among other species (Katayama et al., 2020; Syme, 2019). The mild or early stages of KD are less frequently diagnosed because the affected cats may remain asymptomatic (Cobrin et al., 2013; Lobetti & Lambrechts, 2000), until the damage is severe, leading to delayed presentation to a clinical setting. This is futher worsened by our reliance on insensitive diagnostic tests which are more suited to detect the later stages of KD. These traditional tests, such as serum creatinine (SCr) or blood urea nitrogen (BUN), require almost 75% of the renal mass to become dysfunctional before these markers are elevated to levels that alert clinicians to the presence of renal damage.

De Loor et al. (2013) notes “kidney injury can be present in the absence of kidney function” (p. 998, para 1). This aptly describes the limitation associated with the use of conventional markers that are elevated over the reference range only when the GFR is affected and this allows cases of subclinical injury to go undiagnosed (Bland et al., 2014; Sargent et al., 2021; Vaidya, Ferguson, Collings, et al., 2008; van den Berg et al., 2018). This severe limitation prevents effective treatment of kidney damage or dysfunction in its early stages, primarily

since subclinical active renal injury is associated with increased mortality risk (Gumbert et al., 2020). Therefore, we must use markers that can identify kidney injury long before we see any change in GFR, i.e., before 75% of the nephrons become dysfunctional.

2.4 ACUTE KIDNEY INJURY (AKI)

Synonym: **Acute renal failure (ARF)**

Acute Kidney Injury is usually characterized by abrupt renal dysfunction (Chen et al., 2020; Sargent et al., 2021), set off by ischemic or toxicant-induced renal parenchymal injury that develops within three months (Cowgill, 2016; Kovarikova, 2015; Vaidya, Ferguson, & Bonventre, 2008). It is caused by "rapid hemodynamic, filtration, tubulointerstitial or outflow injury to the kidneys leading to accumulation of metabolic toxins (uremia toxins) and dysregulation of fluid, electrolyte, and acid-base balance" (Cowgill, 2016, p. 1).

Acute Kidney Injury is presumed to have a high mortality rate (Cowgill, 2016) of ~ 50% across species (Dunaevich et al., 2020; Worwag & Langston, 2008). As mentioned before, the main limitation faced in a clinical environment is a delay in detection (Cobrin et al., 2013; Kovarikova, 2015; Vanmassenhove et al., 2013) which can prove to be critical, as early diagnosis can either reverse the damage or cease its progression (Senior, 2017).

Intrinsic AKI can originate from either glomerular or tubular injury, with ischemia being the most common cause of the latter (Kanagasundaram, 2015). As different areas of the kidney have varying levels of susceptibility to injury, due to the unique vasculature and metabolic needs of the tubular cells, the minimum threshold for inciting renal injury is not known.

Prevalence

Eatroff (2020) indicated that due to the lack of information on AKI and its various possible aetiology, the exact prevalence of the condition cannot be determined. Furthermore, the current dilemma, with the use of conventional diagnostic tests (Segev, 2018a, 2018b), has led to underestimating the true prevalence of AKI in companion

animals. The mortality rate for AKI-affected individuals was 47% in a retrospective study by Worwag and Langston (2008), and the prognosis is generally poor (Dickerson et al., 2017).

Pathophysiology

Acute Kidney Injury develops through four distinct phases of injury, i.e., initiation, extension, maintenance, and recovery (Eatroff, 2020; Ross, 2011). It is at the initiation phase that occurs immediately after the injury, when interventional therapy is most effective at reversing the damage. However, we fail to detect kidney injury at this stage due to a delay in admission, absence of any clinical signs or seemingly ordinary results found upon screening. The extension phase is characterized by the continued series of pathological changes leading to cell death (Ross, 2011). It progresses to the maintenance phase, where scarring or irreversible tubular lesions are formed, resulting in tubular dysfunction. This is the stage when clinicopathological signs, such as azotemia (Ross, 2011), are apparent (Chen et al., 2017). Reparative mechanisms in the recovery stage are characterized by hypertrophy of nephrons which helps compensate for the loss of the function of damaged nephrons (Grauer, 1996) and this change is irreversible. Improving screening tests that can identify the initiation phase of AKI would significantly improve the prognosis for kidney injury.

The kidneys are more susceptible to injury when compared to other organs due to their unique anatomic vasculature (Jepson, 2016). The pathophysiology involves (Lobetti & Lambrechts, 2000; Ross, 2011) vasoconstriction of the AA in response to injury, reduced renal blood flow (RBF), reduced energy stores (ATP), the effect of pro-inflammatory modulators and tubular cell damage; these adaptive changes can lead to apoptosis (Grauer, 1996; Vaidya, Ferguson, & Bonventre, 2008) and desquamation of brush border cells. Histopathological study revealed that AKI is characterized by cell death/necrosis, and intact cells were morphologically altered. The changes observed after apoptosis include the presence of cytoplasmic blebs and reduced brush border of the PCT cells (Schmiedt et al., 2016).

Cellular damage results in increased intracellular calcium levels, which sets off

the activation of several other catabolic enzymes (Ross, 2011). Ischemia in the kidneys stimulates nitric oxide synthase enzyme. The resulting increase in nitric oxide can react with other breakdown products such as superoxide to form reactive compounds, affecting transport proteins and intercellular adhesion junctions, thereby disrupting tubular reparation and recovery (Ross, 2011). Further, the action of inflammatory mediators can worsen hypoxia (Vaidya, Ferguson, & Bonventre, 2008), further worsening the severity of the damage. This can lead to an imbalance in the solute levels, such as increasing sodium ions. The RAAS is stimulated, causing the vasoconstriction of the AA, resulting in lowered GFR (Ross, 2011). Thus, the inherent mechanisms of the kidney may be involved in a self-perpetuating cycle of renal injury and fibrosis.

Risk Factors

The risk factors for AKI include: age, renal ischemia (Grauer, 2005; Kanagasundaram, 2015; Schmiedt et al., 2016) or reperfusion injury (Eatroff; Keir & Kellum, 2015; Lobetti & Lambrechts, 2000), subclinical inflammation, pre-existing CKD (Long et al., 2016), nephrotoxin-induced injury, or dehydration, hypovolemia, fluid overload, hypovolemia, hypotension, infarction, underlying disorders, medications (Chertow et al., 1997), infectious disease and/ or sepsis (Senior, 2017). One potential prerenal cause for AKI is decreased or altered renal blood perfusion due to intraoperative hypotension resulting from prolonged surgery/ anaesthesia (Grauer, 1996; Senior, 2017).

It was found that ischemia is a common risk factor for AKI in dogs (Mishra et al., 2003). An experimental study that induced renal ischemia for 15 or 30 mins in cats found signs of atrophy and inflammation of varying severity in the tubular sections of nephrons through histopathology at 120 days, with warmer body temperature at the time of episode being at higher risk of renal injury even when it is within the physiological range (Dickerson et al., 2017). This suggests that both the duration, as well as the severity, of ischemia along with the body temperature may influence the level of renal damage.

Perioperative renal ischemia is less pronounced when compared to ischemia in other organs and vessels in humans (Chertow et al., 1997). The renal tubules function in an

environment with low oxygen tensions (Chakrabarti et al., 2012) and are, therefore, susceptible to hypoxia. Hypoxia can result in increased production of free radicals, which cause summative oxidative damage. The risk is further increased by their high metabolic demands (Jepson, 2016). In support of this, experimentally induced unilateral renal ischemia (60 mins) was shown to induce adaptive fibrotic changes over 70 days, with major changes occurring in the tubular regions, in both, the corticomedullary tubular sections and the Bowman's capsule (Schmiedt et al., 2016). The previous study observed significant hyperplastic, regenerative and/or necrotic pathology of the epithelial cells in the corticomedullary tubular sections by days 3 and 6 post-ischemia. Thus, the PCT (straight) and loop of Henle (ascending limb) are more likely to undergo maladaptive, possibly chronic changes when subjected to reduced perfusion.

Decreased renal perfusion is associated with decreased cardiac output, oncotic pressure, increased blood viscosity or decreased prostaglandin formation. A study in the dog model showed that increasing renal perfusion through a venous shunting procedure resulted in a reversal of renal damage and proved that an adequate amount of oxygen is essential for re-establishing the GFR (Morales et al., 2002). These studies demonstrate that a minimal level of oxygen must be maintained within the kidneys for the vital functioning of nephrons.

Markers sensitive to renal damage rather than a change in function, are better suited to diagnose AKI (Segev, 2018a). Clinical signs may be intense, and this condition may involve multiple organ systems such as the pancreas, lungs and GIT (Segev, 2018b). Current grading and sub-grading of AKI are done following the guidelines set by IRIS (Cowgill, 2016), as shown in Table 1. These guidelines are based on the values of classical markers such as serum creatinine (SCr). These guidelines help determine the prognosis of the case and plan the treatment regimen accordingly.

Table 1 IRIS AKI grading criterion (Cowgill, 2016).

AKI Grade	Blood Creatinine	Clinical Description
Grade I	< 1.6 mg/dl (< 140 µmol/l)	**Non-azotemic AKI:** - Documented AKI:(historic, clinical, laboratory or imaging evidence of AKI, clinical oliguria/anuria, volume responsiveness) and/or - Progressive non-azotemic increase in blood creatinine ≥ 0.3 mg/dl (≥ 26.4 µmol/l) within 48 hrs - Measured oliguria (<1 ml/kg/hr) or anuria over 6 hrs
Grade II	1.7-2.5 mg/dl (141-220 µmol/l)	**Mild AKI:** - Documented AKI and static or progressive azotemia - Progressive azotemic: increase in blood creatinine; ≥ 0.3 mg/dl (≥ 26.4 µmol/l) within 48 hrs, or volume responsiveness - Measured oliguria ((<1 ml/kg/h) or anuria over 6 hrs
Grade III	2.6-5 mg/dl (221-439 µmol/l)	
Grade IV	5.1-10 mg/dl (440-880 µmol/l)	**Moderate to severe AKI:** - Documented AKI and increasing severities of azotemia and functional renal failure
Grade V	> 10 mg/dl (> 880 µmol/l)	

Clinical signs

The signs observed in an AKI-affected animal include polyuria/ oliguria, depression, vomition, polydipsia, anorexia, and hypothermia. Seizures and muscle fasciculations may be seen in severe cases with uremic nephropathy (Senior, 2017). Polyureic AKI, i.e., in animals who have lost their ability to concentrate the ultrafiltrate, are more likely to have both glomerular and tubular injury.

Serum biochemistry findings include observing an increase in serum creatinine (SCr), blood urea nitrogen (BUN), phosphate, potassium, along with a decrease in calcium; Upon urine examination, the presence of active sediment with casts and inflammatory cells is indicative of AKI (Senior, 2017).

As mentioned before, transient AKI can act as a precursor for KD later in life (Dunaevich et al., 2020; Vaidya, Ferguson, & Bonventre, 2008), as depicted in Figure 6. The auto-regulatory capacity of the kidneys fails once AKI sets in (Kanagasundaram, 2015). Unregulated renal pressure may place the tubular sections of the nephron in a vulnerable position. Even a slight change in the typical structure and/or function of renal tissue, such as delayed repair (Vaidya, Ferguson, & Bonventre, 2008), can set off a cycle of sustained or progressive damage. Furthermore, the inflammatory, fibrotic changes brought about by the damage tend to remain after repair (Schmiedt et al., 2016), leading to increased susceptibility to renal injury in the future (Kirita et al., 2020). Ongoing or episodic bouts of AKI progressing to CKD only become clinically symptomatic once the compensatory mechanisms of the kidneys are incapacitated (Chen et al., 2020).

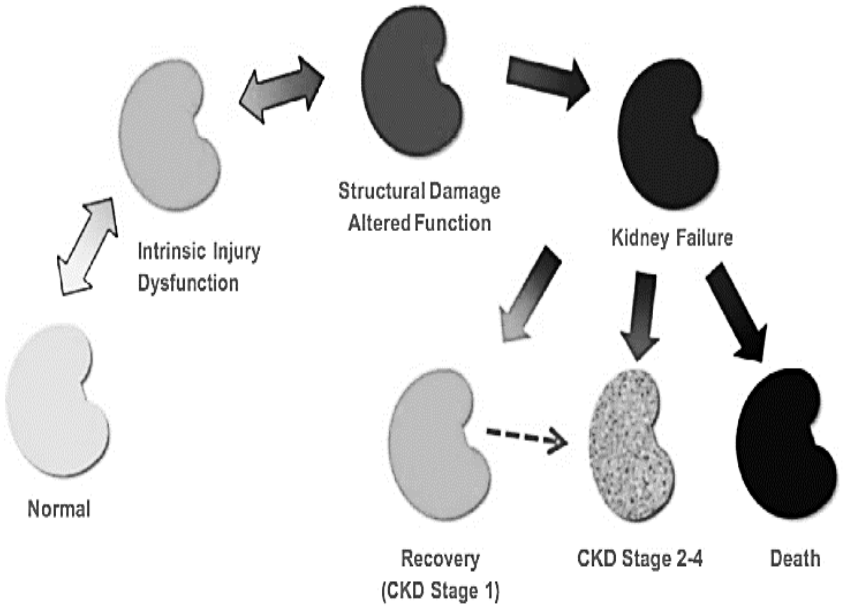


Figure 6 Spectrum of AKI and CKD pathophysiology.

Mild damage to a normal kidney induced by a change in the renal environment can result in acute kidney injury. This can progress to irreversible chronic kidney damage. Reprinted from Cowgill et al. (2016).

The progression of AKI to CKD should be prevented as early as possible, especially since AKI can be potentially reversed, while the changes brought on by chronic fibrosis are irreversible (Chen et al., 2020). If kidney injury could be diagnosed early on, the pathological process can then be delayed or reversed with appropriate treatment and rehabilitation, inevitably improving the patient's quality of life. However, we require sensitive and specific, markers to aid in the detection and diagnosis of early KD. Such development can be introduced through focused species-specific studies to compare and introduce improved, validated renal biomarkers.

2.5 CHRONIC KIDNEY DISEASE (CKD)

Chronic Kidney Disease is a progressive disorder involving the kidneys that develops over a long period, typically over three months (Dunaevich et al., 2020), characterized by altered renal structure and function (Jepson, 2016; Sargent et al., 2021). The resulting damage may be irreparable. Unlike AKI, CKD can be either acquired or congenital in origin (Antunes Ribeiro et al., 2020; Reynolds & Lefebvre, 2013).

Prevalence

The prevalence of CKD increases with age (Biourge et al., 2020; Conroy et al., 2019; Geddes, 2013; Jepson, 2016; Kovarikova, 2015; Reynolds & Lefebvre, 2013; Roura, 2019) and is estimated to affect ~ 80% of cats over fifteen years of age, while it is reported to affect ~ 1-3% of the population across other age groups (Conroy et al., 2019; Paepe & Daminet, 2013; Roura, 2019). CKD has not been reported to be gender specific. Similar to AKI, the insensitivity of our current diagnostic tests have probably undervalued the true prevalence of CKD in the population (Conroy et al., 2019).

Risk factors

The factors influencing AKI also influence CKD development (Jepson, 2016). An interplay of several factors such as age, drugs, diet, genetic/hereditary factors, comorbidities

(infectious/inflammatory, neoplastic), a recent history of general anaesthesia, hypertension, proteinuria, vaccination, scarring, and active injury is associated with CKD development (Chen et al., 2020). The Dunaevich et al. (2020) study on dogs reported that 45% of the CKD cases had no known aetiology, while approximately 7% of all the CKD cases had an ischemic origin. The delayed diagnosis makes identifying CKD risk factors and aetiological causes challenging (Roura, 2019).

Pathophysiology

A histopathological study revealed CKD models to be characterized by hyperplastic arteriosclerosis, tubulointerstitial inflammation and fibrosis (Chakrabarti et al., 2013), followed by tubular mineralization in later stages (Antunes Ribeiro et al., 2020; Bland et al., 2017; Grauer, 2021). The autoregulatory system of the kidneys engages in the progression of CKD by inducing fibrotic and sclerotic changes in the glomerulus (Coleman & Elliott; Jepson, 2016). The uninjured functional nephrons adapt to compensate for the loss in function by undergoing hypertrophy, while the glomerulus remains in a constant state of hypertension and hyperfiltration (Biourge et al., 2020).

These fibrotic changes affect the kidneys' filtration capacity, eventually decreasing the GFR once the compensatory mechanisms fail. Glomerular damage results in improper filtration of metabolic wastes and increased leakage of charged or high molecular weight (HMW) proteins into the ultrafiltrate, while tubular damage impairs the resorptive processes. This results in an accumulation of unfiltered toxic metabolites in the bloodstream with electrolyte imbalance as well as increased quantities of various molecules in the ultrafiltrate and, subsequently, the excreted urine.

The increasing quantity of nitrogenous wastes (urea) in blood has lasting toxic consequences on the organ systems and this elevation is translated into increased BUN values in serum samples. Persistent uremia can injure the cellular components of blood, resulting in a decreased lifespan of erythrocytes. This, along with a decrease in the renal production and secretion of erythropoietin, leads to anaemia (Antunes Ribeiro et al., 2020). Several pathophysiological and haematological changes can, thus, be observed with the progression of KD. These changes, however, are revealed upon biochemical

examination only once most of the renal nephrons have been damaged, which finally potentiates clinical signs such as lethargy, vomition and other uremic effects.

Clinical Signs

The clinical signs of CKD are generally non-specific, made more difficult by the cat's ability to mask the illness. Signs that may be observed include anorexia, lethargy, weight loss, polydipsia, polyuria, vomition and/or diarrhoea (Conroy et al., 2019; Dunaevich et al., 2020).

Cat owners usually fail to observe changes in behaviour and other symptoms until they become overtly evident over time (Segev, 2018b), as was observed in a study by Conroy et al. (2019), where 66.6% were presented after the appearance of clinical signs. An alarming 24.8% of the cats diagnosed with CKD in the same study were asymptomatic. Some of the early outward manifestations of KD may be mistaken for signs of ageing (Robertson et al., 2018). This misconception is concerning since clinical symptoms appear only once a significant mass of the kidneys is damaged, primarily when the autoregulatory and compensatory mechanisms of the kidneys fail to maintain normal function (Reynolds & Lefebvre, 2013). Educating the owners on KD, along with its associated signs, is vital for improved screening and detection of the condition.

Grading

Kidney dysfunction in humans is clinically identified through proteinuria, hypertension, and azotemia (de Souza Rodrigues et al., 2017). In tandem with this, CKD has been classified into four stages by the International Renal Interest Society (IRIS) based on SCr and symmetric dimethylarginine (SDMA) concentrations, as displayed in Table 2. They are further sub-grouped based on systolic arterial pressure (SAP), urine protein to creatinine ratio (UP:C) and urine specific gravity (USG). As per the IRIS guidelines (similar to AKI), CKD staging helps ascertain the diagnosis/prognosis and plan the treatment protocol (Antunes Ribeiro et al., 2020; Paepe & Daminet, 2013).

Table 2 IRIS Staging of CKD (IRIS CKD Pocket Guide; IRIS Staging of CKD, 2019).

Stage	SCr* (mg/dl)	SDMA† (µg/dl)	UP:C‡ (Substage)	SAP§ mmHg (Substage)	Comments
1	**< 1.6 (< 140 µmol/l)**	**< 18**	Non-proteinuric < 0.2	Normotensive < 140 (minimal risk of target organ damage).	Non-azotemic; normal to slightly increased blood SDMA; with/-out change in urine concentrating ability, abnormal renal palpation or imaging findings, proteinuria, abnormal biopsy results; serial increase in SCr or SDMA; Persistent SDMA value > 14 µg/dl;
2	**1.6-2.8 (140-250 µmol/l)**	**18-25**	Borderline proteinuric 0.2-0.4	Prehypertensive 140-159 (low risk of target organ damage).	Normal to mild azotemia; mild increase in SDMA; Clinical signs may be present or absent.
3	**2.9-5.0 (251-440 µmol/l)**	**26-38**		Hypertensive 160-179 over 1-2 weeks (moderate risk of target organ damage).	Moderate azotemia: absence of clinical signs is categorized as early stage 3 and clinical systemic signs as late stage 3.
4	**> 5.0 (> 440 µmol/l)**	**> 38**	Proteinuric > 0.4	Severely hypertensive ≥ 180 for more than 1-2 weeks (high risk of target organ damage).	Uremic crisis (severe azotemia) and systemic clinical signs.

* Serum creatinine
† Symmetric dimethyl-arginine
‡ Urine protein creatinine ratio
§ Systolic arterial pressure

2.6 RISK FACTORS

Several studies have been performed to identify the risk factors and aetiology for renal disease. However, the inability to identify the early stages of KD also prevents the diagnosis of the aetiology and other inciters for most clinical cases (Syme, 2019; Vanmassenhove et al., 2013). Therefore, it is essential to perform focused studies to determine all the major causal links to kidney damage. The current hypothesis suggests that even subtle insults caused by drugs, hypoxia, or ischemia could precipitate kidney injury. The perioperative environment, including surgical stress (Motayagheni et al., 2017), carries the risk of altered cardiovascular function, increasing the risk of renal hypoperfusion. With the surging commonality of elective surgeries for small animals, conscious use of anaesthetics is essential to prevent or reduce the risk of injury to the kidneys.

2.6.1 General Anaesthesia and Surgery

Several possible complications arise with the use of general anaesthetics and surgical procedures as an invariable consequence of their depressor effects. To list a few: hypovolemia, hypoxemia, decreased cardiac output and hypotension, all of which can affect vital organ function/structure (Duke-Novakovski et al., 2016; Mama & Rezende, 2015), and in the case of kidneys, possibly incite AKI (Eatroff et al., 2012). Perioperative AKI is induced surgically through hypoperfusion and inflammation (Gumbert et al., 2020). This type of injury is usually transient and generally goes undetected due to both the absence of overt clinical signs and the insensitivity of conventional point-of-care tests.

Cats are susceptible to anaesthetic complications due to their innate physiology (Brodbelt, 2010; Brodbelt et al., 2007; Robertson et al., 2018). Levy et al. (2017) found that cats undergoing the spay/neuter procedure were at a higher risk of mortality (five-fold) when compared to dogs, with Brodbelt et al. (2007) reporting a 0.24% risk of anaesthesia-induced mortality in cats. It was observed in a study by Bland et al. (2017) that histopathology of renal sections of healthy controls (cats without KD) had evidence of mild injury possibly linked to sedation/anaesthesia or euthanasia. Similarly, Chen et al. (2019), as well as Worwag and Langston (2008), observed general anaesthesia-induced AKI in cats. The latter

retrospective study identified AKI onset (based on serum biochemistry) in two cats, with the cause being pinned on the recent use of anaesthesia.

Senior or geriatric cats are more susceptible to anaesthesia-induced changes in respiratory and cardiovascular function (Robertson et al., 2018). These transient systemic changes can affect the blood supply to different organ systems, especially the kidneys. Furthermore, the metabolically active medullary components of the kidneys are susceptible to ischemia-induced pathology as the renal perfusion across the cortex and medullary region differ, as mentioned before. To re-emphasize, the cortex receives approximately 85-90% of the RBF, while the medulla receives around 7% of it (Mama & Rezende, 2015; Sjaastad et al., 2016).

The inadvertent effects of general anaesthesia on the cardio-respiratory system are described below.

Intraoperative systemic hypotension is observed when the mean arterial pressure (MAP) falls below 62 mmHg, or systolic arterial pressure (SAP) falls below 87 mmHg (Robertson et al., 2018; Ruffato et al., 2015). It is a common complication (Gaynor et al., 1999) seen in almost 3% of the cats subjected to general anaesthesia (McMillan & Darcy, 2016). A minimum SAP of 80 mmHg or a MAP of 60 mmHg is essential to ensure vital organ perfusion (Duke-Novakovski et al., 2016, p. 435; Mama, 2021), primarily since renal autoregulation works only when the arterial BP is within a specific range, as previously shown in Figure 5.

When the arterial BP falls, the systemic autoregulatory mechanisms prioritize securing sufficient perfusion to the CNS and myocardium, decreasing renal perfusion. Therefore, general anaesthesia and surgery, causing hypotension with/-out hypovolemia (Grauer, 1996), can increase the risk of renal injury and dysfunction (Kongara et al., 2009). Furthermore, when the renal autoregulatory mechanism fails to perform (Mama & Rezende, 2015), the now compromised RBF, if found to persist, may worsen the risk of tubular and glomerular injury in the kidneys through the action of vasoconstrictors (on AA), aggravating deteriorative processes such as fibrosis (Gumbert et al., 2020). A study by Worwag and Langston (2008) retrospectively identified 2 cases (cats) that had suffered from AKI, post a hypotensive-hypovolemic episode.

Hypoventilation**,** a consequence of anaesthesia (Robertson et al., 2018), was reported in ~ 9.7% of cats in a teaching hospital (McMillan & Darcy, 2016). A decrease in oxygen levels can intensify renal tubule injury as these cells are highly active.

Hypothermia is a complication associated with anaesthetic use across all age groups in cats (Brodbelt et al., 2007), with almost 7% of the caseload in a teaching hospital experiencing it according to a survey (McMillan & Darcy, 2016). It can delay drug clearance (Robertson et al., 2018). Thus, the adverse effects (if any) of these drugs on the respiratory and cardiovascular systems can persist until they have been eliminated from the body. Drug accumulation could result in mild renal injuries, as well.

Anaesthetic agents

Most anaesthetics cause changes in systemic physiological parameters such as BP, heart rate (HR) and respiratory rate (RR); however, these changes are short-lived. The transient nature of these changes, along with the constant peri-operative monitoring, ensures that the cardiovascular and respiratory functions are maintained at levels that minimize the risk of organ damage (Mama & Rezende, 2015). Knowing that even mild injury could be a precursor for more lethal damage, we must improve our understanding of the interaction between various anaesthetics and the renal system.

Pre-anaesthetic medication such as phenothiazines (e.g., acepromazine) and subsequent general anaesthesia using volatile anaesthetics (e.g. isoflurane) can cause significant vasodilation with cardiac depression that may inevitably decrease cardiac output as well as MAP (Gumbert et al., 2020). A fall in the MAP below 60 mmHg can lead to the failure of the renal autoregulatory mechanism leading to decreased RBF (hypoperfusion) and potentially damaging the nephrons if the circumstances persist. This can embroil the kidneys into a state of progressive damage.

Isoflurane is a commonly used inhalant anaesthetic used to maintain anaesthesia. Isoflurane allows for faster recovery (Sano et al., 2018); however, it can cause dose-dependent hypotension (Ramsey, 2011, p. 182), reduced cardiac output and stroke volume (Mazzaferro & Wagner, 2001), and respiratory depression (Poterack et al., 1991; Ramsey,

2011). Poterack et al. (1991) observed that when isoflurane was administered at 3% in cats, the HR decreased by 50%, SAP by 61%, MAP by 70% and abdominal aortic blood flow by 71%. This decrease in abdominal blood output may influence renal perfusion and lead to sustained pathological changes.

Opioids, such as buprenorphine, butorphanol and morphine, are combined with other anaesthetics for their analgesic/sedative properties (Robertson et al., 2018). The duration of action for buprenorphine (4-6 hr) and morphine (2-4 hr) is longer in cats than in other animals due to the lack of a hepatic-origin metabolic enzyme (Ramsey, 2011). Thus, these opioids remain in systemic circulation for an extended period. These drugs may have a depressor effect on HR, cardiac output, and BP (Mazzaferro & Wagner, 2001).

Acepromazine is a phenothiazine that causes sedation by depressing the CNS (Ramsey, 2011) and affects BP (Mazzaferro & Wagner, 2001). It can cause hypotension (Mazzaferro & Wagner, 2001; Ramsey, 2011; Robertson et al., 2018; Schwarz et al., 2014) by inducing systemic vasodilation, i.e., reduced vascular resistance. It has been shown to exacerbate hypotension when used in adjunct with low-level isoflurane in dogs (Sinclair & Dyson, 2012). A study on cats sedated with acepromazine and alfaxalone undergoing spay procedure reported hypotension in 71% of the cases, stabilized with fluid therapy (Schwarz et al., 2014). The use of acepromazine in combination with opioids (butorphanol) to sedate cats can reduce BP through the effect of vasodilation (phenothiazine-induced) by 30 minutes (Costa et al., 2021).

Dexmedetomidine is an α_2-agonist that has sedative, muscle relaxative and analgesic properties (Ramsey, 2011). It affects the cardiovascular system, thereby reducing heart rate (40%), stroke index and cardiac output (60%); however, it also causes vasoconstriction, inadvertently causing an increase in BP, systemic vascular resistance, and blood glucose (Pypendop et al., 2011; Robertson et al., 2018). We generally observe a biphasic effect on BP, i.e., an initial increase followed by a decrease in BP. It causes a dose-dependent decrease in MAP (Mazzaferro & Wagner, 2001). It was shown to have adverse hemodynamic effects when given to isoflurane-anaesthetized cats (Pypendop et al., 2011).

When dexmedetomidine is combined with ketamine, it provides short-duration anaesthesia in cats and analgesia/sedation with opioids. It is quickly metabolized and, thus, the animal recovers faster from anaesthesia. The combination of Dexmedetomidine with alfaxalone and butorphanol was shown to reduce BP, diastolic arterial pressure (DAP) and MAP, in cats after thirty minutes (Cremer & Ricco, 2018). The combination of dexmedetomidine with ketamine and butorphanol in healthy cats was shown to decrease the partial pressure of oxygen and, in turn, increase the risk of hypoxemia, i.e., when the partial pressure of oxygen falls below 80 mmHg (Cremer & Ricco, 2018).

Ketamine is a dissociative anaesthetic agent (sympathomimetic) with analgesic properties and a quick recovery rate (Fish, 2008, p. 9). It has positive ionotropic-chronotropic effects on the cardiovascular system (Da Silva Mello et al., 2019; Fish, 2008, p. 10; Sano et al., 2018), along with broncho-dilatory effects (Cremer & Ricco, 2018). It is reported to increase the MAP in cats (Da Silva Mello et al., 2019; Sano et al., 2018).

Premedicated induction using a combination of dexmedetomidine, ketamine and butorphanol (DKT), administered intramuscularly (IM), produces rapid sedation, analgesia and anaesthesia. This protocol is commonly used for minor procedures such as spays or neuters. Its rapid onset makes it an excellent option to streamline surgeries for places with a high volume of cases, as seen in shelters (Ko & Berman, 2010). A combination of DKT premedicated induction (IM), followed by inhalant anaesthesia to maintain the depth of anaesthesia during surgeries such as ovariohysterectomy (OHE) provides smooth induction, maintenance, and recovery. The main disadvantage of using this anaesthetic combination is the associated drop in MAP, where there is no control over the depth of anaesthesia after IM administration of DKT (Ko et al.2009).

Alfaxalone is a neuro-steroid known for its dose-dependent properties such as rapid induction and short-action (Fish, 2008, p. 11). Its use can cause respiratory depression at high doses (Cremer & Ricco, 2018) and severe hypotension in cats (Fish, 2008). Some of the adverse effects of this agent include ataxia, muscle fasciculations, paddling and vomiting during recovery in cats when used as the sole anaesthetic agent.

The occurrence of any of the above-mentioned systemic changes in response to anaesthetic drugs and surgery, resulting in decreased oxygen deliverance, increased oxidative damage, and more, can cause renal lesions that may not be clinically apparent (Katayama et al., 2019); however, these subclinical changes may play a role in the pathophysiology of progressive KD. This hypothesis was supported by Lobetti and Lambrechts (2000), who suggested that while most organs may tolerate transient hypotension, it is not so with the kidneys. Any renal structural and functional change can lead to irreversible damage (Grauer, 1996).

A study on female dogs undergoing routine spay procedures found signs of active tubular damage postoperatively. The damage was detected by noting the presence of renal casts on urine sediment examination and an increase in the concentration of Gamma-glutamyl transferase/GGT, a relatively new renal marker, within 24 hrs postoperatively (Lobetti & Lambrechts, 2000). Interestingly, in this study, the older renal markers such as SCr and BUN remained within the reference range, suggesting that novel markers might be a more sensitive or an early indicator of mild renal structural damage.

What is important to note is that this increase in renal marker concentration supports the theory that general anaesthesia and surgery are capable of causing asymptomatic (externally) renal damage that remains undiagnosed when we use the traditional renal markers to monitor for KD. A study by Chen et al. (2019) reported AKI-induced by general anaesthesia in two cats, diagnosed by the steady increase in SCr value by 0.3 mg/dl within 48 hrs; however, these incidences might have been a more severe form of renal injury.

These studies provide evidence to suggest that general anaesthesia and surgery can cause acute injury to renal tissue, which, as deduced from the literature cited above, can go undetected with the use of our current panel of renal markers in a clinical setting. These renal changes may be identified by using more sensitive markers such as GGT and other structure-specific markers rather than function-sensitive ones.

2.6.2 Intravenous Fluid Administration

Intravenous (IV) fluid administration during general anaesthesia and surgery is recommended to maintain normovolemia and normotension (Zuurbier et al., 2002). It increases venous return, thereby preventing hypotension and reducing the risk of renal hypoperfusion (Brodbelt, 2010; Gumbert et al., 2020; Lobetti & Lambrechts, 2000; Robertson et al., 2018; Sinclair & Dyson, 2012). The administration of fluids in mouse models increased MAP without affecting HR (Zuurbier et al., 2002). Similarly, fluid resuscitation in dogs with hypotension helped increase the SAP to the normal range with no significant effect on heart rate (Powell, 2013). The administration of fluid during surgery has also reduced recovery time (Griffin et al., 2016).

Interestingly, studies by Brodbelt et al. (2007) and Brodbelt (2010) observed that cats receiving fluids were at a higher risk (4-fold) of anaesthetic death than those who didn't. But variables such as fluid overloading, presence of comorbidities, surgical procedure, systemic dysfunction and age must be considered to determine the actual effect of perioperative fluid therapy (Brodbelt, 2010). Griffin et al. (2016) highlighted the guidelines for neuter shelters which mention that while peripheral venous access should remain available for all subjects during surgery, IV fluids should be administered only in high-risk patients.

Excess fluids can cause complications such as overloading the circulatory system (Brodbelt et al., 2007), leading to hypervolemia (Sano et al., 2018), oedema/effusion, and disruption of homeostasis. The Lobetti and Lambrechts (2000) study on dogs reported that intraoperative IV fluid is not essential in elective surgeries such as ovariohysterectomy (OHE). However, there were signs of active kidney damage that were undetected by traditional markers in the study. This study did not compare the effect of IV fluid administration in control animals. These signs of ongoing, transient renal damage require further study to determine whether it could have long-lasting effects, i.e., to know whether fluid administration is reno-protective in function or reno-offensive.

2.7 DIAGNOSIS OF RENAL DYSFUNCTION

An ideal renal marker would have all the properties listed in Box 1 (Chen et al., 2019; Cianciolo et al., 2016; Cobrin et al., 2013; Hokamp & Nabity, 2016; Kovarikova, 2015, 2018; Quimby, 2015; Segev, 2018a):

Box 1 Properties of an ideal renal marker

- High sensitivity and specificity
- Present in urine and/or serum
- Non-invasive
- Allow for early and rapid detection
- Structure-specific (tubular or glomerular KD)
- Determine the severity of damage
- Monitor progression of KD
- Provide information on the reparative process
- Help determine the aetiology
- Evaluate prognosis/possible outcome
- Inexpensive and readily available as a point-of-care assay
- Determine the effectiveness of treatment
- Validated use
- Low intra-individual variability
- Constant production and plasma concentration
- No protein-binding, tubular secretion/resorption without catabolism, extra-renal clearance
- Not affected by extra-renal factors such as age, muscle mass, diet etc.

2.7.1 Conventional Indicators of Renal Dysfunction

The current diagnostic tests used to detect KD involve analysing blood and urine parameters (Hokamp & Nabity, 2016). Serum creatinine (SCr), blood urea nitrogen (BUN), and SDMA are indirect GFR indicators measured using blood samples. Urinary parameters analysed while screening for kidney dysfunction include urine protein creatinine ratio (UP:C) and USG. Aside from the tests mentioned here, geriatric cats (> 14 yrs old) can be monitored for progression of renal dysfunction by analyzing serum phosphorus (sP), serum calcium (ionized), and testing the urine concentrating ability (isosthenuria) of the cats (McGrotty, 2008).

Apart from the blood and urine tests, imaging techniques such as ultrasonography can support the diagnosis based on the kidneys' size, architecture, and echogenicity. An additional observation noted was the decrease in urinary pH associated with AKI and CKD

compared to healthy cats (Jing et al., 2020). The gold standard for diagnosing CKD is the renal biopsy, which reveals renal tubular tissue fibrosis and other morphological abnormalities upon histopathological sectioning and examination (Antunes Ribeiro et al., 2020; Cianciolo et al., 2016; Segev, 2018b). It must be noted that changes in the traditional renal markers do not provide information on the renal injury but rather indicate renal dysfunction (Vaidya et al., 2008).

2.7.1.1 Direct GFR Indicators

Estimation of GFR is the most accurate and sensitive index of renal function since it is "directly proportional to functional renal mass" (Cobrin et al., 2013; De Loor et al., 2013; Finch, 2014; Heiene & Lefebvre, 2013; Kovarikova, 2015; Sargent et al., 2021; Von Hendy-Willson & Pressler, 2011). The GFR is measured by monitoring the plasma clearance rate using compounds such as inulin (gold standard), exogenous creatinine or iohexol, all of which are inert molecules that are neither reabsorbed nor secreted by nephrons and can, thus, be used to monitor the functional capacity of kidneys. This ensures that the plasma clearance remains strongly correlated to renal clearance (Kovarikova, 2018; Von Hendy-Willson & Pressler, 2011).

However, the methods used to determine GFR are time-consuming, complicated, expensive, and impractical for clinical use (Cobrin et al., 2013; Von Hendy-Willson & Pressler, 2011), wherein rapid diagnosis is crucial for ensuring a better prognosis. The use of GFR is further limited by the effect of extra-renal factors such as "patient signalment, circadian variation, hydration status, diet and use of sedation" (Von Hendy-Willson & Pressler, 2011). The estimation of GFR would prove to be much more useful were it more accessible, with improved assays that could be used to determine the GFR immediately.

Unlike in humans, using an estimated GFR (eGFR) formula has proven unreliable in cats, partly because of the play of multiple unknown factors which affect GFR, leading to an underestimated eGFR (Finch et al., 2018; Geddes, 2013). By improving our understanding of the risk factors associated with kidney damage in cats, eGFR formulas can be updated and used as a tool for predicting KD.

2.7.1.2 Indirect GFR Indicators

Endogenous SCr, BUN and now SDMA are commonly used renal markers that are inversely proportional to GFR, i.e., an increase in these markers signifies a reduced renal function. They are easier to measure when compared to GFR and are, thus, preferred tools to monitor kidneys in a clinical setting. They can be analyzed at point-of-care with the right equipment and are inexpensive. Unfortunately, several limitations are associated with the use of these conventional markers. These biomarkers are influenced by several extrarenal factors such as hypovolemia, diet, underlying comorbidities and altered filtration leading to inaccurate GFR estimation (De Loor et al., 2013). Details on each of these markers are described below.

Serum Creatinine (SCr)

Creatinine is a byproduct of enzyme-independent phosphocreatine metabolism in the muscle (Geddes, 2013; Lefebvre et al., 2015) and is considered a marker of renal function (Segev, 2018a). It is presumed to be produced at a constant rate and is excreted by the kidneys through filtration as well as tubular secretion (Moran & Myers, 1985), without re-absorption (Lefebvre et al., 2015).

SCr is currently a universally accepted renal biomarker (Segev, 2018a). It is a good indicator of long-term survival in cats with AKI and CKD (Chen et al., 2020; Moran & Myers, 1985). The risk of mortality increases with acute elevation in SCr in AKI-affected individuals (Kanagasundaram, 2015). Unfortunately, SCr is an imperfect marker (Hokamp & Nabity, 2016; Kovarikova, 2015; Quimby, 2015) because its elevated levels are observed only once substantial renal damage has occurred.

Serum creatinine has been observed to remain within the reference range until approximately 75% of the nephrons are non-functional (Hokamp & Nabity, 2016; Kovarikova, 2015), as seen in Figure 7 (Stage three). This is a severe constraint that comes with the use of SCr, especially when it comes to diagnosing early or mild KD. Mishra et al. (2003) found that when subclinical ischemic renal injury was induced in a mouse model,

the SCr levels remained similar to that of the control animals, unlike other newer renal biomarkers (Neutrophil gelatinase-associated lipocalin).

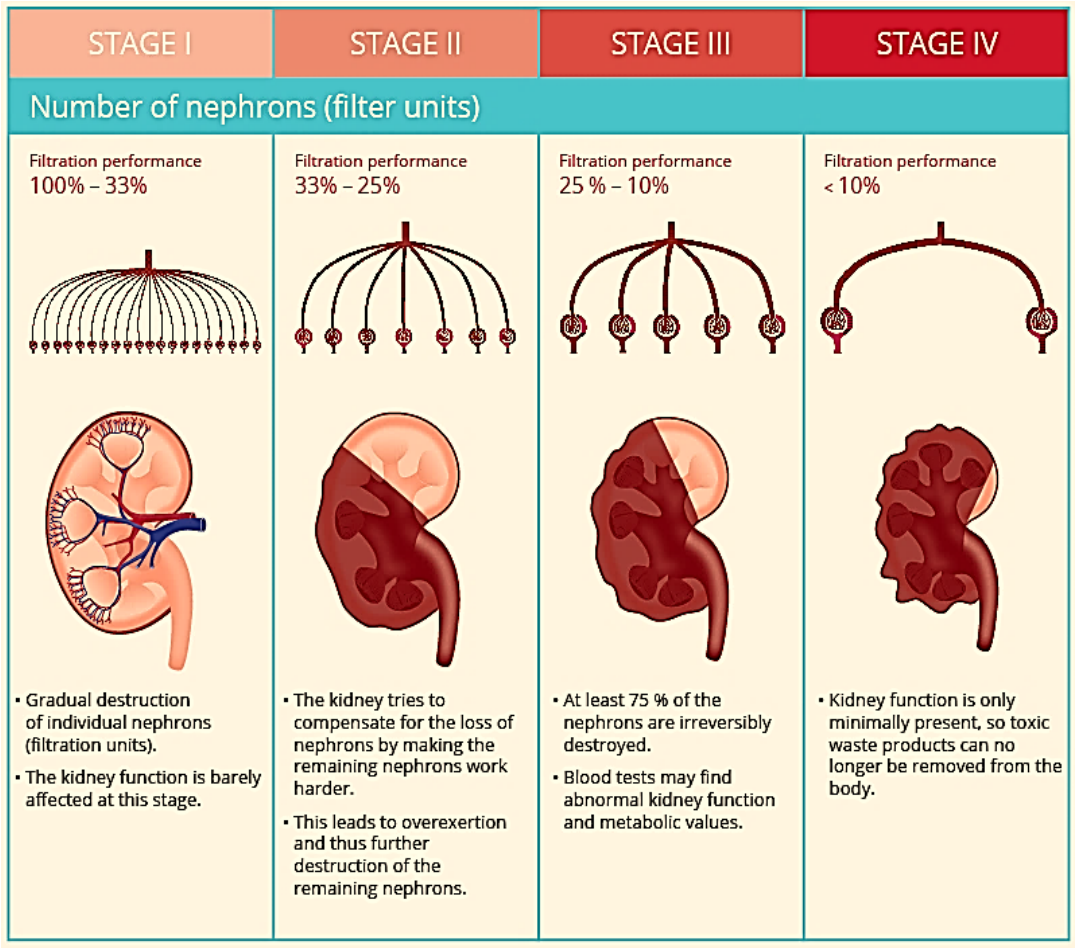


Figure 7 Renal filtration performance across the stages of CKD.

This chart shows the decreasing renal filtration ability and nephronal functionality with increasing severity of CKD. Image reprinted from Boehringer Ingelheim International.

Serum creatinine has a curvilinear, exponential relation with GFR (Geddes, 2013; Lefebvre et al., 2015; Sargent et al., 2021), as shown in Figure 8. In the initial stages of KD, an abrupt fall in GFR will only see minimal changes in SCr concentration (Segev, 2018a; Vaidya, Ferguson, & Bonventre, 2008). However, in the later stages, even slight changes in GFR can result in a significant elevation in SCr concentration (Kovarikova, 2018; Vaidya, Ferguson, & Bonventre, 2008). The SCr concentration is highly increased in

more severe forms of AKI, where the renal function is significantly reduced (Scheemaeker et al., 2020). Furthermore, its concentration is affected by several extra-renal factors.

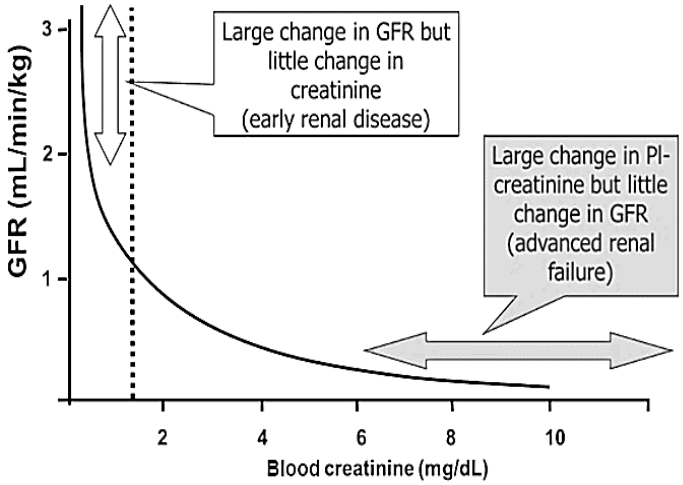


Figure 8 Curvilinear relation between SCr and GFR as seen in dogs (Lefebvre et al., 2015).

In the early stages of renal disease, even if the GFR were to fall drastically, SCr would remain within normal limits due to the compensatory and adaptive mechanisms of the kidney. However, in the advanced stages of renal damage, even a minor change in GFR would see a large change in SCr as the compensatory mechanisms fail.

The limitations associated with the use of SCr to monitor kidney function are listed in Box 2 (Finch, 2014; Hall et al., 2014; Hokamp & Nabity, 2016; Lefebvre et al., 2015; McBride et al., 2019; Pressler, 2015; Quimby, 2015; Segev, 2018a; Vaidya, Ferguson, & Bonventre, 2008).

Box 2 Limitations associated with SCr

- Not indicative of early stages of renal dysfunction
- Does not increase until at least 75% of the renal mass is dysfunctional
- Does not provide information on the site of lesion/damage
- Does not provide information on the severity of the disease until a steady state of dysfunction is maintained
- Wide reference range
- High individuality in small companion animals
- Variations across animal breeds and analytical laboratories
- Influenced by extra-renal factors like muscle mass and age
- Unreliable for monitoring kidney function in cachexic or wasting cats as well as in kittens.
- Influenced by diet, hydration status and blood volume of the subject.
- Circadian variation (cats).

The use of SCr is being reviewed for optimized utilization of the biomarker. Previous research has suggested that even slight variations in SCr, within the reference range, could be indicative of a significant drop in GFR and, thus, kidney function (as cited in Hokamp et al., 2016; Hokamp & Nabity, 2016). Therefore, serial or trending measurement of SCr in an individual animal (fasted) could provide more rapid information on reducing function.

The exception for SCr use is in wasting individuals with end-stage renal damage, where even serial measurements would prove unreliable as creatinine levels would be affected by the increased catabolism in the muscles. Furthermore, serial measurement requires frequent sampling and knowledge of the baseline SCr for the individual, which might prove cumbersome for pet owners.

The reliability of SCr may be improved with the introduction of age- and breed-specific reference ranges (Hokamp & Nabity, 2016). IRIS board members have suggested that persistent upper range values of SCr in normovolemic cats hint at a renal dysfunction and have recommended monitoring these individuals closely (Grauer, 2019). Therefore, while SCr is useful in monitoring KD in its later stages, its use can be improved, and other more reliable early indicators with more characteristics of an ideal marker should be introduced to make up for its limitations.

Blood Urea Nitrogen (BUN)

Urea is produced from the breakdown of ammonia in the liver (Geddes, 2013). It is positively correlated with SCr (Lefebvre et al., 2015) as both are metabolites excreted in urine; however, unlike creatinine, BUN is reabsorbed by the tubules. A steadier rise in BUN than SCr indicates a poor prognosis for the patient (Inaguma et al., 2018). BUN is commonly monitored in conjunction with other biochemical analytes such as SCr, USG, UP:C and SDMA.

While BUN has been shown to have a higher sensitivity than SCr, it has a lower specificity (Finch, 2014), as it is a product of protein catabolism and is, thus, affected by extra-renal factors such as diet, hydration status, biological variability, hepatic function, tubular re-

absorption, and GIT haemorrhage (Finch, 2014). It is, therefore, an unreliable marker for sole monitoring of renal function (Geddes, 2013).

Kidney disease, specifically CKD, is positively correlated with age. An increased prevalence is positively associated with the positive predictive value/PPV (Finch, 2014) and, thus, SCr and BUN can be used for screening purposes in senior cats.

Symmetric Dimethyl-arginine (SDMA)

Methylated arginine is a byproduct of protein metabolism. It is mainly found in three forms within the body: Monomethylarginine (MMA), Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine/SDMA (Chen et al., 2017). SDMA is the most reliable indicator as it is primarily filtered and excreted by the renal system (Brown, 2016). It is an inert protein metabolite (Grauer, 2019) found in several tissues. It is a good addition to the conventional panel of renal markers (Sargent et al., 2021) as it can detect chronic renal dysfunction as early as when ~ 30-40% of the nephrons are affected. SDMA in cats was found to increase over the reference range before SCr in 80.9% of the CKD cases by a mean of 17 months (Hall et al., 2014; Hokamp & Nabity, 2016).

Unlike SCr, SDMA is not influenced by age, weight, body condition (Perez-Lopez et al., 2020), breed, sex, diet, muscle mass (Chen et al., 2017; Segev, 2018a), inflammation, weight loss, comorbidities such as diabetes, or reduced analytical variability (Hokamp & Nabity, 2016; Sargent et al., 2021). SDMA also has a uniform reference range (Hall et al., 2014; Hokamp & Nabity, 2016; Segev, 2018a) and is excreted primarily in urine in unbound form without being subjected to tubular reabsorption. It is believed to be synthesized at a constant rate (Hokamp & Nabity, 2016); these properties make the use of SDMA more advantageous than SCr. However, further studies on the effects of protein catabolism need to be conducted to improve our understanding of its influence on SDMA.

Symmetric dimethyl-arginine shows a positive correlation with SCr and GFR in geriatric cats with KD (Brans et al., 2021; Hall et al., 2014), unlike healthy animals (weaker association). It was found to have a stronger correlation with plasma iohexol clearance

(GFR) than SCr. The sensitivity of SDMA (76-94%) was found to be similar to that of SCr (71-88%); however, its specificity (75-76%) was lower than that of SCr (94-96%). So, the question remains as to whether SDMA is genuinely better than the other classical renal markers in detecting renal dysfunction. Therefore, more studies are required to compare the use of SDMA against other markers.

2.7.1.2.1 Other Variables

Urine Specific Gravity (USG)

Renal injury can result in loss of the ability to concentrate urine, especially when the tubular components of the nephrons are damaged. Therefore, determining the specific gravity of urine, in addition to the use of the conventional renal markers, can help identify renal azotemia (De Loor et al., 2013). If the animal is dehydrated and unable to conserve blood volume through urine concentration, it is likely to be suffering from KD (Watson et al., 2015). Contrary to general expectations, a study on dogs by Antunes Ribeiro et al. (2020) found that the USG did not vary significantly between the different stages of KD. However, cats differ from dogs in their urine concentrating ability.

A study involving thirteen cats presumed to be afflicted with AKI had USG, ranging from 1.005 to 1.065 (Bland et al., 2019). Similarly, a study involving ten cats suffering from CKD found that all had USG < 1.036 (Paepe et al., 2015), however, changes in USG are absent in the early stages of the renal damage because cats are capable of retaining their urine concentrating ability, unlike other species (Geddes, 2013; Paepe & Daminet, 2013). There have been similar observations in studies on cats with KD, with most being isosthenuric, i.e., a USG ranging from 1.007 to 1.015 (Bland et al., 2014; Jing et al., 2020; Paepe & Daminet, 2013).

Serum Electrolytes

Several changes in the serum levels of certain electrolytes such as phosphorus, calcium (Segev, 2018b) and potassium (Paepe & Daminet, 2013) can be seen in AKI and CKD. Hyperphosphatemia is seen in both AKI (Paepe & Daminet, 2013) and CKD; however, this increase in phosphorus levels is more gradual in the latter (Segev, 2018b). It is also correlated with tubular fibrosis (Grauer, 2021). An increase in plasma phosphorus levels by even 1 mg/dl is associated with an increased risk of progression by ~ 41% (Chakrabarti et al., 2012).

Urine protein-creatinine ratio (UPCR or UP:C)

Proteinuria is characterized by fibrosis of interstitial tissue and hypertrophy of the glomeruli (Chakrabarti et al., 2013). Injury to the GFB causes a change in the structure of endothelial cells or podocytes (Menon et al., 2012). Therefore, glomerular damage is one of the causes of proteinuria as it enables the escape of macromolecules from the vascular lumen and into the Bowman's space.

Albumin is a globular protein that is generally absent in urine due to the selective permeability of the glomerulus. The small amount which enters the tubular fluid is reabsorbed in the PCT in healthy animals, and, thus, albuminuria (albumin in the urine) indicates glomerular and/ or tubular damage. Studies in humans have shown that an elevated UP:C is linked to adverse outcomes/poor prognosis (Cianciolo et al., 2016).

Urine protein concentration (UPC) is a quantitative index of total protein in the urine (Eatroff; Paepe & Daminet, 2013) which helps monitor glomerular filtration (Sargent et al., 2021). UP:C was found to vary between the different stages of CKD, with ~ 90% of the cases (dogs) being proteinuric (Antunes Ribeiro et al., 2020). The same study found an increase in UP:C across the stages. An increase in UP:C of 0.1 was associated with a 24% increase in the risk of progression of kidney damage (Chakrabarti et al., 2012). Proteinuria or excess protein in the urine has been suggested to be an inflammatory stimulator that further potentiates the injury.

A UP:C value of > 2.0 indicates glomerular dysfunction (Cianciolo et al., 2016). Cats with borderline proteinuria must be monitored, while those with persistent proteinuria require treatment (Grauer, 2019) as it is associated with the development of CKD (Wang et al., 2017) and mortality (Grauer, 2016). A dog study showed that UP:C was the only traditional renal parameter that was able to detect AKI in parvovirus-affected dogs along with novel biomarkers (van den Berg et al., 2018). However, 95% of the cases had a UP:C below 2.0 which did not help in pinpointing the site of damage, i.e., distinguishing between glomerular and tubular damage.

Limitations with UP:C analysis includes a low specificity (Grauer, 2016; Panboon et al., 2017) as there can be several causes for proteinuria (Cianciolo et al., 2016; Paepe & Daminet, 2013), such as haemorrhage, cystitis, pyuria and systemic or postrenal inflammatory conditions (Geddes, 2013). However, they can be added to the panel of renal indicators or predictors of renal damage as they provide helpful information to aid in timely intervention.

Miscellaneous

While a valuable tool in detecting the presence of kidney injury, urine sediment examination is neither sensitive nor specific when used alone. However, it was found that the presence of renal cell casts in the urine can provide specificity of ~ 90% for AKI (Schinstock et al., 2013). Thus, it is a valuable addition to the diagnostic tools when it comes to examining urine samples.

Ultrasonography may reveal hyperechoic cortices with or without an abnormal appearance in AKI (Segev, 2018b) and contracted kidneys in case of CKD (Segev, 2018b; Wang et al., 2017). These findings can help finalise the diagnosis of KD in clinically ill patients.

Innovative Algorithms

Recent trends in monitoring and screening renal function using artificial intelligence (AI) have proven to help predict renal dysfunction and its progression. An example of one such

AI is RenalTech, which studies the changes in USG, urine pH, creatinine, BUN, and WBC count. It was used to successfully predict CKD in a patient (as cited in Clinician's brief). However, this technology is in its early phases of development.

Conclusion

Early and rapid diagnosis of KD is difficult due to imperfect diagnostic tools as observed with most reported clinical cases, which are diagnosed nearing the end stage where the damage could be irreversible or the treatment options unviable, i.e., renal replacement therapy (Segev, 2018a). It is pivotal that we develop better renal biomarkers which allow for earlier interventional therapy at stages where KD can be reversed (Segev, 2018a) or can cease/reduce the rate of progression, thereby improving the patient's quality of life while at the same time reducing both the mortality and the morbidity rate (Hokamp & Nabity, 2016; Kovarikova, 2015). Further, improvement in this area will help narrow down the aetiological causes of KD (Pressler, 2015). This would greatly help ensure reduced exposure to the inducers and reduce the incidence of KD.

2.7.2 Novel Indicators

Identifying novel renal biomolecules in urine at elevated concentrations in individuals suffering from kidney damage is now gaining momentum in the renal research field, as it has the potential to identify structural damage in its early stages of pathological changes. The protein levels in the urine of a healthy animal are maintained at a low level, and an increase in specific protein levels can be attributed to either glomerular and/or tubular dysfunction. When the GFB is damaged (glomerular damage), it becomes porous, leading to leakage of plasma proteins into the ultrafiltrate. In tubular dysfunction, the tubular cells fail to reabsorb the filtered protein, which can be seen when the transport receptors (megalin, cubilin) are overloaded (De Loor et al., 2013) or when the epithelium is damaged. Therefore,

the resulting increase in these proteins in urine can provide a clearer idea of the site of the damage.

Tests designed to identify these proteins are more specific than the traditional markers as the former are intrinsic to renal structure, unlike the latter, which reflect the clearance rate (renal function). Several human and animal studies have observed increments in these proteins even before SCr elevation (Segev, 2018a; van den Berg et al., 2018). Serum/plasma biomarkers of renal injury are suggested to be less sensitive than urinary biomarkers (Pressler, 2015), especially since urinary proteins are intrinsic to renal structure and metabolism.

Generally, the presence of high molecular weight proteins (> 100 kDa) or Intermediate-Molecular Weight proteins (IMW; 40-100 kDa) in urine signifies glomerular damage and is observed as overt proteinuria; whereas the presence of low molecular weight proteins (< 40 kDa) or enzymes in urine is indicative of tubular damage (Hokamp & Nabity, 2016), which is observed as mild proteinuria.

Advantages of using novel urinary biomarkers include (De Loor et al., 2013): early detection of kidney injury (acute, stable or progressive state), determining the pathological region in the nephrons, as well as improving our understanding of kidney damage along with improved profiling of the different stages and grades of KD.

2.7.2.1 Urinary Tubular Proteins

The renal tubules are metabolically active sites of secretion, reabsorption, and production in the nephron. However, these regions are at a higher risk of damage. Upon injury, the tubular epithelial cells undergo pathological changes such as desquamation, cell rupture, or cell death, impairing their normal function. These changes result in impaired re-absorption (in PCT), the release of intracellular proteins and increased protein turnover (Cianciolo et al., 2016), leading to an increase in urinary tubular protein concentration that is generally absent or present in low quantities in urine.

The impairment of tubular reabsorptive function allows for an increase in Retinol Binding Protein (RBP), and Neutrophil-gelatinase Associated Lipocalin (NGAL) concentrations in urine. Increased protein turnover or up-regulation results in increased Kidney Injury Molecule-1 (KIM-1) and NGAL concentrations, while sloughing off of damaged tubular epithelial cells and cellular contents leads to increased luminal N-Acetyl beta-d-Glucuronidase (NAG) enzyme and KIM-1 concentrations in urine. These processes resulting in increased levels of these proteins in urine can help detect tubular dysfunction.

Most urinary biomarkers are normalized to urinary creatinine (uCr) concentrations to account for variations caused by urine flow rate. The kidneys regulate the water content in the tubular fluid according to the body's needs. Thus, the concentration of any biomarker depends on the excretion rate of urine, its volume, concentration, and flow rate. It is surmised that UCr excretion remains constant for an individual in a steady state (Kovarikova, 2015; Waikar et al., 2010). The biomarker excretion is linearly proportional to UCr excretion (Waikar et al., 2010), and normalization will allow for spot sample collection rather than 24-hour urine collection (Pressler, 2015).

In contrast to animals with a constant UCr excretion rate, Waikar et al. (2010) suggested that standardizing the markers in patients with conditions affecting the GFR rate may lead to inaccurate estimation of biomarker excretion. Therefore, this would not be recommended in those animals with inconsistent UCr or GFR values, such as in AKI (Boyd et al., 2019; Waikar et al., 2010). However, subclinical active damage may not have as potent an effect on GFR and UCr excretion; therefore, its use in determining the excretion rate of a biomarker may provide an accurate description of renal structure. Further studies are required to ascertain the need to normalize urinary biomarkers in a mild kidney injury state.

Kidney Injury Molecule-1 (KIM-1)

Synonyms: Hepatitis A virus-cell receptor-1 (HAVCR-1), T-Cell Immunoglobulin-1 (TIM-1), Cochlear Injury molecule-1 (CIM-1)

Kidney Injury Molecule-1 is a transmembrane glycoprotein (phosphatidylserine receptor; LMW) found primarily in the dedifferentiated cells (Vaidya, Ferguson, & Bonventre, 2008) of the straight segment (S3) of PCT. Interestingly, the S3 segment of PCT lies in the outer medulla and is, thus, majorly supplied by venous blood from the vasa recta (Seldin & Giebisch, 1985), making these tubular segments more susceptible to hypoxic injury than the other segments of PCT. The cells of the S3 have a higher number of peroxisomes and lysosomal vesicles, and a lower number of mitochondria (small).

The S3 cells are more susceptible to ischemic insult (Bland et al., 2017; Schmiedt et al., 2016). It has been suggested that KIM-1 plays a role in cell adhesion. It is physiologically upregulated for phagocytic and apoptotic cell clearance mechanisms (Gibbs et al., 2019), explaining its high energy profile. It is also said to be involved in the regenerative capacity of the kidney (Lim et al., 2013).

The KIM-1 concentration in urine was similar across neutered and intact cats, with healthy individuals having consistently low levels of urinary KIM-1 when analyzed using a lateral flow assay kit (Bland et al., 2019). A previous study by Bland et al. (2017) supported this finding as immunohistochemistry (IHC) of renal segments revealed a complete absence or faint staining (KIM-1 indicative) in S3 of healthy cats. The low KIM-1 levels in healthy cats have been attributed to normal turnover, thereby regularly releasing the protein into the luminal fluid.

Interestingly, Bland et al. (2017) revealed that in cats with KD, IHC staining was intense three days post-exposure to renal ischemia across the tubular cells and within the tubular lumen, supporting the finding that KIM-1 levels are increased in urine in response to tubular injury. However, the increase in KIM-1 may be proportional to the severity of damage (desquamation or cell death), with milder injuries having a steadier increase in KIM-1.

Kidney Injury Molecule-1 is being used as a biomarker in human studies to monitor renal damage as its levels increase by 3 to 100-fold in injured cells. Similar results have been observed in cats (Bland et al., 2014) with correlation to the severity of the disease and, thus, are aptly described as "sensitive tissue indicators of AKI", but not for CKD (Bland et al., 2014; Bland et al., 2017), similar to what was observed in human medicine (Vaidya, Ferguson, & Bonventre, 2008).

Bland et al. (2019) found that cats with sepsis and post-renal AKI were more likely to show a transient increase in KIM-1 levels, likely due to the increased pressure/damage to the tubules. It was suggested that a serial comparison of KIM-1 for the same individual would prove to be more informative when used to identify damage since the KIM-1 values were found to overlap, in some cases, between the healthy cats and those suspected of AKI or CKD.

Kidney Injury Molecule-1 was not correlated to SCr; however, it was implied that the protein might be influenced by urine flow, type and severity of the tubular injury, and renal perfusion (Bland et al., 2019). Further, it was shown to identify renal damage in cats who had SCr within the reference range (Bland et al., 2014). Thus, KIM-1 appears to have promising results in determining renal structural damage, especially AKI (three-fold increase) and can be used to monitor the transition of AKI to CKD. However, more studies are required to determine the cutoff values and validate various kits, especially the non-invasive LFA spot kit (Bland et al., 2019).

Neutrophil Gelatinase-Associated Lipocalin (NGAL)

Synonyms: Lipocalin 2 (LCN2), Siderocalin, Uterocalin and 24p3 (Giasson et al., 2011)

Neutrophil Gelatinase-associated Lipocalin is a glycoprotein (LMW) with reno-protective properties (Gibbs et al., 2019; Kuwabara et al., 2009). It is found within the cytoplasmic granules of neutrophils and other tissues (Segev, 2018a). It maintains the innate immune system as an acute-phase protein (Giasson et al., 2011) by binding to siderophores, effectively removing free iron to prevent bacterial growth (Gibbs et al., 2019). This

mobilization of iron molecules into cells prevents the formation of free radicals, thereby allowing NGAL to function as an antioxidant. While NGAL has been identified in serum and urine, urinary NGAL (uNGAL) is more sensitive to renal damage than the former (Wang et al., 2017).

Several forms of NGAL have been discovered: 25 kDa monomer, 45-50 kDa dimer and NGAL/matrix metalloproteinase-9 (NGAL/MMP-9) heterodimer complex (Hokamp & Nabity, 2016; Wu et al., 2019). The monomeric molecule is associated with renal injury (renal tubular cells), while the dimeric protein is primarily released by neutrophils. The heterodimer is found to occur with either one of the conditions and with hematuria.

It has been suggested by Kuwabara et al. (2009) that the increase in uNGAL of mice is primarily caused by impaired renal re-absorption due to tubular damage, aside from its up-regulation. There was an increase in a specific mRNA expression in the ascending loop of Henle (a susceptible portion of nephron) in mice with post-renal AKI, lasting for two weeks post-exposure. Similarly, Mishra et al. (2003) found a positive correlation between the dose-duration of an ischemic episode in mouse and rat models with uNGAL, produced by the PCT cells, thereby supporting the hypothesis that uNGAL elevation is proportional to the level of injury.

The concentration of uNGAL can be used to monitor pathology in the GFB and tubular component of the nephron (Kuwabara et al., 2009). This biomarker may be more sensitive to the site of the lesion and a possible early predictor of renal injury. It may also be capable of identifying and monitoring early renal damage as well as CKD progression in humans, lab animals and dogs.

In the mouse study by Mishra et al. (2003), uNGAL was detected as early as 4-6 hours post-exposure to subclinical ischemic renal injury in mice. In contrast, the SCr levels remained the same as that of the control animals. uNGAL was even detected two hours after the ischemic episode in severe renal ischemia. The time taken for an increase in uNGAL concentration is related to the duration of ischemia. Similarly, in a review on renal markers, Kovarikova (2015) stated that uNGAL was expressed in significant amounts in animal models suffering from a renal injury caused by ischemia. uNGAL was elevated, unlike

SCr, in dogs with IRIS grade I AKI compared to the control group in a study by Scheemaeker et al. (2020).

A three-fold increase in human uNGAL levels was reviewed to predict AKI (Boyd et al., 2019), similar to what is expected for KIM-1. Cats with AKI and pyuria had the highest urine NGAL creatinine ratio/UNCR (Wu et al., 2019). UNCR was found to precede the increase in SCr by a median of 2 days in gentamicin-induced AKI in dogs with no comorbid conditions (Palm et al., 2016).

Palm et al. (2016) showed that uNGAL is specific to kidney injury and can even detect the recovery stage of AKI in dogs. It may be an early marker of AKI in dogs (Segev, 2018a; Segev et al., 2013), capable of differentiating the azotemic and non-azotemic forms of KD. This biomarker was also found to detect AKI in those dogs with parvovirus, unlike the traditional markers (van den Berg et al., 2018).

Like in dogs, cats with AKI have been shown to have significantly high UNCR values when compared to CKD-affected and healthy cats (using Western blot analysis with rabbit anti-canine Ab). Cats with CKD have higher values when compared to healthy cats (Wu et al., 2019). The evidence mentioned above suggests that uNGAL analysis might help determine different stages and conditions of KD.

Upon study, uNGAL was shown to be a useful marker in predicting CKD progression, especially in its later stages (3 and 4) in cats (Wang et al., 2017). There is a direct correlation between uNGAL and UNCR, with the latter being a better prognostic indicator of CKD.

Increases in monomeric uNGAL have been associated with BUN, serum Phosphorus and SCr, with AKI-affected cats having higher concentrations of the same when compared to those with CKD. The dimeric form is related to UTI, while the heterodimer was also related to renal disease (Wu et al., 2019). Interestingly, both the monomeric and dimeric forms were absent in healthy cats.

However, some studies have shown that uNGAL concentration increases in inflammatory conditions (neutrophil influx) such as pyuria in humans and cats (Vaidya, Ferguson, & Bonventre, 2008; Wu et al., 2019). This increase could signify both neutrophil concentration

in locally inflamed tissue leading to upregulation of NGAL and/or as a predictor of undetected renal damage such as pyelonephritis (Segev, 2018a). There is a slight probability of renal disease going undetected in lower urinary tract disease cases (Cowgill et al., 2016); however, this requires further study to know if there is a link between lower tract disorders and renal damage.

Studies have used immunoassays (Sandwich ELISA) with anti-canine NGAL Antibodies as they have been found to positively cross-react with feline proteins (~ 74% similar to canine NGAL protein) and have been reported to be successful (Wang et al., 2017). Further studies must be conducted to determine and validate assays for cat urine samples specific to monomeric uNGAL (renal tubular injury) in conditions involving the kidneys.

Retinol Binding Protein (RBP)

Retinol Binding Protein is a lipocalin (LMW) synthesized primarily in the liver (Vaidya, Ferguson, & Bonventre, 2008). It is closely involved with Vitamin A1 (retinol) transport, and it circulates in the bloodstream either in its bound or unbound form (Segev, 2018a). RBP is bound to transthyretin (55 kDa), and this form constitutes ~ 90% of the total RBP circulating in the body. The unbound form enters the tubular fluid, is catabolized, and then reabsorbed by the PCT cells (De Loor et al., 2013; Hokamp & Nabity, 2016; Kovarikova, 2015; Vaidya, Ferguson, & Bonventre, 2008). The absence of reports on urinary RBP (uRBP) in healthy cats can be, due to, either the complete re-absorption of RBP present in the ultrafiltrate or because of urine concentrations below the sensitivity of the assays used in these experiments.

The study by Raila et al. (2001) observed RBP in PCT cells in the sub-apical cytoplasmic vesicles of the epithelial PCT cells of healthy cats on IHC, similar to KIM-1. This biomarker has been reported to be synthesized at a constant rate in humans (Vaidya, Ferguson, & Bonventre, 2008), uninfluenced by other factors in contrast to SCr and BUN. Its presence in urine indicates early tubular damage (Segev, 2018a). It is a more sensitive marker of PCT

damage than the NAG enzyme, another novel renal marker, in humans (Vaidya, Ferguson, & Bonventre, 2008).

The presence of uRBP is attributed to an alteration in the endocytic capacity of the PCT cells (van Hoek et al., 2008). A study in dogs with X-linked hereditary nephropathy by Nabity et al. (2012) noted RBP to be the sole indicator to increase with disease progression. It was correlated with KD progression (AKI and CKD), proportional to the severity of tubular injury (Hokamp et al., 2016).

The study by van Hoek et al. (2009) used uRBP to identify CKD in hyperthyroid cats. Furthermore, they used the biomarker to show the effectiveness of hyperthyroidism treatment in reversing suspected renal damage and has been used as an indicator of reversible tubular dysfunction. Failure of re-absorption could also be due to excess proteins in the tubular fluid, thus, resulting in saturation of the tubular endocytic mechanism.

Human RBP sandwich ELISA (Immunodiagnostik AG, Germany) has been vetted for cat sample analysis (Perez-Lopez et al., 2020; van Hoek et al., 2008). Studies have shown RBP levels to be undetectable in the urine of healthy cats using Western blot (Hokamp & Nabity, 2016). Polyclonal rabbit-human uRBP Ab commercial sandwich ELISA was used in the study by van Hoek et al. (2009).

The concentration of uRBP remains uninfluenced by body condition and urinary pH (Perez-Lopez et al., 2020; Vaidya, Ferguson, & Bonventre, 2008). However, its concentration is unreliable in conditions such as haematuria and proteinuria. Furthermore, RBP in cats with AKI or CKD, without any underlying comorbidity, must be studied to determine its potential as a marker of tubular damage and determine the influence of Vitamin A deficiency on marker concentration.

2.7.2.2 Enzymuria

Enzymuria essentially occurs when there is an increase in enzyme concentrations in urine due to the damage of the brush-border cells in the nephron (Rankin & Valentovic, 2014). These elevated concentrations can signify cellular damage and, thus, can act as a prognostic indicator of organ dysfunction or mild injury.

N-acetyl β-D-glucosaminidase (NAG) enzyme

N-acetyl β-D-Glucosaminidase enzyme is a lysosomal enzyme (HMW) found in cells, sera, and urine. It plays an integral role in the catabolism of glycoproteins/glycosaminoglycans in the renal tubular epithelium (Panboon et al., 2017; Pressler, 2015). It is present in copious amounts within the nephron's PCT cells (Jepson et al., 2008; Kovarikova, 2015) as a result of increased lysosomal turnover (De Loor et al., 2013). Although NAG is found in other tissues as well, however, due to its HMW, serum NAG is not filtered into the tubular fluid.

There are two forms of the isoenzyme-, i.e., NAG A and NAG B, both of which are present in the kidneys of cats (Sato et al., 2002), although higher elevation is observed with the latter in response to KD. NAG-A is intra-lysosomal in location, and NAG-B is found in the lysosomal membrane. While KD increases urinary NAG-A and NAG-B concentration, the primary element released at the tubular injury is NAG-B (Geddes, 2013; Sato et al., 2002). Experimentally induced AKI (sulfonamide) in cats showed an increase in NAG index (urinary NAG levels normalized to creatinine) within 24 hours, suggesting it to be a sensitive indicator of renal tubular damage (Sato et al., 2002).

When the renal parenchyma is damaged, particularly the tubular brush border cells, the NAG enzyme is released into the tubular lumen and excreted in concentrations two to threefold above the baseline (Grauer, 1996), similar to the other novel markers. Urinary NAG (uNAG) provides information on structural damage rather than functional impairment. NAG index was higher in cats with azotemia than those without and was decreased in cats

treated for azotemia (Lapointe et al., 2008). This reveals that it is a good marker for determining the effectiveness of treatment.

There was a weak correlation between NAG Index with SCr (Jepson et al., 2010; Sato et al., 2002) and BUN. The NAG index was shown to increase before the latter two in cats with CKD (Sato et al., 2002). Studies in humans (Vaidya, Ferguson, & Bonventre, 2008), dogs and cats have shown that this elevation in NAG activity can occur before changes in SCr are observed (Bishop et al., 1991). Similarly, an increase in urinary NAG activity was found to precede the elevation of SCr and UP:C in several renal conditions (Kovarikova, 2015), with a constant level of excretion in the mid and late stages of KD (Hokamp et al., 2016).

The NAG index in cats is assumed to rise due to lysosomal activity, stimulated by proteinuria rather than tubular damage in cats suspected of CKD (Hokamp & Nabity, 2016; Jepson et al., 2008, 2010). This assumption is plausible since a positive correlation was found between log UP:C and log NAG index in conditions affecting the urinary bladder, although values remained within the assumed reference range for cats (Panboon et al., 2017; Sato et al., 2002). A similar significant correlation between log NAG index and log UP:C was observed in studies by Jepson et al. (2008), Bishop et al. (1991) and Jepson et al. (2010).

The NAG activity is influenced by (Jepson et al., 2008, 2010; Uechi et al., 1998): alkaline urine (pH > 8.0), sample storage conditions, and method of analysis. Urea was found to negatively impact NAG activity in humans (Vaidya, Ferguson, & Bonventre, 2008). This biomarker is uninfluenced by age, sex (Jepson et al., 2008; Lapointe et al., 2008; Sato et al., 2002) and temperature. Circadian variation in uNAG was absent in cats (Panboon et al., 2017; Uechi et al., 1998).

Several studies have used colourimetric assays to evaluate NAG levels in cats with CKD, and this has helped in the validation of the assay (Jepson et al., 2008, 2010; Lapointe et al., 2008; Panboon et al., 2017; Sato et al., 2002; Uechi et al., 1998). Further studies will help determine the cutoff values for NAG and prepare spot analysis techniques.

Table 3 Summary of renal markers. Modified from Hokamp and Nabity (2016).

RENAL MARKERS	PRODUCTION SITE	MOLECULAR WEIGHT	STRUCTURAL/FUNCTIONAL INDICATOR	VALUES IN CATS	AKI/CKD	NON-RENAL INFLUENCES
SCr	Muscle	113 Da	Functional indicator (indirect GFR)	< 140 µmol/L (IRIS guidelines)	Both	Muscle mass, breed, age, analytical method, diet, hydration, blood volume, individual variation.
BUN	Hepatocytes	60 Da	Functional indicator (indirect GFR)	14-36 mg/dl	Both	Diet, tubular re-absorption in response to homeostasis, biological variation.
Albumin (negative active phase protein)	Hepatocytes	66-69 kDa	Glomerular damage (Structural indicator)	-	CKD	High BP, cardiac or liver conditions; age, body mass; dehydration, stress, exposure to cold.
SDMA (methylated amino acid)	Nucleated cells	202 Da	Functional indicator (indirect GFR)	< 15 µg/dl	CKD	Biological variation.
KIM-1 (transmembrane glycoprotein)	PCT cells of nephron (S3 segment)	30 kDa	Tubular damage (involved in regeneration & upregulation)	Undetected in healthy cats. KIM-1 ratio: 4.8-8.8[a]	AKI sensitive indicator	-
NGAL (glycoprotein)	Neutrophils, kidney, stomach, lungs, small intestine, pancreas, prostate gland, thymus	25 kDa	Tubular damage (decreased re-absorption & reno-protective upregulation).	uNGAL ranges from 0.46[c]- 0.95[d] ng/ml in cats. UNCR (*10^{-6}) ranges from 0.17[‡]-0.23[§] in cats.	AKI and CKD progression (stages 3 and 4).	Inflammation (UTI/pyuria); neoplasia; hematuria, carcinoma, lymphoma;

[a] Bland, S. K., Cote, O., Clark, M. E., DeLay, J., & Bienzle, D. (2014, Sep-Oct). Characterization of kidney injury molecule-1 in cats. *J Vet Intern Med, 28*(5), 1454-1464. https://doi.org/10.1111/jvim.12428

[c] Wu, P. H., Hsu, W. L., Tsai, P. J., Wu, V. C., Tsai, H. J., & Lee, Y. J. (2019, Aug 27). Identification of urine neutrophil gelatinase-associated lipocalin molecular forms and their association with different urinary diseases in cats. *BMC Vet Res, 15*(1), 306. https://doi.org/10.1186/s12917-019-2048-9 -

[d] Wang, I. C., Hsu, W. L., Wu, P. H., Yin, H. Y., Tsai, H. J., & Lee, Y. J. (2017, Jan-Feb). Neutrophil Gelatinase-Associated Lipocalin in Cats with Naturally Occurring Chronic Kidney Disease. *JOURNAL OF VETERINARY INTERNAL MEDICINE, 31*(1), 102-108. https://doi.org/10.1111/jvim.14628

RENAL MARKERS	PRODUCTION SITE	MOLECULAR WEIGHT	STRUCTURAL/FUNCTIONAL INDICATOR	VALUES IN CATS	AKI/CKD	NON-RENAL INFLUENCES
			GFB damage (mice)[b]	In dogs, uNGAL ranged from 0.4-11 ng/ml and UNCR ($*10^{-8}$) from 1-46[e]	UNCR: AKI > CKD > Normal	
RBP	Liver (principal source), kidney, lungs, stomach, brain, spleen, heart, skeletal muscle	21 kDa	Decreased tubular re-absorption and/or glomerular damage	Undetected in the urine of healthy cats. Hyperthyroid cats with KD: 10.6 ± 8.2 µg/L[f].	AKI/CKD; Monitor progression of CKD.	Hematuria; pyuria/bacteriuria;
NAG (lysosomal enzyme)	PCT and other cells	150 kDa	Glomerular leakage or increased lysosomal turnover in tubular cells	NAG activity ranged from 1.06[g]- 22.1[h] U/L. NAG index ranging from 1[i]- 13‡‡ U/g.	AKI	Alkaline urine; hematuria; hemoglobinuria;

[b] Kuwabara, T., Mori, K., Mukoyama, M., Kasahara, M., Yokoi, H., Saito, Y., Yoshioka, T., Ogawa, Y., Imamaki, H., Kusakabe, T., Ebihara, K., Omata, M., Satoh, N., Sugawara, A., Barasch, J., & Nakao, K. (2009, Feb). Urinary neutrophil gelatinase-associated lipocalin levels reflect damage to glomeruli, proximal tubules, and distal nephrons. *Kidney Int, 75*(3), 285-294. https://doi.org/10.1038/ki.2008.499

[e] Steinbach, S., Weis, J., Schweighauser, A., Francey, T., & Neiger, R. (2014, Mar-Apr). Plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) in dogs with acute kidney injury or chronic kidney disease. *J Vet Intern Med, 28*(2), 264-269. https://doi.org/10.1111/jvim.12282

[f] van Hoek, I., Meyer, E., Duchateau, L., Peremans, K., Smets, P., & Daminet, S. (2009, Sep-Oct). Retinol-Binding Protein in Serum and Urine of Hyperthyroid Cats before and after Treatment with Radioiodine. *JOURNAL OF VETERINARY INTERNAL MEDICINE, 23*(5), 1031-1037. https://doi.org/10.1111/j.1939-1676.2009.0364.x

[g] Jepson, R. E., Vallance, C., Syme, H. M., & Elliott, J. (2010, Feb). Assessment of urinary N-acetyl-beta-D-glucosaminidase activity in geriatric cats with variable plasma creatinine concentrations with and without azotemia. *Am J Vet Res, 71*(2), 241-247. https://doi.org/10.2460/ajvr.71.2.241

[h] Uechi, M., Uechi, H., Nakayama, T., Wakao, Y., Ogasawara, T., Takase, K., & Takahashi, M. (1998, Sep). The circadian variation of urinary N-acetyl-beta-D-glucosaminidase and gamma-glutamyl transpeptidase in clinically healthy cats. *J Vet Med Sci, 60*(9), 1033-1034. https://doi.org/10.1292/jvms.60.1033

[i] Kongara, K., Cave, N., Weidgraaf, K., & Rao Dukkipati, V. S. (2020, Sep). Effect of non-steroidal anti-inflammatory drugs on glomerular filtration rate and urinary N-acetyl-beta-D-glucosaminidase activity in cats after dental surgery. *Vet Anaesth Analg, 47*(5), 631-636. https://doi.org/10.1016/j.vaa.2020.04.014

2.8 CONCLUSION

As summarized by the review, there is a great need for newer and improved diagnostic measures to detect early renal injury to reduce the morbidity and mortality caused by KD, determine its true prevalence, improve the prognosis along with patient care, and the owner's confidence in treatment (as cited in Clinician's brief). We require markers that can help identify the stages of KD that generally go unnoticed, such as stage 1 CKD (Chen et al., 2019) and the initial phase of AKI. Furthermore, an improved panel of renal biomarkers would greatly help to improve our understanding of putative factors (Chen et al., 2017), the development of kidney dysfunction (Gibbs et al., 2019), determining the aetiological causes and drug safety assessment studies (Vaidya, Ferguson, & Bonventre, 2008).

With the increasing prevalence of KD in small companion animals, the common factors must be scrutinized to identify the root cause (-es). The growing regularity of sedation/anaesthetic drug use and elective surgeries in veterinary practice leads to questions regarding its safety in the long run. Studies on anaesthetics are performed for various reasons: identifying anaesthetic agents, such as those which are organ-protective (Motayagheni et al., 2017) and those correlated to perioperative mortality (Steagall, 2015). Such studies are paramount for selecting safe agents that have lower odds of causing a negative outcome.

Bound by their limitations, the conventional markers cannot provide the required information on perioperative renal damage. Using improved/novel markers can provide new information that can be applied to determine the renal threshold and safer anaesthetic protocols (Gibbs et al., 2019) that will ensure that renal damage doesn't exceed that threshold.

No one marker of renal damage can provide all the required information on kidney dysfunction, damage or survivability (King et al., 2007). The use of a panel of selective renal markers (SCr, SDMA, BUN, UPC, sP, USG) will, thus, provide a better assessment of kidney function. Hokamp et al. (2016) found conventional markers to perform on par with specific novel biomarkers in detecting renal damage in cats. However, it must be noted

that the novel biomarkers help in gleaning information that traditional markers do not provide. We need markers to provide early, accurate, and more detailed information on the injury's type, status, severity, and location. For example, SCr or SDMA for function and KIM-1 for AKI, and NAG for determining the effectiveness of treatment.

While novel markers are still being studied to determine their usefulness in monitoring the kidneys, the results do seem promising. Further research in these grey areas could develop the point-of-care analysis of samples to determine novel marker concentrations, as was done for SDMA. Research into identifying and categorizing renal markers for differing purposes, such as screening or determining the efficacy of treatment, will enable more efficient handling of KD cases. Cowgill et al. (2016) theorized that SCr and SDMA might be better indicators of steady-state CKD, while novel biomarkers may prove ideal for identifying active injury in progressive CKD.

Compared to studies in dogs, there is a paucity in the number of studies conducted on cats. Some variations have been observed between species. Thus, further research is required to help identify new and improved biomarkers, validate commercial assays (Segev, 2018b), and introduce point-of-care assays for all species.

The effect of different renal diseases on these renal markers must be analyzed to help improve our understanding of pathological and diagnostic development. Similarly, the variations observed in renal markers when individuals with KD also suffer from other maladies such as hyperthyroidism, diabetes mellitus, metabolic disorders, neoplasia etc., must be determined.

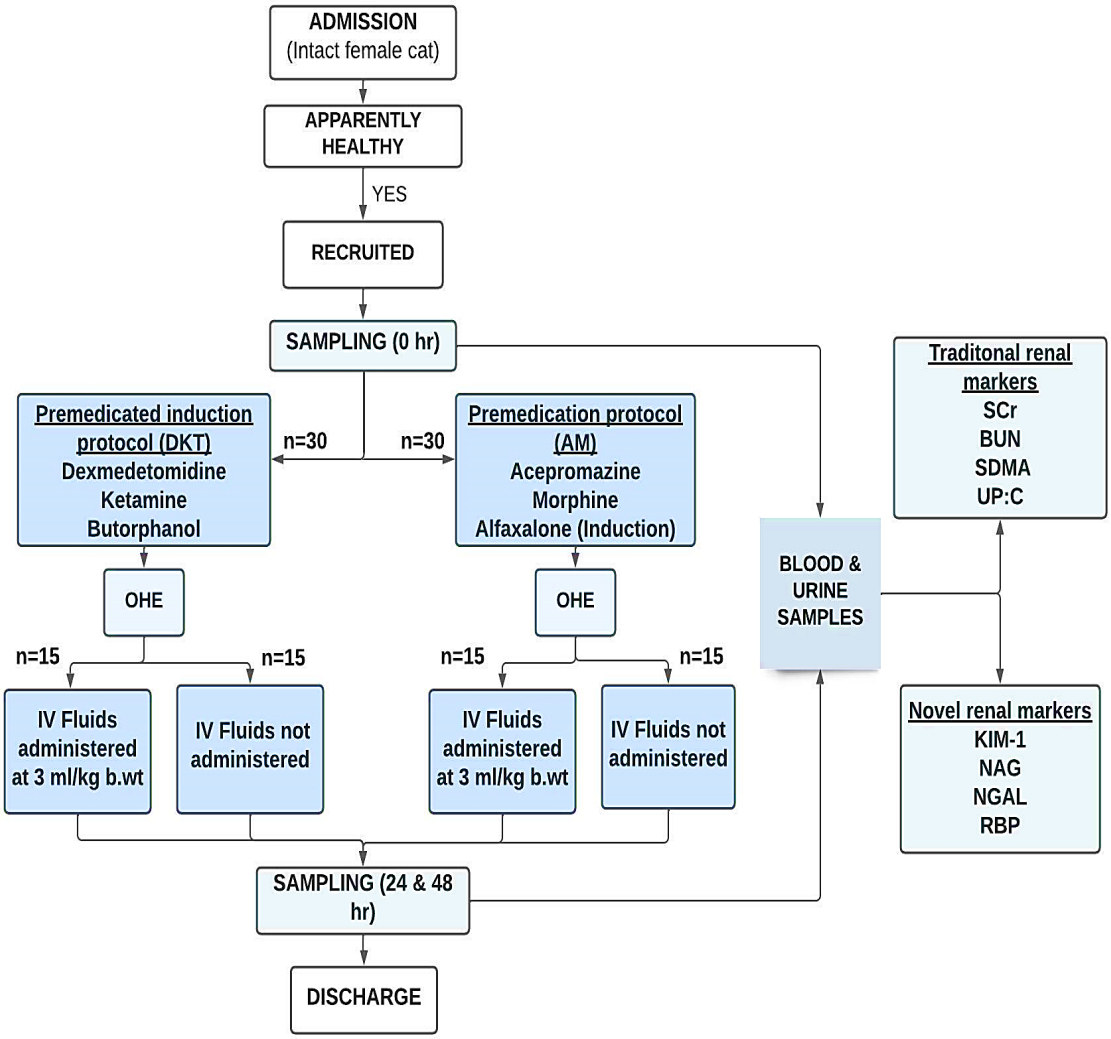
2.9 RESEARCH QUESTIONS

This thesis examines the effects of general anaesthesia and IV fluid administration on both traditional and novel renal markers in apparently healthy adult cats undergoing routine ovariohysterectomy. Two commonly used anaesthetic premedication and induction drug protocols were assessed in this study: (1) acepromazine, morphine and alfaxalone and (2) dexmedetomidine, ketamine and butorphanol.

3 MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

In this randomised controlled trial, 60 female cats, admitted to Massey University Veterinary Teaching Hospital for elective ovariohysterectomy (OHE), were recruited as subjects after obtaining an informed and written owner consent. The cats were randomly allocated to one of four treatment groups (n=15/group), as shown in Flowchart 1. Each treatment group was given a specific combination of premedication and induction agents before the spay procedure, with or without intravenous fluids.



Flowchart 1 Experimental design of this study.

Our study employed some of the most commonly used anaesthetic agents (induction agents) in New Zealand for cats, as reported by Sano et al. (2018) and Gates et al. (2020). Each group received one of two anaesthetic protocols, with or without intravenous fluids throughout anaesthesia and surgery, as summarized in the flowchart and Table 4. The two anaesthetic protocols adopted for the study are as follows: for the AM protocol, we used Acepromazine (Acezine 2; 2 mg ml^{-1}; Ethical agents Ltd., New Zealand), Morphine sulphate injection BP (Hospira Australia Pty. Ltd., Australia) 5 mg ml^{-1}, and Alfaxalone (Alfaxan; 10 mg ml^{-1}; Jurox, New Zealand). For the DKT protocol, we used Dexmedetomidine hydrochloride (Dexdomitor; Zoetis, New Zealand), Ketamine hydrochloride, and Butorphanol (ilium Butorgesic; 10 mg ml^{-1}; Troy Animal Healthcare, Australia). The depth of anaesthesia was maintained during surgery using isoflurane.

Blood (serum) and urine samples were collected from the cats at three different time points, i.e., 0 hr (before premedication), 24 and 48 hrs postoperative. The serum samples were analyzed for renal markers such as SCr, BUN, and UP:C. The urine samples were analyzed for novel renal markers such as KIM-1, NAG, NGAL and RBP for all cats. The 0 hr samples served as a baseline for comparisons to study the changes in renal marker concentrations and determine the resulting renal functional/structural changes, if any, after the routine operative procedure performed under general anaesthesia. These procedures are explained in detail below. The study was approved by the Massey University Animal Ethics Committee (protocol no 20/34).

3.2 STUDY ANIMALS

Primary inclusion criteria for the study participants were as follows: intact female cats that weighed over 1.5 kg, aged between 3 months and 7 years, easy to handle, had no history of medical problems or medications, and had an American Society of Anesthesiology (ASA) grade 1 on examination, i.e., with no underlying medical or behavioural problems. On arrival at the VTH, a detailed physical examination was conducted on each cat, and their body condition was scored on a 9-point scale. Examinations and sample collections were conducted by final year B.V.Sc students under the supervision of a veterinarian (CG/KK)

and/or veterinary technician. Some of the cats were in various stages of pregnancy. However, this variable was not recorded for the study.

The healthy cat subjects were randomly allocated to four experimental groups (n=15 cats/group) using Random Number Calculators (QuickCalcs; GraphPad Software, CA, USA), which provided a randomization table. The premedication/induction protocol and administration of fluids during surgery differed between the treatment groups, as shown in Flowchart 1.

3.3 PERIOPERATIVE PROCEDURE

3.3.1 Pre-Operative Preparations

The cats were fasted for at least 12 hours with ad-lib water access until pre-anaesthetic medication. Before premedication, blood samples (min. 2 ml) were aseptically collected from a peripheral vein (jugular, cephalic or medial saphenous vein). Urine samples (min. 5 ml) were collected via cystocentesis with the cats held in lateral or dorsal recumbency during the process. The details on sample collection are given in **Section 3.4**.

The cats were placed in separate, closed cages in a safe, environmentally controlled room with good ventilation. Each unit contained a soft blanket, an igloo, and a litter tray. A Feliway diffuser (Feliway; Ceva Sante Animale, France), which mimics the action of the feline facial pheromone, was used to handle stress. Paper tags were used to identify the subjects.

As shown in Table 4, the anaesthetic protocol for each animal was based on the treatment group decided, as mentioned before, randomly.

Table 4 Premedication and anaesthetic induction protocols used in the study.

Experimental group (n=15 cats/group)	Abbreviation	Pre-anaesthetic medication	Anaesthetic induction	Intraoperative IV fluid administered (3ml/kg b.wt./hr)
1	AM-IV	**Acepromazine** (0.03mg/kg, IM); **Morphine** (0.3mg/kg, IM)	Alfaxalone (IV to effect)	+[a]
2	AM-no IV	**Acepromazine** (0.03mg/kg BW, IM); **Morphine** (0.3mg/kg BW, IM)	Alfaxalone (IV to effect)	--
3	DKT-IV	**Dexmedetomidine** (10µg/kg BW, IM); **Ketamine** (10mg/kg BW, IM); **Butorphanol** (0.2 mg/kg BW, IM)	--	+[a]
4	DKT-no IV	**Dexmedetomidine** (10µg/kg BW, IM); **Ketamine** (10mg/kg BW, IM); **Butorphanol** (0.2 mg/kg BW, IM)	--	--

The cats in Groups 1 (AM-IV) and 2 (AM-no IV) were premedicated with the pre-anaesthetic agents (AM protocol) administered into the lumbar epaxial muscles, as mentioned in Table 4. Once they were adequately sedated, the surgical site and the

[a] Lactated ringers'/Hartmann's solution administered

venipuncture site were aseptically prepared. A 22G (0.9 × 25 mm) IV catheter (BD Insyte; Becton Dickinson Infusion Therapy Systems Inc., USA) was placed in either the cephalic vein or medial saphenous vein for both anaesthetic induction/maintenance and fluid administration, for those eligible, during surgery. Anaesthesia was induced with Alfaxalone (to effect), which was administered around 30-45 minutes after premedication. Lignocaine (2%, 0.1 ml) was sprayed on the larynx to prevent spasms before an appropriately sized ET tube was inserted. After confirming the tube placement in the trachea, the cuff was inflated using an empty syringe, and 100% oxygen was supplemented (pre-oxygenation) before transferring the cat to the adjacent surgical chamber. Gel lubricant was applied onto the cornea to prevent dryness-associated post-op complications such as keratitis.

The cats in Groups 3 (DKT-IV) and 4 (DKT-no IV) received the DKT protocol with doses as mentioned in Table 4. These drugs were drawn into one syringe and injected into the lumbar epaxial muscles. Once the cats were adequately sedated, the same procedure for intubation, IV catheterisation, and surgical site preparation was followed as described above for the AM treatment groups.

3.3.2 Operative Procedure

The sedated subjects were carefully transferred from the anaesthesia preparation room to the adjacent operating theatre and placed in dorsal recumbency on the operating table. The required instrumentation was attached to record intraoperative variables (details given below). The surgical site was aseptically scrubbed using Chlorohexidine 4% solution (contact time 5 mins), followed by methylated spirits (remove excess scrub), and then draped with a single fenestrated cloth barrier. The drape was secured using Backhaus towel clamps. The OHE was performed via the ventral midline approach by final-year veterinary students under the supervision of an experienced teaching veterinarian.

Anaesthesia was maintained using the inhalant anaesthetic, isoflurane (Attane; Bayer Animal Health, New Zealand) in 100% oxygen delivered via a non-rebreathing system, and the cats breathed spontaneously during surgery. The isoflurane concentration was adjusted accordingly to maintain the cat under the surgical plane of anaesthesia (Stage III, plane 2). The cats' heart rate (HR), respiratory rate (RR), peripheral capillary oxygen saturation

(SpO_2), BP (indirect oscillometric method) and airway gases such as end-tidal concentration of isoflurane were measured using a multifunction monitor (Carescape B650; GE Healthcare, Finland), along with a Blood Pressure Monitor (Petrust BP Monitor; Petrust, Taiwan). The readings were recorded every five minutes. The intraoperative data were collected from the administration of isoflurane until the end of surgery and the disconnection of anaesthesia.

Rectal temperature was also regularly recorded during anaesthesia using a digital thermometer, and body temperature was maintained using a forced-air warming system (Bair Hugger; 3M Company, USA). All cats in groups 1 and 3 received Hartmann's solution (Baxter International Inc, Australia) at 3ml/kg throughout anaesthesia and surgery. The cats in groups 2 and 4 did not receive IV fluids during the procedures.

3.3.3 Post-Operative Care

Buprenorphine (Buprelieve; 10 ml; 0.3 mg ml^{-1}; Jurox, NZ) was administered at 0.03 mg/kg b.wt once the cats were placed in closed cages with warming blankets until they recovered completely from anaesthesia. The ET tube was removed once the laryngeal swallowing reflex was observed. Antimicrobials were not administered as the surgery was conducted in an aseptic manner.

A nutritionally balanced commercial diet, i.e., Hill's Maintenance Diet dry or wet (free choice, thrice/day) and clean water, ad libitum, was provided once the cats recovered from general anaesthesia. Food and water bowls (ad lib) remained accessible until the next meal. Each subject was observed for 48 hours from admission. The analgesic (buprenorphine) was administered (TID) at 0.03 mg/kg until they were discharged from the hospital with additional analgesic if they still showed signs of pain.

3.4 SAMPLE COLLECTION

Sample collection was performed using protocols designed to minimize stress to the patient, including gentle restraint and towel wraps as needed. Blankets and towels used to

assist with restraint were sprayed with Feliway, which has been shown to reduce stress in cats and ease the handling process (Pereira et al., 2016).

Most of the samples were collected without sedation. Aggressive cats (n=4) had their samples collected immediately post-induction at 0 hr and for 24 and 48 hrs, placed in traps for sedation with dexmedetomidine, as advised by Griffin et al. (2016). The antidote/reversal agent (atipamezole) was administered (IM) immediately after the samples were collected for those sedated at 24 and 48 hrs.

3.4.1 Blood

Each subject's blood (~ 2 ml) was drawn from a peripheral vein (jugular, cephalic or saphenous vein) at three different time points, i.e., at 0 (baseline), 24 and 48 hrs., either directly by using a sterile 3 ml single-use leur lock syringe (Maxima; Henry Schein Inc., Germany) with a 23 G 0.6× 16mm disposable needle (Kruuse, Denmark) or by using a winged infusion set 23 G (Kruuse Butterfly; Kruuse, Singapore). The site for venipuncture was clipped and scrubbed with an antiseptic before percutaneous insertion of a sterile needle into the vein. Manual compression (moderate intensity) was applied to the forelimb or hindlimb proximal to the site. The blood samples were drawn into the syringe using the push-pull technique described by Barr et al. (2017). Once the blood was drawn into the syringe, the needle was removed, and the blood was pushed into an open red top vacutainer. The sample, labelled accordingly, was sent to IDEXX Laboratory immediately for biochemical analysis (SCr, BUN, and SDMA).

3.4.2 Urine

Urine samples were collected by cystocentesis (either through manual palpation of the bladder or ultrasound guidance) using a 5 ml sterile syringe (BD 5 ml syringe; BD, Singapore) or immediately after the urine was voided into a non-absorbent litter tray. The litter trays were placed in the cages before sampling (2 hr), and these samples were used if we were unable to draw urine via cystocentesis. The collected urine was transferred into five Safe-Lock Tubes 2ml (Eppendorf reaction tubes; Eppendorf AG, Germany) and labelled appropriately. One aliquot of urine was sent to IDEXX for urine analysis after

collection (UP:C, urine creatinine/UCr and UTP). The four other aliquots were placed in ice (0 °C) until they were transferred to a -80 °C Lab freezer, usually within an hour after collection.

3.5 ANALYTICAL METHODOLOGY

Three (RBP, NGAL, KIM-1) of the four urinary renal markers were quantitatively assayed using immunodiagnostic tests, which include a lateral flow assay (LFA) and various types of enzyme-linked immunosorbent assays (ELISA) that are capable of directly detecting the level of the specific antigen, in this case, various renal tubular proteins. The fourth urinary marker (NAG) was measured using a colourimetric assay.

3.5.1 Kidney Injury Molecule-1 (KIM-1)

Lateral flow assay (LFA)

We adopted the lateral flow assay method described by Bland et al. (2019) to analyze the levels of urinary KIM-1 in the study samples.

Principle

Lateral flow assay, an immunochromatographic test, works on the principle of detecting the antigen of interest present in the urine sample using an antigen-specific monoclonal antibody (Callahan & Yates, 2014, p. 302). It relies on the capillary movement of the sample, across a porous strip, towards a line of unbound gold-labelled monoclonal antibodies, which function as the detector (Bland et al., 2019; Tizard, 2013, p. 502). If present in the sample, the antigen will bind to the antibody to form mobile complexes that flow unidirectional across the strip until they are trapped by a line of immobilized capture antibodies, i.e., monoclonal antibody at 2 mg/ml (Bland et al., 2019). A positive test would be observed as a red line on the strip that would not be visible with negative samples. The presence of a positive control line in the strip allowed us to assess the reliability of each strip.

Instrumentation

The lateral flow strips (fKIM-1 LF; University of Guelph, CN; Bioassay Works, USA), which comprised of a sample pad, a gold-conjugate pad, a cellulose membrane, and an absorbent pad, installed into cassettes (Bland et al., 2019) were used to analyze the urine samples (0, 24 and 48 hr). An optical reader, CUBE Lateral Flow Reader (Manual Cube Flat- V1.7; BioAssay Works, USA), working at a wavelength of 525nm, was used to read the optical density (OD) for each assay.

Procedure

The urine sample was analysed for KIM-1 following the procedure described by Bland et al. (2019). The frozen urine samples (n = 175), stored at -80 °C, were thawed to room temperature and vortexed before performing the KIM-1 analyses. The LFA cassettes were removed from their foil pouch and placed on a flat surface once they had reached room temperature. Five µl of urine sample was added to 95 µl of dilution buffer (BioAssay Works, USA) in a 1.5 ml Eppendorf tube. The test sample (100 µl) was then added to the sample well of the LFA cassette (labelled for identification). The sample movement across the cassette was mediated by capillary action. The colourimetric cassette showed two lines of red colour in the cassette window if the sample is positive for KIM-1, i.e., the red line close to the sample well belongs to the test sample and the line in the control region to the control reagent (Internal positive control: recombinant KIM-1). Each cassette was read after 15 mins using the cube reader. The Cube reader measured the optical density (OD) of the control (C) and test (T) strips in the cassette. The readings were recorded for each urine sample. The C:T ratio and the raw OD values were taken to provide data on KIM-1 levels in the urine samples.

3.5.2 N-Acetyl β-D Glucosaminidase (NAG) Enzyme

Colourimetric assay

Several studies have used colourimetric assays to evaluate NAG levels in cats with CKD (Jepson et al., 2008, 2010; Lapointe et al., 2008; Panboon et al., 2017; Sato et al., 2002;

Uechi et al., 1998). The N-Acetylglucosaminidase Assay (CS0780-1KT; Sigma-Aldrich Co., USA) used in this study had been used and validated by Jepson et al. (2010) to detect the NAG concentrations in the urine of our study cats.

Principle

The assay works on the principle of enzymatic hydrolysis of the substrate 4-Nitrophenyl N-acetyl-β-D-glucosaminide in an acidic environment, resulting in the release of p-nitrophenol. When the acidic solution is turned basic, the p-nitrophenol assumes its ionic form, measured using an EIA (Enzyme immunoassay) plate reader at 405 nm.

Sample preparation

The urine samples (n = 173) were taken out of the -80 ^{0}C freezer after 5-6 months of storage. Once adequately thawed, the samples were centrifuged (Centrifuge 5415 D; Eppendorf AG, Germany) at 1.5 rcf for five mins. The samples were divided into different batches for different plates. There were 29 samples for plate one, plate two, plate three, plate four and plate five, while there were 28 samples for plate six.

Assay procedure

The substrate, standard, stop solutions, and NAG control enzyme was prepared according to the manufacturer's instructions. The former two were equilibrated in an incubator at 37 °C for several minutes. 10 µl of each urine sample (n = 29) was added to the wells of 96 well plates (Tissue culture plate 96 well; Jet Bio-fil, China) in triplicates. Then 90 µl of the substrate solution was added to the wells containing the urine sample, using a multichannel pipette, to make up a total of 100 µl solution. 300 µl of the standard solution was pipetted into three empty wells, respectively. The blank solution consisted of 100 µl of the standard solution pipetted into three empty wells. The positive control solution was prepared using 98 µl of substrate solution and 2 µl of NAG control enzyme. It was added to the last three empty wells on the plate. The plate was then covered and gently agitated to allow for proper mixing of solutions in each well before being placed in the incubator (37 °C) for 30 mins. After 15 mins of incubation, 2 µl of NAG control enzyme was added to the positive control wells as it was not exhibiting the expected colour. Stop solution (200 µl) was added to each

well apart from those containing the standard solution. A microplate reader (VersaMax Microplate reader; Molecular Devices LLC, USA) was used to read the plate at 405 nm. The plate readings were acquired using the SoftMax Pro Software (Molecular Devices LLC, USA). The same steps were repeated for the remaining 5 Biofil plates, with each sample being pipetted in triplicate.

NAG enzyme activity (U/ml) was calculated using the formula:

$$\textbf{Units/ml} = \frac{(\mathbf{A_{405}sample - A_{405}blank}) \times \mathbf{0.05} \times \mathbf{0.3} \times \mathbf{DF}}{\mathbf{A_{405}standard} \times \mathbf{time} \times \mathbf{Venz}},$$

where A_{405}sample is the averaged absorbance of the sample at 405 nm, A_{405}blank is the absorbance of the blank (405 nm), 0.05 is the µmole/ml of 4-nitrophenol in the standard solution, 0.3 is the final volume in millilitres (ml) of the 96-well plate reaction after adding the stop solution, DF is the enzyme dilution factor, A_{405}standard is the absorbance of the standard solution at 405 nm, time in minutes and the V_{enz} is the volume of the sample in ml. The calculations were performed using Microsoft Excel. The derived marker concentration was then expressed in Units/L.

3.5.3 Neutrophil Gelatinase-Associated Lipocalin (NGAL)

3.5.3.1 Trial 1

Principle

Dog NGAL ELISA kit (KIT 043; BioPorto Diagnostics, Denmark), with a 0.56 pg/ml detection limit for dog samples, was used to attempt to detect cat uNGAL. The manufacturer observed cross-reactivity between canine-specific antibodies and cat NGAL. This commercial kit worked on the principle of sandwich ELISA, i.e., used to detect and quantify a specific antigen using two sets of antibodies, an immobilized capture antibody (Anti-dog NGAL Mouse Monoclonal Antibodies) and a detection antibody (Biotin Labelled Mouse Monoclonal Antibody). The antigen (NGAL), if present in the urine sample, will bind to the capture antibody coated on the bottom of the microwell in a microtiter plate to form a complex. The detector antibody recognizes and attaches to the same antigen, forming a sandwiched complex of two antibodies with an antigen in between. The presence of the detector antibody (fixed complex) allows colour formation after washing once the HRP-conjugated streptavidin (which binds to biotin) is added to the peroxidase substrate (TMB).

Sample preparation

Urine samples (n = 42) were taken out of storage after five-six months for a trial run (avoid repeated freezing and thawing cycles) to check the validity of the kit in quantifying uNGAL levels in cats. The samples were centrifuged at 1.5 rcf for five mins to separate the cellular debris.

Assay procedure

The supernatant (12.5 µl) from the first 21 samples was added to the plate wells (Tissue culture plate 96 well; Jet Bio-fil, China), while another 21 samples (25 µl) were added to the remaining wells. The Diluent (237.5 µl) was pipetted into each of the first 21 wells (containing 12.5 µl of the first 21 samples), while 225 µl of Diluent was added to the remaining 21 wells. 250 µl of the standard calibrators (0, 10, 40, 200, 400) was added to the wells, while 250 µl of the diluent solution was added to the last two wells on the last row of the plate. A multichannel pipette (Transferpette-8, Germany) was set at 100 µl and used to

draw the solutions from the plate in duplicate order into the wells of another plate (microwell plate, 96 pre-coated wells; BioPorto Diagnostics, Denmark). The plate was then gently agitated after covering it with the lid. It was then incubated for an hour at room temperature on a shaking platform (IKA-VIBRAX-VXR; Electronic, NZ). The wells were washed three times with the wash buffer.

A wait time of one min was followed between the last two washes. The dog NGAL-antibody (100 µl) was added to each well, and then the lid was placed on the plate. The plate was incubated at room temperature for an hour. The wells were washed as described before. HRP-Streptavidin conjugate (100 µl) was added to each well, and then the plate was covered, followed by incubation at room temperature for 60 mins with routine agitation. The wells were washed as described before. 100 µl of tetramethylbenzidine (TMB) substrate was pipetted into each well, and the clock was started as the first well was filled. The subsequent formation of blue colour seemed promising. The plate was covered and then incubated in a dark room for ten minutes. A 100 µl of stop solution (sulfuric acid) was added to each well, changing the colour of the solution in the wells from blue to yellow, and the plate was agitated for 20 mins. The plate was read within 30 mins using a microplate reader (VersaMax Microplate reader; Molecular Devices LLC, USA) at 450 nm absorbance value. The readings were recorded using the SoftMax Pro Software (Molecular Devices LLC, USA).

The absorbance values of the standards (calibrators) were used to construct a standard curve using the four-parameter logistic (4PLC) curve-fitting software in Prism (GraphPad; USA) after subtracting the value of the blank from each. The curve was used to quantify the levels of NGAL for each sample. However, the values for the diluted and undiluted samples were not in tandem, and, thus, further work using this kit was discontinued.

3.5.3.2 Trials 2 and 3

For this attempt, four frozen samples were used to check the reliability of two kits, i.e., the Rat Lipocalin-2 (LCN2) ELISA Kit (ERLCN2 96 tests Invitrogen, ThermoFisher Scientific, USA) with a 30 pg/ml minimum detectable limit for rat lipocalin, and the Mouse Lipocalin-

2 (LCN2) ELISA Kit (EMLCN2 96 tests Invitrogen; ThermoFisher Scientific, USA) with a minimum detection limit of 0.02 ng/ml for mouse NGAL, in quantifying the uNGAL levels in cats.

Principle

Both the kits worked on the principle of solid-phase sandwich ELISA meant to quantify the levels of mouse and rat NGAL, respectively. Due to the unavailability of validated commercial cat-specific kits, we attempted to test the cat urine samples with these kits.

The rat LCN2 kit relies on using the rat lipocalin-2 antibody (coating the wells) to capture the antigen in the sample (NGAL). The detector antibody (rat lipocalin-2 biotin conjugate) binds to the exposed sites on the antigen (captured by the first antibody). The subsequent addition of HRP- streptavidin (enzyme-linked streptavidin) and the TMB substrate allows for the colour reaction, which can be measured and read to interpolate the antigen levels. The mouse LCN2 kit works on the same principle, with the simple difference of using mouse-specific antibodies.

Sample preparation

Four urine samples were thawed to room temperature and then centrifuged at 25.8 rcf for two mins. The samples were diluted to different concentrations and labelled accordingly, i.e., undiluted (100 µl of the sample), 1:5 (60 µl of sample and 240 µl of assay diluent), 1:10 (30 µl of sample and 270 µl of assay diluent) and 1:20 (15 µl of sample and 285 µl of assay diluent).

Assay procedure

Rat LCN2 ELISA assay kit

The standards (std) were prepared according to the instructions provided by the manufacturer. The standards were of the following concentrations, i.e., 8000 pg/ml (std 1), 3200 pg/ml (std 2), 1280 pg/ml (std 3), 512 pg/ml (std 4), 204.8 pg/ml (std 5), 81.92 pg/ml (std 6), 32.77 pg/ml (std 7) and 0 pg/ml (blank).

Each of the standards (100 µl) was added to the appropriate wells of two plate strips (rat lipocalin-2 antibody-coated wells, 96-well plate; ThermoFisher, USA). We added 100 µl of the undiluted and diluted samples, separately, to the empty wells of the plate. The plate was then covered with a seal and incubated at room temperature for 2.5 hours on the shaking platform. The plate was washed four times periodically with the prepared wash buffer, and the plate was blotted on clean paper towels between each wash. The prepared biotin conjugate (100 µl) was added to each well before being covered and then incubated on the shaking platform for an hour.

The wells were washed following the same protocol as described before. The prepared streptavidin-HRP solution (100 µl) was added to each well, and then the cover was placed over it. The plate was placed on a shaking platform and incubated (room temperature) for 45 mins. The plate was washed, as mentioned before. Once the TMB substrate (100 µl) was added to each well, the substrate would turn blue. The plate was covered and then incubated at room temperature, on a shaking platform, in the dark for 30 mins. Stop solution (50 µl) was added to each well, and the plate was then gently agitated. The solution in the wells turned yellow. The plate was read (450 nm) using a microplate reader (VersaMax Microplate reader; Molecular Devices LLC, USA). The curve fitting software (4 PLC Prism; GraphPad, USA) generated a standard curve, based on all readings after subtracting the background absorbance, and calculated the uNGAL values.

Mouse LCN2 ELISA kit

The standards were prepared according to the instructions provided by the manufacturer. The standards were of the following concentrations, i.e., 100 ng/ml (std 1), 25 ng/ml (std 2), 6.25 ng/ml (std 3), 1.563 ng/ml (std 4), 0.391 ng/ml (std 5), 0.098 ng/ml (std 6), 0.024 ng/ml (std 7) and 0 ng/ml (blank).

Each of the standards (100 µl) was added to the appropriate wells of two plate strips (Mouse Lipocalin-2 antibody-coated wells, 96-well plate; ThermoFisher, USA). We added 100 µl of the undiluted and diluted samples, separately, to the empty wells of the plate. After

covering the plate, the plate was kept for incubation at room temperature for 2.5 hours on the shaking platform. The plate was washed four times with the prepared wash buffer before blotting on clean paper towels. The prepared biotin conjugate (100 µl) was added to each well before being covered with an adhesive cover, followed by incubation on the shaking platform for an hour.

The wells were washed following the same protocol as described before. The prepared streptavidin-HRP solution (100 µl) was added to each well, and then the adhesive plate cover was placed over it. The plate was placed on a shaking platform and incubated at room temperature for 45 mins. The plate was washed, as mentioned before. As soon as the TMB substrate (100 µl) was added to each well, the substrate would turn blue. The plate was covered and then incubated at room temperature, on a shaking platform, in the dark for 30 mins. Stop solution (50 µl) was added to each well, and the plate was then gently agitated. The solution in the wells turned yellow. The plate was read (450 nm) using a microplate reader (VersaMax Microplate reader; Molecular Devices LLC, USA). The curve fitting software (4 PLC Prism; GraphPad, USA) generated a standard curve, based on all readings after subtracting the background absorbance, and calculated the uNGAL values.

The interpolated readings for the samples were not in tandem between the diluted and undiluted samples. Therefore, the use of these kits was discontinued due to their unreliability in quantifying the uNGAL in cats. This suggests that the rat and mouse LCN2 antibodies may not have sufficient cross-reactivity with the cat uNGAL protein.

3.5.3.3 Trial 4

A Cat Neutrophil gelatinase-associated lipocalin ELISA (MyBioSource, USA) with a sensitivity of 0.24 ng/ml, and good intra-/ inter-assay precision was used.

Instrumentation and principle

The sandwich ELISA kit provided a 48-well plate, pre-coated with cat NGAL antibodies. If NGAL is present in the urine sample, the antigen will bind to the antibody and form a complex with the subsequently added biotinylated cat NGAL antibody. A colour reaction

will be observed post-addition of the HRP-Streptavidin conjugate and the substrate. This colour reaction can be read and analysed to determine the level of NGAL in the sample.

Sample preparation

Fresh frozen samples (n = 42) were brought to room temperature and centrifuged at 3000 rpm for 20 minutes per the kit manufacturer's instructions. The supernatant (100 µl) for each sample was pipetted into the wells of the ELISA plate (MBS1602657; MyBioSource.com). Standard solutions were prepared as instructed by the kit manufacturer: 80 ng/ml (std 5), 40 ng/ml (std 4), 20 ng/ml (std 3), 10 ng/ml (std 2), 5 ng/ml (std 1).

Assay procedure

Each standard (50 µl) was added to the plate's standard wells (Cat NGAL pre-coated 48 well plate; MyBioSource, USA). The samples (40 µl) were added to the sample wells in the plate, followed by the addition of an anti-NGAL antibody (10 µl) to the same wells. Streptavidin-HRP (50 µl) was added to the sample and standard wells (except blank). The plate was agitated and then incubated for 60 mins at 37 °C. The plate was then washed five times with the wash buffer.

The substrate solution A (50 µl) was added to every well, followed by the addition of substrate solution B to the same wells. The sealed plate was then incubated for 10 mins at 37 °C in the dark. 50 µl of the stop solution was then pipetted into each well, wherein a change of colour from blue to yellow was observed. The plate was read (450 nm) using a microplate reader (VersaMax Microplate reader; Molecular Devices LLC, USA).

A standard curve was generated, and the levels on NGAL were quantified using curve fitting software (4 PLC MyAssays Online; MyAssays Ltd.), with further corroboration of the readings using GainData (Arigo Biolaboratories; Arigo Biolab. Corp., NZ). Further, the concentrations were finalized using GraphPad Prism 9 Software (GraphPad, USA).

3.5.3.4 Final run

Since Trial 4 showed promising results, the same Cat Neutrophil gelatinase-associated lipocalin ELISA kit (MyBioSource, USA) with a sensitivity of 0.24 ng/ml was employed to

determine the urine NGAL levels for all the cats in the study. Samples were taken out of storage and allowed to thaw to room temperature before centrifuging at 15 rcf for 20 mins. The procedure followed here was the same as described before, replicated on two separate Cat NGAL pre-coated plates (96 well microwell strip plates), each prepared using freshly prepared standards. A standard curve was plotted using Prism software, and the values for the uNGAL concentration were derived using the curve fitting software.

3.5.4 Retinol Binding Protein (RBP)

3.5.4.1 Trial 1

Principle

The Retinol binding protein 4 EIA Kit (RAB0414; Sigma-Aldrich, USA) kit, having a sensitivity of 460 pg/ml and good intra-/inter-assay precision, works on the principle of competitive enzyme immunoassay using an anti-rabbit secondary antibody pre-coated plate (RAB0414A:96 wells; Sigma-Aldrich, USA) to quantitate the levels of RBP4 protein.

The commercial kit used in this study uses antibodies to detect hapten molecules (Tizard, 2013). The antigens in the test sample and the simultaneously added enzyme-labelled antigens (biotinylated RBP4 peptide) compete to bind to the antibodies (anti-RBP4 detection antibody), which are complexed with anti-rabbit secondary antibody coated on the microwells. The bound biotin-labelled antigen gives a colour reaction to adding streptavidin- horseradish peroxidase (SA-HRP). The concentration of RBP4 in the sample will be inversely proportional to the amount of antibody-bound biotin-labelled antigen.

Sample preparation

For plate one, eight (previously un-thawed) frozen samples were taken from storage (-80 °C) after five-six months. The aliquots were centrifuged at 15.8 rcf for two mins. The samples were not diluted, and the instructions were followed as directed by the manufacturer for situations where undiluted samples are used.

Procedure

The reagents were kept on ice until the time of use. The pre-coated commercial plate was allowed to reach room temperature. Two of the eight strips provided in the plate were used for this trial run, to ensure that the kit was reliable before replicating the test with the remaining aliquots. The anti-RBP4 antibody (100 µl) was prepared as instructed and added to each well. The plate was incubated for 90 mins at room temperature with gentle shaking. The wells were emptied and washed four times using the prepared wash buffer. 100 µl of each standard (1000 ng/ml, 100 ng/ml, 10 ng/ml, 1 ng/ml, 100 pg/ml), positive control and

the sample was added to the appropriate wells. One well was filled with the assay diluent (100 µl) to act as the blank for the assay. The plate was covered and incubated for 150 mins at room temperature with gentle shaking. The plate was washed as described before. 100 µl of HRP-streptavidin (prepared) was added to each well and then incubated at room temperature for 45 mins, with gentle shaking. The plate was washed as described above. The TMB substrate (100 µl) was added to each well, and then the plate was incubated for 30 mins in the dark (room temperature) with gentle shaking. The stop solution (50 µl) was added to each well. A microplate reader (VersaMax Microplate reader; Molecular Devices LLC, USA) was used to read the plate at 450 nm.

A standard curve was plotted using Prism software (4PLC; GraphPad), incorporating the percentage absorbance of the standards and the standard concentrations, to interpolate the RBP4 values for the samples. Percentage absorbance was calculated for each standard and the samples using the formula,

$$\textbf{\% absorbance} = \frac{(\boldsymbol{B - Blank\ OD})}{(\boldsymbol{B0 - Blank\ OD})},$$

where B = OD of sample/standard, B_0 = OD of zero standards.

The plate 1 readings appeared accurate, so plates 2 and 3 were prepared for testing. For plates 2 and 3, frozen samples (n = 161) were taken from storage (-80 °C), centrifuged at 15.8 rcf for two mins, and the same procedure was followed carefully, except for one difference. The samples for plates 2 and 3 were diluted four-fold as instructed by the manufacturer.

Upon plotting the standard curve and interpolation of the values for the samples using Prism software (GraphPad), a discrepancy was noted for the diluted samples. This led us to believe that the kit was unreliable for quantifying RBP4 concentration in cat urine.

3.5.4.2 Trial 2

The Cat Retinol Binding Protein 4 (RBP4) sandwich ELISA kit (MBS048954; MyBioSource, USA) with a sensitivity of 0.1 µg/ml and a detection range that spans from

3.12-100 µg/ml, was used as the previous kits had proved to be unreliable. We sought to use feline RBP4-specific commercial kits this time around. To date, no literature source has used feline RBP-specific ELISA kits until now.

Sample preparation

Urine samples (n = 164) were taken from storage at -80 ^{0}C and thawed to room temperature before centrifuging at 15 rcf for 20 mins. We attempted to limit the number of thaws (preferably to one).

Procedure

The contents of the kits (2 microstrips 96 well plates, and reagents) were allowed to reach room temperature before use. The blanks were left empty as instructed by the manufacturer, while 50 µl of each standard (S1 3.125 µg/ml, S2 6.25 µg/ml, S3 12.5 µg/ml, S4 25 µg/ml, S5 50 µg/ml and S6 100 µg/ml) was added to the standard wells of both the plates. Each sample (50 µl) was added to the sample wells of both plates. The HRP-Conjugate reagent (100 µl) was added to all the wells, except for the blank, in both the plates. The plates were subsequently covered and incubated at 37 °C for one hour. The wells were washed four times using the prepared wash buffer. 50 µl of chromogen solution A was added to every well, followed by the subsequent addition of 50 µl of chromogen solution B (TMB). The plates were immediately covered with aluminium foil. The plates were incubated for 15 mins at 37 °C. 50 µl of stop solution was added to every well. A microplate reader (VersaMax Microplate reader; Molecular Devices LLC, USA) was used to read the plates at 450 nm.

Professional curve fitting software (GraphPad Prism) was used to plot the standard curve. The levels of the analyte were calculated subsequently.

3.5.5 Dipstick Analysis

While frozen urine samples are unsuitable for sediment examination, that doesn't hold true for dipstick analysis (Reppas & Foster, 2016). The urine samples were analysed using commercially available dipstick strips (CombiScreen 11SYS Plus; Analyticon

Biotechnologies AG, Germany). Every strip had several test pads installed on them, each impregnated with different chemical reagents. These reagents react to certain compounds that give semi-quantitative results in urine (Gribbles). Dipstick strips measure different urinary parameters such as glucose, bilirubin, urobilinogen, ketones, protein, cell counts (red and white blood cells), nitrite, ascorbic acid, protein, blood, USG, and pH. The WBC, USG and nitrite readings are not accurate for cats (Reppas & Foster, 2016). The urine samples used for NAG analysis (brought to room temperature) were used for dipstick analysis using the drip method, and the results, after testing, were recorded.

3.6 STATISTICAL ANALYSIS

All analyses in the results section were carried out using RStudio Version 1.2.5042 (RStudio Inc., USA).

3.6.1 Descriptive statistics

Descriptive data were generated for all study variables, and the distributions of all numerical variables were assessed for normality through visual observation of frequency histograms. Parametric tests (t-test) were used to establish any difference in variables (weight, heart rate, respiratory rate, temperature) at baseline between the different treatment groups. A non-parametric test (Wilcoxon) was conducted to establish any statistically significant difference in age between the treatment protocols.

For variables such as heart rate (HR), mean arterial pressure (MAP), systolic arterial pressure (SAP), temperature (Temp) and oxygen saturation (SpO_2) in the anaesthetized cats, the minimum threshold was taken as 100 bpm for HR, 60 mmHg for MAP, 80 mmHg for SAP, 36.4 °C for Temp and 94% for SpO_2. The mentioned values are the lower limits for the respective reference ranges below which the animal may be at increased risk of mild renal pathology due to the inability of the kidneys to autoregulate renal blood flow if the conditions persist for some time. These values were considered while dividing the numerical intraoperative variables into categorical variables.

3.6.2 Inferential statistics

This study was conducted specifically to determine whether using either of the two different pre-operative anaesthetic drug protocols, with or without intraoperative fluid administration, causes significant changes in the traditional and novel renal biomarkers. ANOVA was used to compare the change in renal marker values over the different time points between and within treatment groups, followed by Posthoc Tukey test when the differences were found to be significant (p-value < 0.05).

We then attempted to construct a series of linear regression models to account for the influences of potential confounders (such as age, weight, and intraoperative variables) on changes in renal biomarker values between time points between the different treatment groups. A univariable screen was first performed to identify variables associated with the outcome at a p-value < 0.20 for potential inclusion in the multivariable model. They were then fit into the multivariable model using a backwards stepwise approach, sequentially removing variables with the highest p-value above 0.05. However, only the univariable associations remained significant at a p-value < 0.05.

Further attempts were made to explore multivariable associations by converting the continuous outcome variables into binary variables. Changes in the renal biomarkers in a direction that indicated potential structural damage was considered a ‘positive’ outcome in the models.

4 RESULTS

4.1 DESCRIPTIVE STATISTICS

60 intact female cats with a mean age of 17.44 months ± 16.8 months (median: 11, min: 3, max: 81) and weight of 3 kgs ± 0.74 kg (median: 3.02, min: 1.25, max: 4.5), respectively, were recruited for the study. Table 5 and Table 6 summarize the baseline values recorded during the physical examination for all the cats. While some of the cats were in the various stages of gestation, pregnancy was not recorded as a variable in this study.

Table 5 Baseline demographic data of the study population.

	Mean ± Sd	Median	Range
Age (months)	**17.44 ± 16.80**	11	11-81
Weight (kg)	**3.00 ± 0.74**	3.02	1.25-4.5
HR (bpm)	**182.24 ± 34.4**	180	104-240
RR (bpm)	**51.97 ± 16.08**	48	20-96
Temperature (°C)	**38.5 ± 0.6**	38.6	36.9-39.6

As shown in Table 6, the mean body weight was uniform across the treatment groups (p-value < 0.05). The mean age appears higher for the DKT treatment groups, especially DKT-IV; however, the difference between the treatment groups was not statistically significant (p-value < 0.05). All the recorded parameters (HR, RR, temperature) were within the reference range with no significant difference between the groups (p-value < 0.05).

Table 6 Baseline demographic data of the study cats across the treatment groups. [*]

	AM-no IV	DKT-no IV	AM-IV	DKT-IV
Number of cats	15	15	15	15
Age (months)	**14.23** ± **14.61** (3, 10, 48)	**17.71 ± 21.32 (**4, 10, 81)	**17.03 ± 13.21 (**3.5, 12, 48)	**21.04 ± 18.40 (**3.5, 16.5, 72)
Body weight (kg)	**2.77 ± 0.94 (**1.52, 3, 4.18)	**3.00 ± 0.58 (**1.88, 2.95, 4.3)	**3.05 ± 0.61 (**1.29, 3.15, 3.9)	**3.18 ± 0.8 (**1.25, 3.3, 4.5)
HR (bpm)	**181.33 ± 40.62 (**120, 180, 240)	**186.36 ± 30.62 (**110, 180,224)	**190.8 ± 33.56 (**104, 200, 240)	**168.67± 31.89 (**116, 184, 200)
RR (bpm)	**47.2 ± 16.44 (**20, 50, 64)	**47.5 ± 13.60 (**28, 46, 72)	**61.6 ± 13.37 (**36, 62,66)	**50.56 ± 18.16 (**39, 44, 96)
Temperature (°C)	**38.29 ± 0.78 (**37.2, 38.2, 39.6)	**38.55 ± 0.2 (**37.7, 38.6, 39.2)	**38.65 ± 0.64 (**36.9, 38.7, 39.5)	**38.48 ± 0.35 (**38.1, 38.4, 39.1)

[*] Values presented as mean ± sd (min, median, max), where applicable.

4.2 INFERENTIAL STATISTICS

Table 7 and Table 8 summarize the analyzed values for the intraoperative variables recorded for the study population as a whole and across treatment groups, respectively. The mean MAP (m-MAP), mean SAP (m-SAP) and mean DAP (m-DAP), mean temperature (m-Temp), mean isoflurane% (m-isoflurane) and mean oxygen saturation (m- SpO_2) were analysed by using the means of all the recorded values for intraoperative variables for each cat. The minimum value for each variable (abbreviated as l-MAP, l-SAP, l-DAP, l-Temp, and l-SpO_2) was analysed using the lowest value recorded for each cat. Only statistically significant findings are mentioned here.

4.2.1 Intraoperative variables (Study Population)

The anaesthetic duration did not differ significantly across the treatment groups (p-value > 0.05), as shown in Figure 9. HR, m-MAP, m-SAP, m-Temp, and m-SpO_2 were within the reference range for anaesthetized cats in the study population. The oxygen saturation (SpO_2) was at 96.10 ± 2.16%, End-tidal Carbon dioxide ($ETCO_2$) at 37.61 ± 7.16 mmHg, heart rate (HR) at 153.7 ± 24.73 bpm and respiratory rate (RR) at 8.68 ± 7.48 bpm. They were all within the reference range.

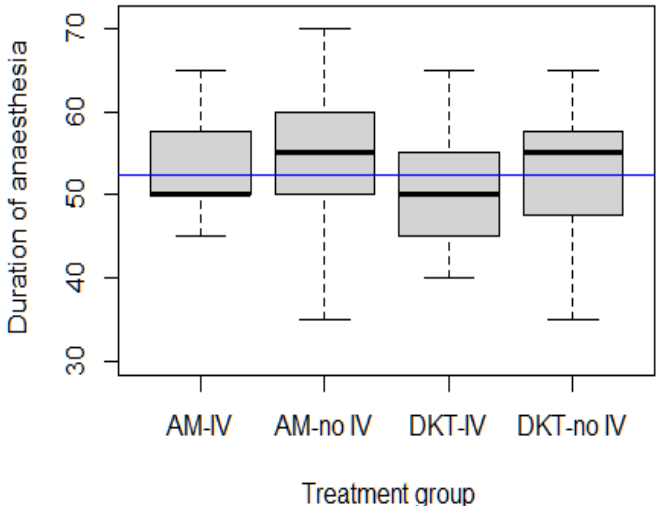


Figure 9 Anaesthetic duration for the study groups.

This boxplot graph depicts the variation in anaesthetic duration across the four treatment groups. The blue line represents the mean duration of anaesthesia for the study population.

As shown in Table 7, the animals were normotensive with an m-SAP of 110.89 ± 15.37 mmHg and l-SAP (at the lowest) of 91.73 ± 16.56 mmHg for the anaesthetized study population. Other variables such as m-MAP (79.62 ± 15.61 mmHg), m-temp (36.97 ± 0.69 °C) and m-DAP (62.61 ± 16.7 mmHg), were all within the acceptable limits for the whole study population.

Table 7 Intra-operative data of the study population.

Variables	Mean ± Sd	Median	Range
Anaesthetic Duration (mins)	52.42 ± 8.80	50	25-70
Duration of surgery (mins)	40 ± 8.78	40	20-50
Mean HR (bpm)	153.7 ± 24.73	153.5	89.4-200.4
Mean RR (bpm)	18.68 ± 7.48	18.67	6-42.56
Mean ETCO2(mmHg)	37.61 ± 7.16	35.61	26-55.6
Mean Et isoflurane %	0.88 ± 0.23	0.91	0.23-1.4
m-MAP (mmHg)	79.62 ± 15.61	78	59.22-122
m-SAP (mmHg)	110.89 ± 15.371	107.35	85.78-148.67
m-DAP (mmHg)	62.61 ± 16.7	57.11	39.2-108.4
m-Temp (°C)	36.97 ± 0.69	36.92	35.7-38.38
m- SpO2 (%)	96.10 ± 2.16	96.50	88.67-99.86
m-Isoflurane (%)	0.99 ± 0.33	1	0.37-1.84
Maximum Isoflurane %	0.64 ± 0.32	0.5	0.5-2.5
l-MAP (mmHg)	62.8 ± 12.63	61	38-109
l-SAP (mmHg)	91.73 ± 16.56	91	50-135
l- DAP (mmHg)	45.49 ± 11.83	45	25-88
l-SpO2 (%)	92.57 ± 4.96	94	78-99
l-Temp (°C)	36.55 ± 0.81	36.6	33.7-38.2

4.2.2 Intraoperative variables (Treatment Groups)

Table 8 presents the details of various variables recorded intraoperatively across the treatment groups. Figure 10 and Figure 11 display various recorded intraoperative data across treatment groups. It was observed that the mean duration of anaesthesia (Figure 9) and surgery were not significantly different between the treatment groups, with the means ranging from 50-55 mins ($t= 1.558$, p-value < 0.05) and 36-43 mins, respectively.

Table 8 Intra-operative data of the study cats across the treatment groups*.

Variables	AM-no IV	DKT-no IV	AM-IV	DKT-IV
Anaesthetic Duration (mins)	**55 ± 8.66** (35, 55,70)	**51.33 ± 9.15** (35, 55, 65)	**53.33 ± 6.72** (45, 50, 65)	**50 ± 10.35** (25, 50, 65)
Duration of surgery (mins)	**42.86 ± 5.67** (35, 40, 50)	**36.43 ± 11.07** (20, 35, 50)	**41 ± 7.42** (30, 40, 50)	**40 ± 10.48** (25, 42.5, 50)
Mean-HR (bpm)	**159.57 ± 17.6** (122.2, 160.77, 188.1)	**135.1 ± 22.76** (89.4, 134.2,170.8)	**166.3 ± 22.92** (135.9, 175.8, 199.2)	**154.04 ± 25.43** (116.3, 152.45, 200.36)
Mean-RR (bpm)	**24.17 ± 5.57** (16.64, 23.4, 42.55)	**12.71 ± 4.82** (6.4, 12.1, 23.8)	**22.78 ± 4.71** (17.7, 22.12, 34.73)	**15.64 ± 5.57** (6, 15.3, 24.3)
Mean $ETCO_2$ (mmHg)	**32.44 ± 2.66** (28.7, 32.1, 38.23)	**44.72 ± 5.82** (33.69, 44.25, 55.6)	**32.61 ± 4.52** (26, 31.5, 42)	**40.68 ± 5.91** (32.33, 40.36, 49.6)
Mean ET isoflurane (%)	**0.98 ± 0.17** (0.66, 1.03, 1.23)	**0.79 ± 0.32** (0.23, 0.77, 1.4)	**0.95 ± 0.16** (0.7, 0.92, 1.24)	**0.81 ± 0.3** (0.53, 0.76, 1.12)
m-MAP (mmHg)	**70.64 ± 8.97** (61.2, 69.95, 95.83)	**87.18 ± 18.64** (68, 81.3, 122)	**71.09 ± 9.96** (59.22, 67.2, 89.09)	**88.88 ± 13.43** (63.33, 87.82, 114.33)
m-SAP (mmHg)	**104.61** ± **11.44** (90.9, 102.7, 138.17)	**112.64 ± 16.77** (94.75, 108.38, 142.43)	**105.61 ± 13.3** (85.77,107.74, 124.2)	**120.71 ± 15.19** (97, 122.37, 148.67)
m-DAP (mmHg)	**52.92 ± 8.95** (39.2, 53.41, 70.33)	**75.72 ± 19.44** (50.64, 69.46, 108.4)	**51.49 ± 8.01** (40.91, 52.22, 66.23)	**71.24 ± 13.4** (47.67, 69.94, 95.67)
m-Temperature (°C)	**36.58 ± 0.55** (35.7, 36.4, 37.82)	**37.38 ± 0.45** (36.72, 37.32, 38.33)	**36.76 ± 0.64** (35.85, 36.59, 38.03)	**37.26 ± 0.76** (35.83, 37.24, 38.38)
m-SPO_2 (%)	**96.23 ± 2.60** (88.67, 96.64, 99.86)	**96.29 ± 1.44** (93.3, 96.18, 98.54)	**96.47 ± 1.96** (92.8, 96.8, 99.33**)**	**95.39 ± 2.50** (90.87, 95.5, 99.18)
l-DAP (mmHg)	**40.43 ± 6.19** (28, 39.5, 52)	**51.69 ± 16.49** (30, 51, 88)	**38.43 ± 4.96** (28, 40, 45)	**51.86 ± 10.2** (25, 52, 72)
l-MAP (mmHg)	**57.42 ± 5.65** (51, 55.5, 70**)**	**66.69 ± 17.26** (41, 62, 109**)**	**57.46 ± 5.56** (50, 55, 68**)**	**69.23 ± 13.63** (38, 71, 97**)**
l-SAP (mmHg)	**88.28 ± 10.34** (70, 89, 110**)**	**89.64 ± 20.45** (50, 92.5, 124**)**	**87.93 ± 10.89** (73, 88.5, 105)	**101.07 ± 19.72** (58, 98, 135**)**
l-Temp (°C)	**36.18 ± 0.56** (35, 36.1, 37.5**)**	**37.02 ± 0.56** (36.2, 36.95, 38.2**)**	**36.19 ± 0.93** (33.7, 36.4, 37.6)	**36.95 ± 0.74** (35.6, 37, 38**)**
l-SPO_2 (%)	**94 ± 5.04** (79, 95, 99)	**91.73 ± 5.05** (78, 93, 97)	**92.8 ± 4.09** (80, 94, 98)	**91.73 ± 5.69** (79, 93, 98)

* Where applicable, values are presented as mean ± sd (min, median, max).

The mean MAP (m-MAP), SAP (m-SAP), temperature (m-Temp), and oxygen saturation (m-SPO_2) were calculated using the mean of all the recorded values for all cats. The lowest MAP (l-MAP), SAP (l-SAP), temperature (l-Temp), and oxygen saturation (l-SPO_2) were calculated using the minimum value recorded for each variable in every cat.

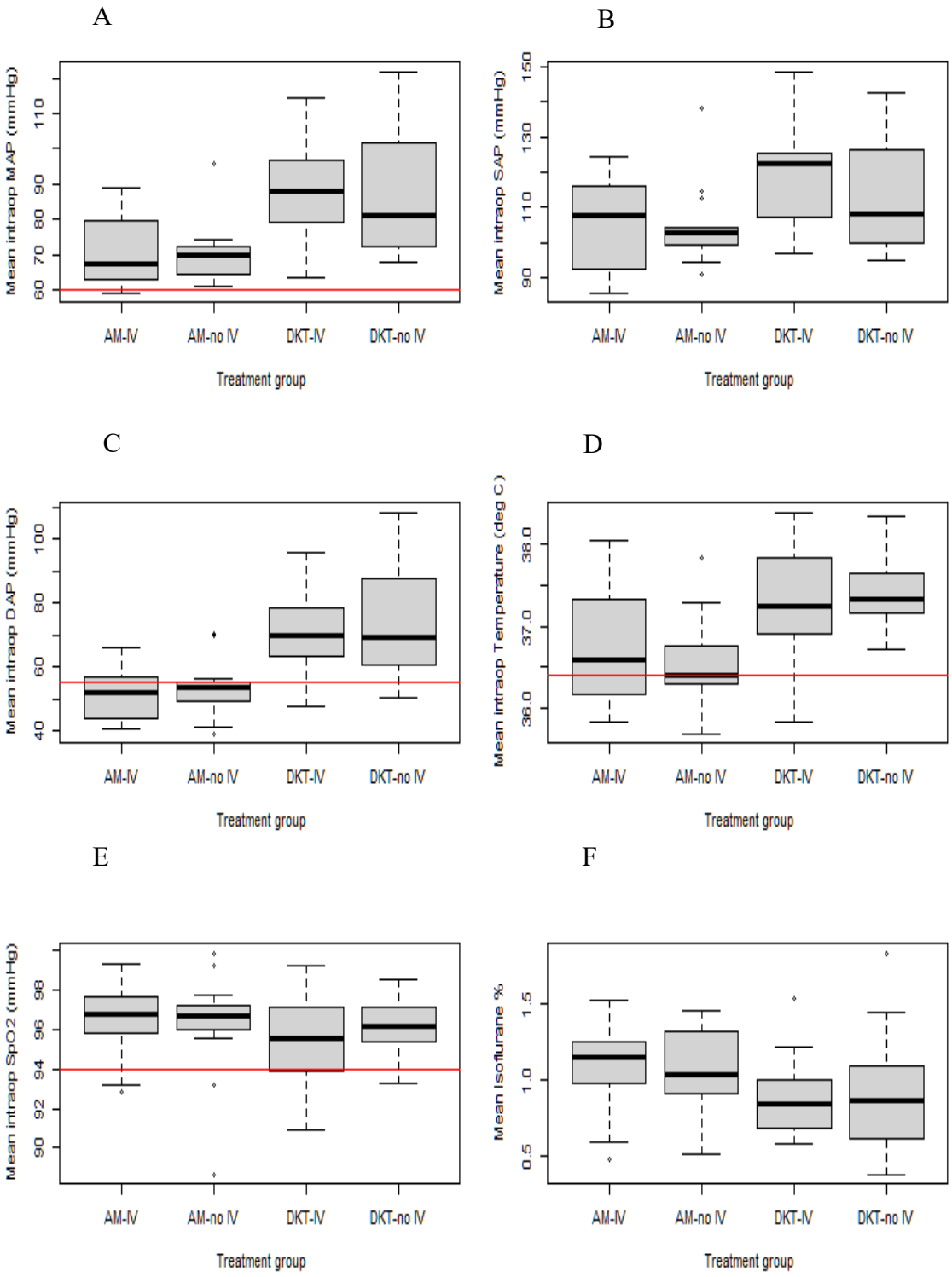


Figure 10 Baseline values of A) m-MAP, B) m-SAP, C) m-DAP, D) m-Temp, E) m-SpO2, and F) m-Isoflurane in different treatment groups.

These boxplots display the five-number summary for the vital parameters recorded (using the means of the recorded variables) for the four treatment groups. The red line represents the lower limit of the reference range for the physiological systemic parameters.

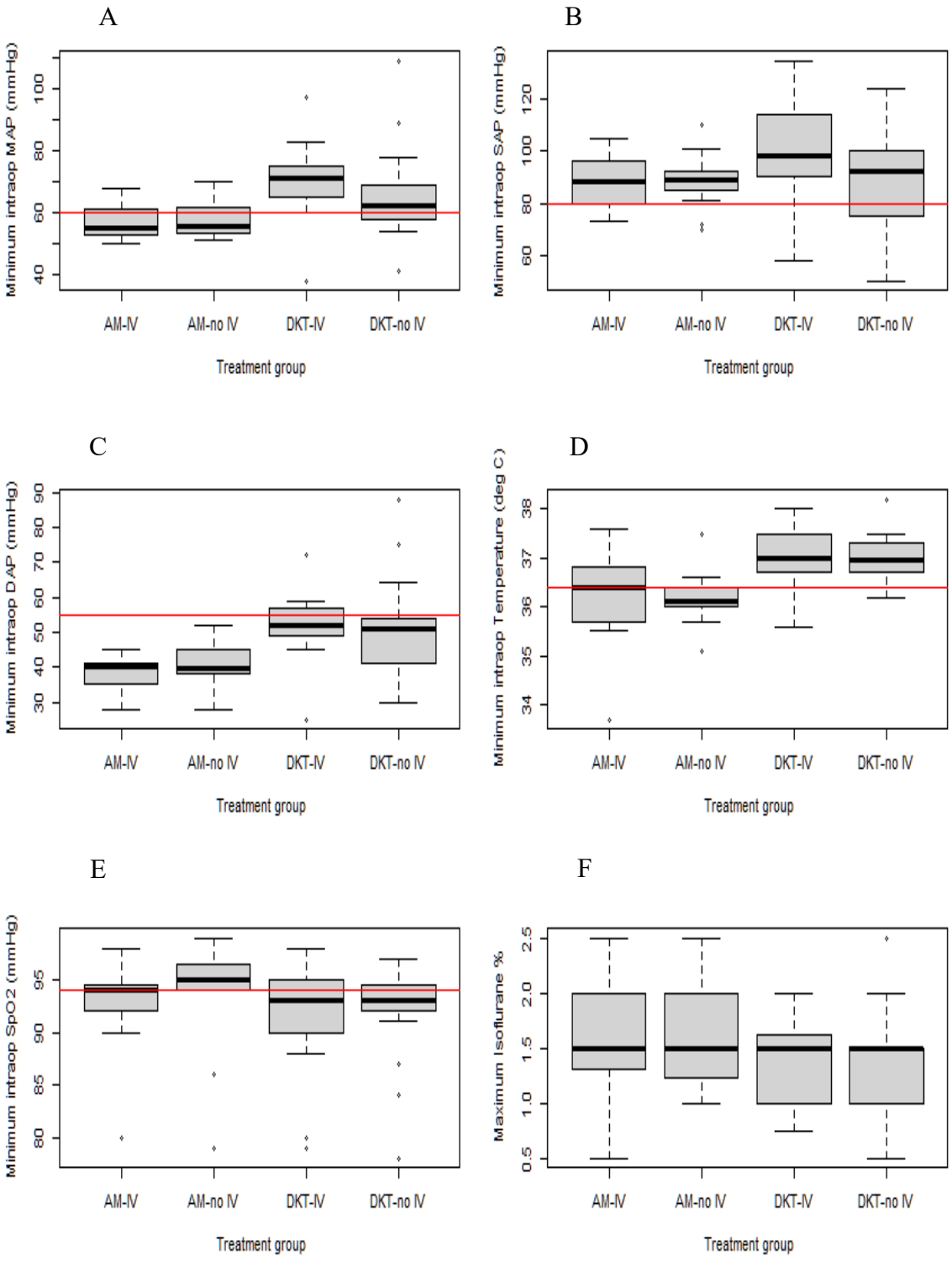


Figure 11 Baseline values of A) l-MAP, B) l-SAP, C) l-DAP, D) l-Temp, E) l-SpO2 and F) maximum Isoflurane percentage in treatment groups.

These boxplots display the five-number summary for the vital parameters recorded (using the minimum values of the recorded variables) for the four treatment groups. The red line represents the lower limit of the acceptable range for the physiological parameters.

Between the two treatment protocols (AM and DKT), variables such as HR, m-MAP, m-SAP, m-Temp, and m-SpO_2 were within the reference range for anaesthetized cats. The differences between those receiving the two protocols (AM and DKT) are summarized in Table 9, while Table 10 shows the differences between the treatment groups.

Table 9 Difference in the intraoperative variables recorded for the treatment protocols* (T.P).

Variable	T.P	Mean ± sd	t-statistic	95% C.I.	p-value
HR (bpm)	AM	162.93 ± 20.37			
	DKT	144.56 ± 25.6	3.08	6.4-30.24	< 0.005
RR (bpm)	AM	23.46 ± 6.37			
	DKT	14.1 ± 5.29	5.9	6.21-12.56	< 0.001
m-MAP (mmHg)	DKT	88.03 ± 15.94			
	AM	70.87 ± 9.3	4.7	9.8-24.5	< 0.001
l-MAP (mmHg)	DKT	67.96 ± 15.29			
	AM	57.44 ± 5.48	3.29	4.01-17.03	< 0.005
m-SAP (mmHg)	DKT	116.68 ± 16.23			
	AM	105.11 ± 12.19	3	3.6-19.27	< 0.005
m-DAP (mmHg)	DKT	73.4 ± 16.41			
	AM	52.21 ± 8.37	5.99	14.04-28.34	< 0.001
l-DAP (mmHg)	DKT	51.78 ± 13.32			
	AM	39.43 ± 5.59	4.45	6.7-17.98	< 0.001
m-Isoflurane (%)	AM	1.07 ± 0.28			
	DKT	0.9 ± 0.35	2.17	0.014-0.34	< 0.05
ET-agent (%)	AM	0.97 ± 0.16			
	DKT	0.8 ± 0.26	2.8	0.05-0.28	< 0.01

(continued)

* AM protocol: acepromazine, morphine and alfaxalone.
DKT protocol. Dexmedetomidine, ketamine, butorphanol.

Table 9 (Continued).

Variable	T.P	Mean ± sd	t-statistic	95% C.I.	p-value
m-Temp (°C)	DKT	37.31 ± 0.63			
	AM	36.67 ± 0.6	3.65	0.3-1	< 0.001
l-Temp (°C)	DKT	36.98 ± 0.66			
	AM	36.19 ± 0.76	3.95	0.39-1.2	< 0.001
m-ETCO$_2$ (mmHg)	DKT	42.7 ± 6.12			
	AM	32.52 ± 3.64	7.82	7.56-12.79	< 0.001

Respiratory rate

Mean RR was observed to be significantly higher for those cats premedicated with AM protocol than those with the DKT protocol (p-value < 0.001), as shown in Figure 12 (A). There was no significant difference in RR mean within the two protocols, i.e., no difference between AM-IV and AM-no IV, and DKT-no IV and DKT-IV.

Heart rate

The DKT protocol groups had a lower mean HR than the AM protocol groups, as shown in Figure 12 (B). Within the treatment groups, we found the drop in HR to be < 20% for all, except in treatment group 4 (DKT-no IV) group where the HR fell from 186.36 ± 30.62 bpm at baseline to 135.1 ± 22.76 bpm.

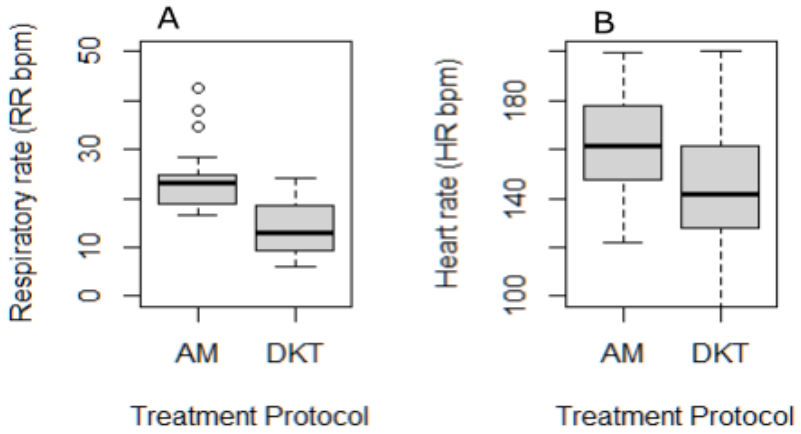


Figure 12 Effect of the treatment protocols on A) RR (bpm), B) HR (bpm).

These boxplots summarize the graphically demonstrate the effect of the treatment protocols on two variables, RR and HR. The study cats who received the DKT protocol had a lower RR and HR than those who received the AM protocol.

Mean arterial pressure

Between the two protocols, the m-MAP was shown to be significantly lower for those who received AM than those who received DKT (p-value < 0.001), as shown in Figure 13 (A). Between the groups, the m-MAP was found to differ statistically (p-value < 0.001), as can be seen in Figure 10 (A). The mean l-MAP recorded for the AM treatment protocol was lower (p-value < 0.005) than for the DKT treatment protocol.

Systolic arterial pressure

As with m-MAP, the values for m-SAP were within normal limits, as illustrated in Figure 10 (B). The mean m-MAP was lower for those cats premedicated with AM than those with DKT (p-value < 0.005). There was no significant difference in mean l-SAP between the AM and DKT protocols (p-value > 0.05).

Diastolic arterial pressure

The mean m-DAP was lower in the AM group than the standard lower limit for DAP, i.e., < 55 mmHg. It was within the normal range for the DKT group. Among those who received AM and DKT, the latter was found to have a higher mean (p-value < 0.05).

Between the treatment groups, the m-DAP was found to significantly differ (p-value < 0.05), as shown in Figure 13 (B). There was a significant difference in mean l-DAP between the AM protocol and the DKT protocol, as seen in Figure 10 (C).

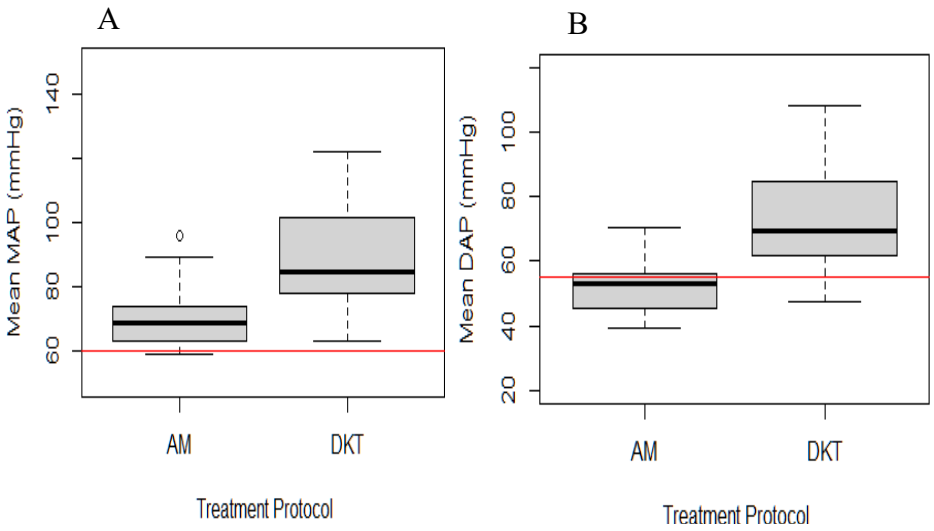


Figure 13 Effect of the treatment protocol on A) m-MAP (mmHg) and B) DAP (mmHg).

These boxplots show that m-MAP and m-DAP were lower for the study cats who received the AM protocol when compared to those who received the DKT protocol. The red line represents the lower limit of the acceptable range for the physiological variable.

Isoflurane administered

As observed in Figure 10 (F), the m-Isoflurane % was higher for those receiving the AM protocol than for the DKT protocol (p-value < 0.05).

End-tidal isoflurane %

The end-tidal agent percentage was higher for the AM group than for the DKT group (p-value < 0.05). Mean ET isoflurane was maintained at > 0.6% (when HR and RR were in the expected range). There was no statistical difference in the mean ET-isoflurane between the treatment groups (p-value > 0.05).

Body Temperature

The m-Temp was within the reference range for all the treatment groups. Interestingly, the AM groups had a lower mean when compared to DKT groups, as seen in Figure 10 (D). There was a significant difference between the l-Temp recorded for those who received AM protocol than the DKT protocol (p-value < 0.05).

Oxygen saturation

The m-SpO_2 was within the reference range with no significant difference between the DKT groups and the AM groups, as seen in Figure 10 (E). There was no statistical difference in l-SpO_2 between, both, the two protocols and between the groups (p-value > 0.05).

End-Tidal CO2

Mean $ETCO_2$ was higher in the DKT groups than in the AM groups (p-value < 0.001). The mean $ETCO_2$ differed between the treatment groups (p-value < 0.01).

Table 10 Difference in the intraoperative variables recorded between the treatment groups.

Variable	Treatment group	Mean ± sd	F-statistic	df	Mean difference	C.I.	p-value
HR (bpm)	***Between the groups***		***5.4***	***3, 56***			***< 0.005***
	AM-IV	166.3 ± 22.92					
	DKT-no IV	135.1 ± 22.76			31.22	9.6-52.8	< 0.002
	AM-no IV	159.57 ± 17.6					
	DKT-no IV	135.1 ± 22.76			24.49	2.87-46.11	< 0.05
RR (bpm)	***Between the groups***		***12.55***	***3, 51***			***< 0.001***
	AM-IV	22.78 ± 4.71					0.02
	DKT-IV	15.64 ± 5.57			7.14	1.17-13.11	
	AM-IV	22.78 ± 4.71					
	DKT-no IV	12.71 ± 4.82			10.07	4.31-15.83	< 0.001
	AM-no IV	24.17 ± 5.57					
	DKT-IV	15.64 ± 5.57			8.5	2.45-14.62	< 0.005
	AM-no IV	24.17 ± 5.57					
	DKT-no IV	12.71 ± 4.82			11.47	5.59-17.34	< 0.001
m-MAP (mmHg)	***Between the groups***		***7.02***	***3, 47***			***< 0.001***

(continued)

Table 10 (Continued).

Variable	Treatment group	Mean ± sd	F-statistic	df	Mean difference	C.I.	p-value
	DKT-IV	88.88 ± 13.43					
	AM-IV	71.09 ± 9.96			17.79	3.82-31.77	< 0.01
	DKT-no IV	87.18 ± 18.64					
	AM-IV	71.09 ± 9.96			16.09	2.12-30.07	0.02
	DKT-IV	88.88 ± 13.43					
	AM-no IV	70.64 ± 8.97			18.24	3.98-32.51	< 0.01
	DKT-no IV	87.18 ± 18.64					
	AM-no IV	70.64 ± 8.97			16.54	2.27-30.8	0.02
l-MAP (mmHg)	***Between the groups***		***3.49***	***3, 47***			***< 0.05***
m-SAP (mmHg)	***Between the groups***		***3.8***	***3, 52***			***0.02***
	DKT-IV	120.71 ± 15.19					
	AM-IV	105.61 ± 13.3			15.1	0.74-29.47	0.04
	DKT-IV	120.71 ± 15.19					
	AM-no IV	104.61 ± 11.44			16.1	1.74-30.47	0.02

(continued)

Table 10 (Continued).

Variable	Treatment group	Mean ± sd	F-statistic	df	Mean difference	C.I.	p-value
m-DAP (mmHg)	***Between the groups***		***12.29***	***3, 51***			***< 0.001***
	DKT-no IV	75.72 ± 19.44					
	AM-IV	51.49 ± 8.01			24.23	10.84-37.62	< 0.001
	DKT-IV	71.24 ± 13.4					
	AM-no IV	52.92 ± 8.95			18.32	5.18-31.46	< 0.005
	DKT-no IV	75.72 ± 19.44					
	AM-no IV	52.92 ± 8.95			22.8	9.4-36.19	< 0.001
l-DAP (mmHg)	***Between the groups***		***6.65***	***3, 51***			***< 0.001***
	DKT-IV	51.86 ± 10.2					
	AM-IV	38.43 ± 4.96			13.43	3.07-23.79	< 0.01
	DKT-IV	51.86 ± 10.2					
	AM-no IV	40.43 ± 6.19			13.26	2.7-23.82	< 0.01
	DKT-IV	51.86 ± 10.2					
	AM-no IV	40.43 ± 6.19			11.43	1.07-21.79	0.02

(continued)

Table 10 (Continued).

Variable	Treatment group	Mean ± sd	F-statistic	df	Mean difference	C.I.	p-value
	DKT-no IV	51.69 ± 16.49					
	AM-no IV	40.43 ± 6.19			11.26	0.7-21.82	0.03
m-Temp (°C)	***Between the groups***		***4.62***	***3, 46***			***< 0.01***
	DKT-IV	37.26 ± 0.76					
	AM-no IV	36.58 ± 0.55			0.68	0.03-1.33	< 0.05
	DKT-no IV	37.38 ± 0.45					
	AM-no IV	36.58 ± 0.55			0.8	0.1-1.5	< 0.02
l-Temp (°C)	***Between the groups***		***4.9***	***3, 46***			***< 0.005***
	DKT-no IV	37.02 ± 0.56					
	AM-IV	36.19 ± 0.93			0.83	0.02-1.63	< 0.05
	DKT-no IV	37.02 ± 0.56					
	AM-no IV	36.18 ± 0.56			0.83	0.01-1.66	< 0.05

(continued)

Table 10 (Continued).

Variable	Treatment group	Mean ± sd	F-statistic	df	Mean difference	C.I.	p-value
m-ETCO2 (mmHg)	***Between the groups***		***23.2***	***3, 56***			***< 0.001***
	DKT-IV	40.68 ± 5.91					
	AM-IV	32.61 ± 4.52			8.07	3.33-12.82	< 0.001
	DKT-no IV	44.72 ± 5.82					
	AM-IV	32.61 ± 4.52			12.11	7.36-16.85	< 0.001
	DKT-IV	40.68 ± 5.91					
	AM-no IV	32.44 ± 2.66			8.24	3.5-12.98	< 0.001
	DKT-no IV	44.72 ± 5.82					
	AM-no IV	32.44 ± 2.66			12.27	7.53-17.02	< 0.001

4.2.3 Baseline (0 hr) renal marker values

Table 11 displays the concentrations of traditional and novel renal markers in the urine and serum samples for the study population. Table 12 summarizes the normalized renal marker values. The baseline renal marker values across treatment groups have been displayed as boxplots in Figure 14, Figure 15 and Figure 16. The USG of the samples ranged from 1 to > 1.030 (1.027 ± 0.01), while the mean urine pH was 5.69 ± 1.11 (ranging from 3-9).

Table 11 Baseline values for various renal markers.

Renal markers	Mean ± Sd	Range	Median
SCr[1] (µmol/L)	75.60 ± 15.29	44.00-110.00	74.00
SDMA[2] (µg/dl)	10.97 ± 3.59	5.00-26.00	11.00
BUN[3] (mg/dl)	8.39 ± 2.05	5.20-18.10	8.10
UTP[4] (g/L)	0.54 ± 0.33	0.09-2.18	0.47
UP:C[5]	0.23 ± 0.12	0.09-0.67	0.19
KIM-1 OD[6]	64.05 ± 20.61	12.20-99.20	50.20
NGAL[7] (ng/ml)	12.77 ± 4.26	3.27-22.66	12.36
NAG activity[8] (U/L)	11.93 ± 11.45	0.92-51.51	8.26
RBP[9] (µg/ml)	38.12 ± 5.32	22.02-48.21	38.66
KIM-1 ratio[10]	2.97 ± 1.72	1.24-9.76	2.38

[1] Serum creatinine
[2] Symmetric dimethylarginine
[3] Blood urea nitrogen
[4] Urine total protein
[5] Urine protein creatinine ratio
[6] Kidney injury molecule-1 optical density
[7] Urine neutrophil gelatinase associated-lipocalin
[8] N-acetyl β-D-glucosaminidase activity
[9] Retinol binding protein
[10] KIM-1 ratio (control OD/test OD)

Table 12 Baseline values for markers normalized to urine creatinine value.

Normalized marker	Mean ± sd	Median	Range
NAG index (U/g)	5.69 ± 5.75	4.27	0.96-28.3
uRBP:Cr	0.06 ± 0.28	0.02	0.01-1.97
UNCR (µg/mmol)	0.83 ± 0.75	0.60	0.09-4.26

As seen in Table 11 and Figure 14, the mean values of the conventional markers, i.e., SCr (75.6 ± 15.29 µmol/L), SDMA (10.97 ± 3.59 µg/dl), and BUN (8.39 ± 2.05 mg/dl) at baseline (0hr) were within the acceptable limits, except UP:C (0.23 ± 0.12).

The renal marker values across all the time points, i.e., baseline (0 hr), 24 and 48 hr values, are summarized as mean ± sd in Table 14 and Table 16 for the study population and across the treatment groups. The data have also been displayed as boxplots in Figure 14, Figure 15 and Figure 16.

4.2.3.1 Traditional Markers

As shown in Figure 14 (A) and (B), the mean SCr and BUN values recorded for the population (blue line) and across the groups fell well within the normal limits (below the red line). The mean SDMA values fell within the normal range for the study population and across the treatment groups (C). The mean UP:C value for the study population revealed borderline proteinuria. There were no significant differences in the mean baseline values of SCr, SDMA, BUN, UTP, urine pH and UP:C (p-value > 0.05). However, there was a difference in UCr outlined in Table 13.

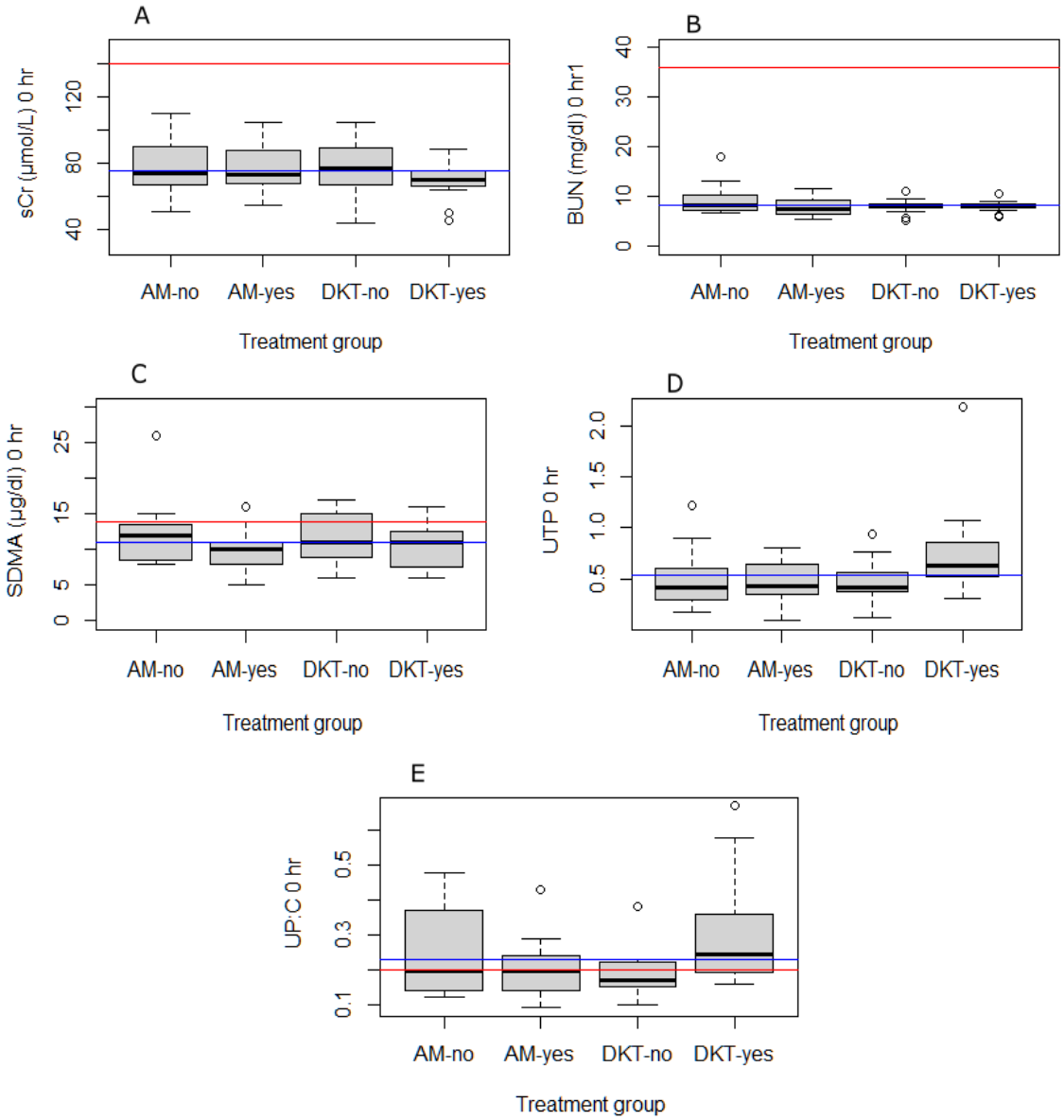


Figure 14 Baseline values (0 hr) for conventional renal markers A) serum creatinine (SCr), B) blood urea nitrogen (BUN), C) symmetric dimethylarginine (SDMA), D) urine total protein (UTP), and E) urine protein creatinine ratio (UP:C) across treatment groups*.

The blue line represents the mean value for the study population, while the red line represents the upper limit for the reference range. As can be seen from the boxplots above, the mean concentration of the conventional markers was within the reference range at 0 hr.

* The treatment groups in the boxplots have been abbreviated as follows:
AM-no stands for AM- no IV group.
AM-yes stands for AM group.
DKT-no stands for DKT-no IV group.
DKT-yes stands for DKT group.

Table 13 Difference in renal marker values at 0 hr between the treatment groups.

Marker	Treatment group	Mean ± sd	F-statistic	df	MD*	95% C.I.	p-value
UCr†	***Between the groups***		***5.02***	***3, 43***			***< 0.005***
	AM-no IV	0.02 ± 0.01					
	AM-IV	0.01 ± 0.01			0.006	0.001-0.01	< 0.01
	AM-no IV	0.02 ± 0.01					
	DKT-no IV	0.01 ± 0.01			0.005	0.0005-0.01	< 0.05

4.2.3.2 Novel Renal Markers

There was no evidence of any statistical difference (p-value > 0.05) in baseline means of KIM-1 OD, KIM-1 ratio, NAG activity (U/L), NAG Index (U/g), RBP (µg/ml), uRBP:Cr, NGAL (ng/ml), and UNCR (µg/mmol) across the treatment groups, as illustrated in Figure 15 and Figure 16.

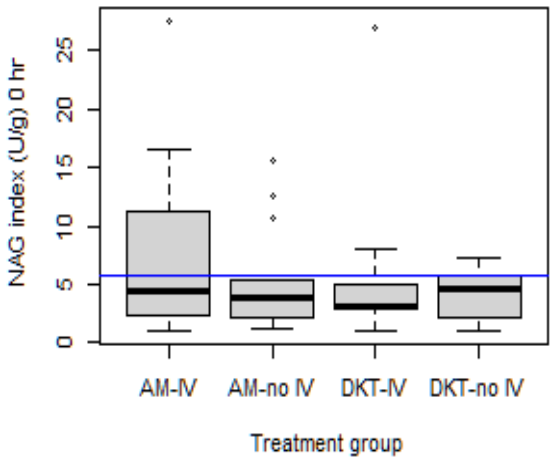


Figure 15 NAG Index (U/g) at baseline (0 hr) across the different treatment groups.

There was no significant difference in mean NAG index concentrations across the treatment groups at 0 hrs as shown in the boxplot. The blue line represents the mean NAG Index value for the study population.

* Mean difference
† µmol/L

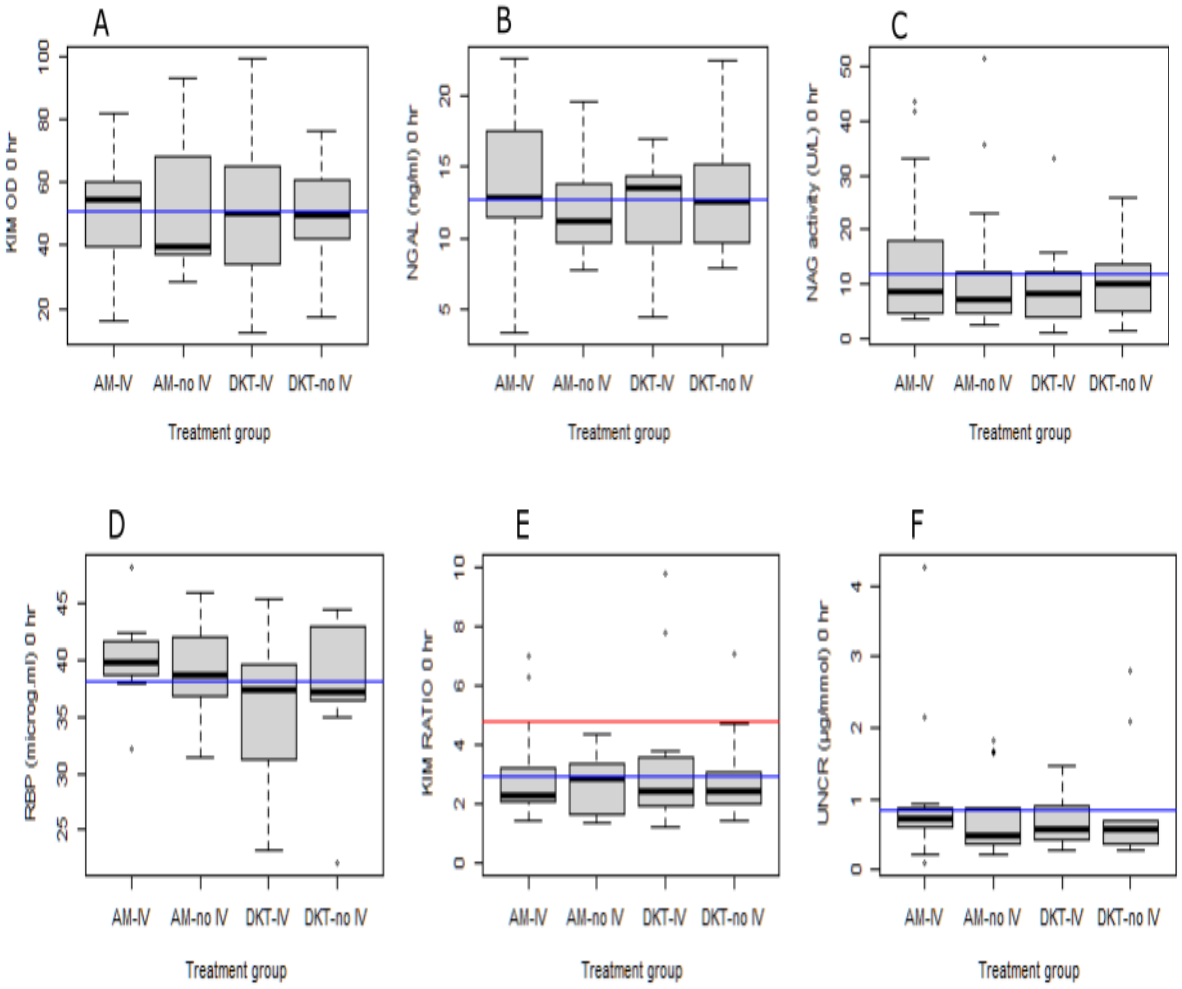


Figure 16 Baseline values (0 hr) for novel renal markers A) KIM-1 OD, B) NGAL, C) NAG activity, D) RBP, E) KIM-1 ratio and F) UNCR across treatment groups.

There was no significant statistical difference in the mean values of the novel markers between the treatment groups at 0 hr as shown in the boxplot above. The blue line represents the mean value for the study population, while the red line represents the estimated upper limit based on other sources (no standardized range available at the time of writing this thesis).

4.2.4 Renal marker values across time-points (Study Population)

Table 14 tabulated the marker values for the study population across the three time points (0, 24 and 48 hr). Urine pH was observed to be 5.39 ± 0.72 (4 - 8) at 24 hrs and 5.15 ± 0.48 (4 - 6.5) at 48 hrs. USG was approximately 1.03 ± 0.007 at 24 hrs and 1.03 ± 0.01 at 48 hrs. However, the USG readings are unreliable as they were recorded using the dipstick method.

Table 14 Renal marker concentration at different time points*.

Renal markers	T0	T24	T48
Classical markers			
SCr (µmol/L)	75.6 ± 15.29	86.52 ± 25.52	93.72 ± 29.01⇑
SDMA (µg/dl)	10.97 ± 3.59	9.04 ± 2.54	8.47 ± 2.59
BUN (mg/dl)	8.93 ± 2.05	7.8 ± 2.35	7.53 ± 1.62
UTP (g/L)	0.54 ± 0.34	0.69 ± 0.42	0.75 ± 0.4
UP:C	0.23 ± 0.12	0.25 ± 0.16	0.25 ± 0.16
Novel markers			
KIM-1 OD	50.81 ± 20.61	57.48 ± 16.55	57.15 ± 18.44
NGAL (ng/ml)	12.77 ± 4.26	11.85 ± 4.28	9.25 ± 3.75
NAG activity (U/L)	11.93 ± 11.45	8.88 ± 5.79	9.20 ± 4.66
RBP (µg/ml)	38.12 ± 5.32	38.73 ± 3.05	37.90 ± 3.93
KIM-1 ratio (C/T)	2.97 ± 1.72	2.29 ± 0.74	2.22 ± 0.73
NAG Index (U/g)	5.69 ± 5.75	3.19 ± 1.83	3.25 ± 1.79
UNCR (µg/mmol)	0.83 ± 0.75	0.53 ± 0.36	0.47 ± 0.60
uRBP:Cr	0.06 ± 0.28	0.01 ± 0.006	0.01 ± 0.01

* T0 : 0 hrs
T24: 24 hrs
T48: 58 hrs
Values presented as mean ± sd.

⇑ significant increase between 48 and 24 hrs

24hrs-0 hrs

While analyzing the changes in renal marker values across time points, we found no significant difference in SCr, SDMA, USG, KIM-1 ratio, RBP, uRBP:Cr, NGAL, and UNCR, between 24 and 0 hrs for the study population (p-value > 0.05). Those that were statistically significant are summarized in Table 15.

48-24 hrs

While comparing the changes in renal marker values across 48 and 24 hours, we observed no significant difference in RBP, USG, uRBP:Cr, NGAL, UNCR and UCr values across 24 and 48 hrs. However, there was a difference in the following traditional and novel renal marker values. Those that were statistically significant are summarized in Table 15.

48-0 hrs

While comparing the renal marker values between 48 and 0 hrs, we found no significant difference in mean SCr, SDMA, BUN, USG, UTP, KIM-1 OD, KIM-1 ratio, RBP, uRBP:Cr, NGAL, and UNCR between 48 and 0 hrs for the study population (p-value > 0.05). Those that were statistically significant are summarized in Table 15.

Table 15 Renal markers with statistically significant difference in values between time-points.

Between time points	Renal marker	F-statistic	p-value
24-0	**BUN (mg/dl)**	6.03	< 0.05
	UCr (µmol/L)	30.46	< 0.001
	UTP (g/L)	13.45	< 0.001
	Urine pH	6.5	< 0.02
	UP:C	27.34	< 0.001
	KIM-1 OD	6.79	< 0.05
	NAG activity (U/L)	23.94	< 0.001
	NAG Index (U/g)	10.03	< 0.005
48-24	**SCr (µmol/L)**	37.45	< 0.001
	SDMA (µg/dl)	6.3	< 0.02
	BUN (mg/dl)	22.24	< 0.001
	UTP (g/L)	33.45	< 0.001
	UP:C	91.35	< 0.001
	Urine pH	5.6	< 0.05
	KIM-1 OD	25.23	< 0.001
	KIM-1 ratio	15.25	< 0.001
	NAG activity (U/L)	15.77	< 0.001
	NAG Index (U/g)	8.7	< 0.005
48-0	**UP:C**	7.55	< 0.01
	NAG activity (U/L)	24.54	< 0.001
	NAG Index (U/g)	16.68	< 0.001
	Urine pH	4.1	< 0.05

4.2.5 Renal marker values (Treatment Groups)

Table 16 enumerates the mean concentration of the various renal markers analyzed for all the treatment groups at the three time points.

Table 16 Renal marker values for the treatment groups across the different time points. [1]

	AM-no IV			DKT-no IV			AM-IV			DKT-IV		
Renal Marker	**T0**	**T24**	**T48**	**T0**	**T24**	**T48**	**T0**	**T24**	**T48**	**T0**	**T24**	**T48**
SCr[2]	77.74 ± 17.02	86.43 ± 26.91	97 ± 38.52	78.47 ± 16.87	83.24 ± 22.1	97 ± 29.77	76.2 ± 15.21	92.34 ± 33.22	94.54 ± 29.08	70 ± 11.52	83.43 ± 18.21	87 ± 18.22
SDMA[3]	12.07 ± 4.58	8.27 ± 1.67	9.47 ± 2.19	11.58 ± 3.44	9.09 ± 2.51	7.7 ± 1.26	9.94 ± 2.87	9.94 ± 3.31	8.87 ± 3.97	10.34 ± 3.18	8.86 ± 2.35	7.86 ± 1.71
BUN[4]	9.32 ± 3.13	7.87 ± 1.55	7.5 ± 1.15	8.1 ± 1.47	7.11 ± 1.44	8.04 ± 1.8	7.98 ± 1.86	8.56 ± 3.97	7.86 ± 2.18	8.19 ± 1.1	7.58 ± 0.98	6.8 ± 0.86
UCr[5]	0.02 ± 0.01	0.02 ± 0.01	0.17 ± 0.17	0.01 ± 0.01	0.01 ± 0.01	0.21 ± 0.25	0.01 ± 0.01	0.01 ± 0.01	0.2 ± 0.15	0.01 ± 0.01	0.01 ± 0.01	0.23 ± 0.21
UP:C	0.24 ± 0.13	0.32 ± 0.22	0.33 ± 0.22	0.2 ± 0.09	0.2 ± 0.09	0.24 ± 0.13	0.21 ± 0.09	0.2 ± 0.06	0.22 ± 0.15	0.32 ± 0.18	0.28 ± 0.19	0.24 ± 0.1
NAG Activity[6]	12.58 ± 14	11 ± 7.9	9.93 ± 4.54	10.5 ± 7.66	6.55 ± 4.45	6.89 ± 2.55	14.78 ± 13.9	8.94 ± 5.14	10.34 ± 4.82	9.47 ± 8.66	9.08 ± 4.48	9.81 ± 5.86
NGAL[7]	11.93 ± 3.52	10.57 ± 4.37	9.56 ± 5.21	13.08 ± 4.4	13.35 ± 5.27	9.21 ± 3.03	13.89 ± 5.18	12.69 ± 3.14	9.2 ± 3.23	12.07 ± 3.82	10.88 ± 3.79	9.02 ± 3.58
RBP[8]	38.91 ± 4.07	38.24 ± 3.64	37.3 9 ±3.4	37.83 ± 5.71	38.72 ± 2.82	38.06 ± 2.45	40.07 ± 3.43	39.73 ± 3.05	38.13 ± 5.64	35.49 ± 6.98	38.3 ± 2.7	38.05 ± 3.97
KIM-1 OD	52.5 ± 22.04	61.03 ± 17.9	55.7 ± 15.41	49.91 ± 17.57	51.14 ± 13.36	60.1 ± 15.26	50.9 ± 20.25	57.5 ± 18.2	66.15 ± 21.19	49.83 ± 24.17	60.47 ± 16.37	60.63 ± 21.34
NAG Index[9]	5.28 ± 4.43	3.25 ± 1.75	3.53 ± 1.36	4.22 ± 2.16	2.43 ± 1.43	2.39 ± 0.96	7.2 ± 7.45	3.64 ± 2.37	3.64 ± 2.5	5.97 ± 7.69	3.39 ± 1.65	3.51 ± 1.88
UNCR[10]	0.78 ± 0.57	0.41 ± 0.26	0.58 ± 1.03	0.83 ± 0.8	0.64 ± 0.42	0.41 ± 0.35	0.99 ± 1.06	0.63 ± 0.28	0.4 ± 0.25	0.71 ± 0.43	0.51 ± 0.47	0.53 ± 0.54
uRBP:Cr	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	0.2 ± 0.59	0.02 ± 0.01	0.02 ± 0.02
KIM-1 ratio	2.68 ± 1.07	2.15 ± 0.59	2.37 ± 0.66	2.89 ± 1.52	2.54 ± 0.8	2.14 ± 0.52	3 ± 1.71	2.32 ± 0.84	2.05 ± 0.81	3.35 ± 2.45	2.16 ± 0.73	2.33 ± 0.93

[1] Values represented as mean ± sd.
[2] µmol/L
[3] µg/dl
[4] mg/dl
[5] µmol/L
[6] U/L
[7] ng/ml
[8] µg/ml
[9] U/g
[10] µg/mmol

4.2.5.1 Group-Wise Differences

An independent t-test was performed to identify significant differences (p-value < 0.05) in mean marker values between the treatment groups, as shown in Table 17.

Table 17 Difference in the renal marker values between the treatment groups across time points.

Marker	Time point	Treatment group	Mean/M.D[*] ± sd	t-statistic	p-value
SDMA	48 hr	AM-no IV (vs)[†] DKT-no IV	9.47 ± 2.19 7.69 ± 1.25	2.5	0.02
UTP[‡]	24 hrs		0.99 ± 0.61 0.54 ±0.26	2.6	0.02
UCr	0 hr		0.01 ± 0.007 0.01 ± 0.003	2.5	0.02
UTP	48-24		0.54 ±0.42 0.11 ±0.27	3	0.006
NAG Index	48 hr		3.53 ± 1.36 2.38 ± 0.9	2.6	0.02
NAG activity	48 hr		9.9 ± 4.5 6.9 ± 2.5	2.2	0.04
SDMA	48-24		1.31 ± 2.84 -1.54 ± 1.75	3	0.007
KIM-1 ratio	48-24		0.22 ± 0.44 -0.4 ± 1	2.2	0.04
KIM-1 OD	48-24		-5.33 ±11.55 8.96 ± 20.55	-2.3	0.03

(continued)

[*] Mean difference
[†] versus
[‡] g/L

Table 17 (Continued).

Marker	Time point	Treatment group		Mean/M.D.[§]±sd	t-statistic	p-value
SDMA	48 hr	AM-no IV DKT-IV	(vs)	9.46 ± 2.18 7.86 ± 1.7	2.12	0.04
SDMA	48-24			1.31 ± 2.84 -0.92 ±2.06	2.29	0.03
UTP	24-0			0.54 ± 0.42 -0.05 ± 0.4	3.52	0.002
UTP	24 hr	AM-no IV AM-IV	(vs)	0.99 ± 0.61 0.48 ± 0.15	3.12	0.007
UCr	0 hr			0.01 ± 0.007 0.005 ± 0.002	3.16	0.007
UNCR	24 hr			0.4 ± 0.3 0.6 ± 0.27	-2.16	0.04
uRBP:Cr	24 hr			0.01 ± 0.006 0.02 ± 0.004	-2.19	0.04
SDMA	24-0			-3.8 ± 4.96 0 ± 4.2	-2.27	0.03
UTP	24-0			0.53 ± 0.42 0.02 ± 0.21	4.03	0.001
KIM-1 ratio	48-24			0.22 ± 0.44 -0.2 ± 0.34	2.84	0.008
KIM-1 OD	48-24			-5.33 ± 11.55 7.6 ± 11.7	-2.94	0.007
SDMA	48-0	AM-IV DKT-no IV	(vs)	-1.1 ± 3.9 -4.15 ± 3.4	2.25	0.03
NAG activity	48 hr			10.3 ± 4.8 6.9 ± 2.5	2.4	0.03
UTP	24 hr	AM-IV DKT-IV	(vs)	0.7 ± 0.23 0.48 ± 0.15	-2.8	0.01

(continued)

[§] Mean difference

Table 17 (Continued).

Marker	Time point	Treatment group	Mean/M.D.*±sd	t-statistic	p-value
UCr	0 hr		0.005 ± 0.002 0.008 ± 0.002	-3.15	0.006
RBP	0 hr		40.06 ± 3.42 35.48 ± 6.97	2.14	0.05
BUN	48-0		-0.12 ± 1.8 -1.45 ± 0.96	2.4	0.02
BUN	48 hr	DKT-no IV (vs) DKT-IV	8.03 ± 1.79 6.79 ± 0.86	2.19	0.04
BUN	48-24		1.36 ± 2.43 -0.82 ± 1.37	2.54	0.02

4.2.6 Effect of treatment group

The treatment groups did not affect the difference in mean NAG activity, NAG index, RBP, NGAL, UNCR, SCr and UCr between different time points, p-value > 0.2 (See Appendix A). The renal markers affected by treatment groups are summarized in Table 18.

* Mean difference

Table 18 Renal markers (mean difference) influenced by treatment groups.

Renal marker across time points	Treatment group	F-statistic	df	MD† (95% C.I.)	p-value
KIM-1 OD 48-24	***Treatment groups***	2.493	3. 53		***0.07***
	AM-no IV				0.04
KIM-1 ratio 48-24	***Treatment groups***	2.35	3, 52		***0.08***
	DKT-no IV				0.02
uRBP:Cr 48-24	***Treatment groups***	1.75	3, 46		***0.17***
SDMA 24-0	***Treatment groups***	2.11	3, 52		***0.11***
	AM-no IV				0.02
SDMA 48-24	***Treatment groups***	2.47	3, 48		***0.07***
	AM-IV				0.03
	DKT-no IV				0.02
BUN 24-0	***Treatment groups***	1.9	3, 52		***0.14***
	AM-IV				0.02
BUN 48-0	***Treatment groups***	2.8	3, 50		***0.049***
	AM-IV				0.03
	DKT-no IV				0.02
BUN 48-24	***Treatment groups***	2.9	3, 46		***0.045***
	AM-IV				0.07
	DKT-IV (vs)			2.18	
	DKT-no IV			(0.005-4.37)	0.05

(continued)

† Mean difference

Table 18 (Continued).

Renal marker across time points	Treatment group	F-statistic	df	MD‡ (95% C.I.)	p-value
UTP 24-0	***Treatment groups***	7.59	3, 43		***< 0.001***
	AM-IV				< 0.001
	DKT-no IV				< 0.005
	DKT-IV				< 0.001
	AM-no IV (vs) AM-IV			0.51 (0.15-0.87)	0.002
	AM-no IV (vs) DKT-no IV			0.43 (0.05-0.81)	0.02
	AM-no IV (vs) DKT-IV			0.58 (0.21-0.95)	< 0.001
UTP 48-0	***Treatment groups***	2.3	3, 48		***0.09***
	DKT-IV				0.01

The treatment groups were associated with the mean difference in KIM-1 OD 48-24[§] (Figure 17 A), KIM-1 ratio 48-24 (Figure 17 B), uRBP:Cr 48-24, SDMA 24-0[**] (Figure 18 A), SDMA 48-24 (Figure 18 B). Post hoc analyses using Tukey's test indicated no significant difference between the treatment groups (p-value > 0.05).

‡ Mean difference
§ Between 48 and 24 hrs
** Between 24 and 0 hrs

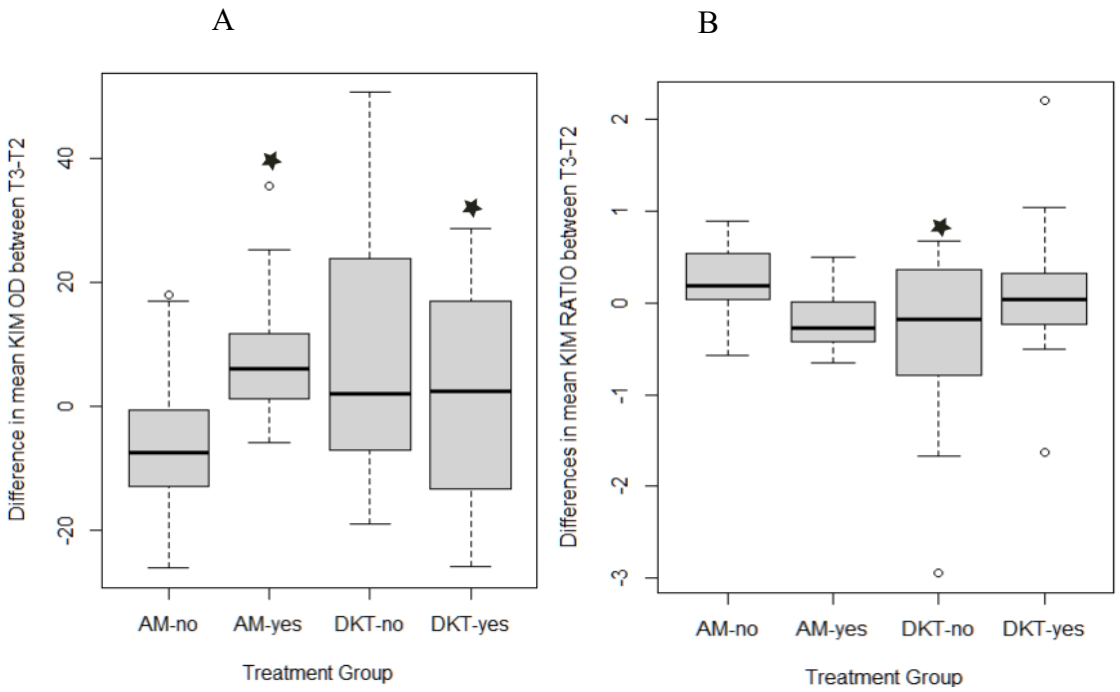


Figure 17 Mean difference in A) KIM-1 OD 48 -24 hrs and B) KIM-1 ratio 48-24

These boxplots summarize the association between the mean difference recorded in the renal markers, KIM-1 OD and KIM-1 ratio, across two time-points against the treatment groups. Those with a significant association are identified by the symbol ★ (p-value < 0.05).

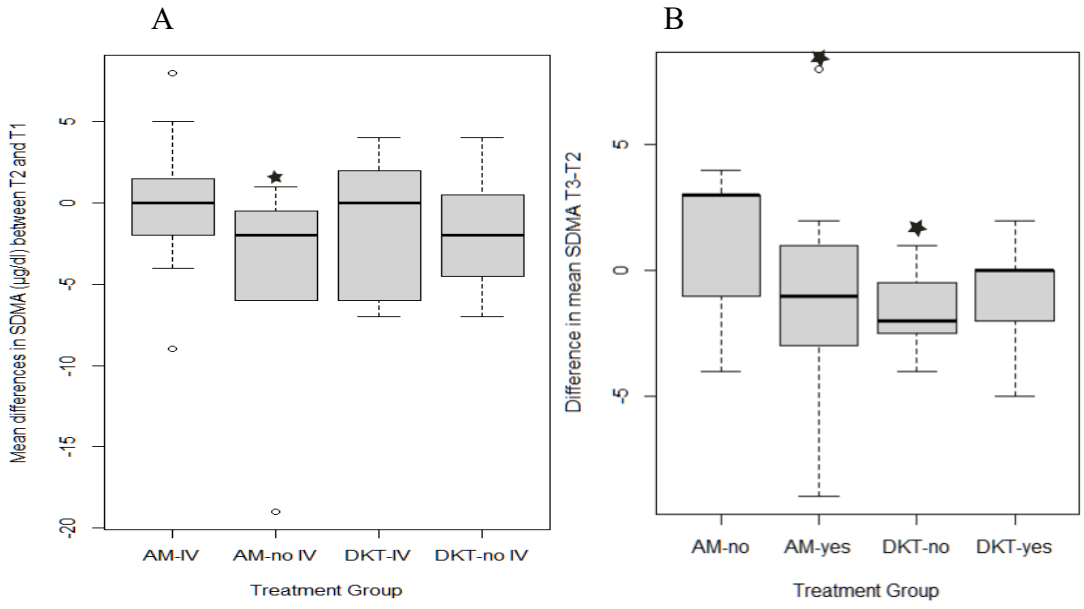


Figure 18 Mean difference in SDMA between A) 24 and 0 hrs; B) 48 and 24 hrs.

These boxplots summarize the association between the mean difference recorded in the renal marker, SDMA, across two time-points against the treatment groups. Those with a significant association are identified by the symbol ★ (p-value < 0.05).

The treatment groups were found to be associated with the mean difference in BUN 24-0 (Figure 19 A), BUN 48-0 Figure 19 B), and BUN 48-24 (Figure 19 C). Post hoc analyses using Tukey's test indicated a very weak difference in mean BUN 48-24 between those in the DKT-no IV group and those in the DKT-IV group (p-value 0.05).

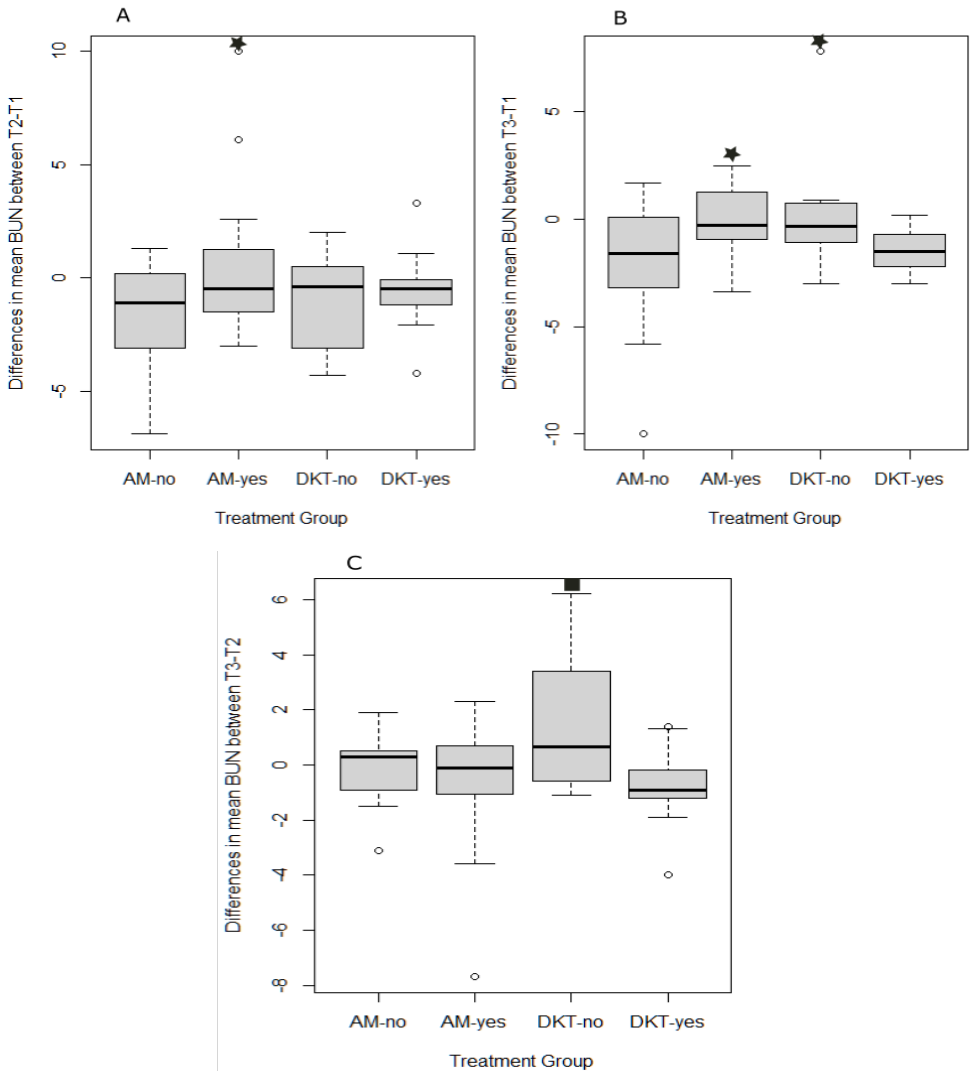


Figure 19 Mean difference in BUN between A) 24 and 0 hrs; B) 48 and 0 hrs; C) 48 and 24 hrs.

These boxplots summarize the association between the mean difference recorded in the renal marker, BUN, across two time points against the treatment groups. Those with a significant association are identified by the symbols ★ (p-value < 0.05) and ■ (p-value < 0.2).

Treatment groups were associated with the mean difference in UTP 24-0 (Figure 20) and UTP 48-0 (Figure 20). Post hoc analyses using Tukey's test indicated no significant difference between the treatment groups (p-value > 0.05).

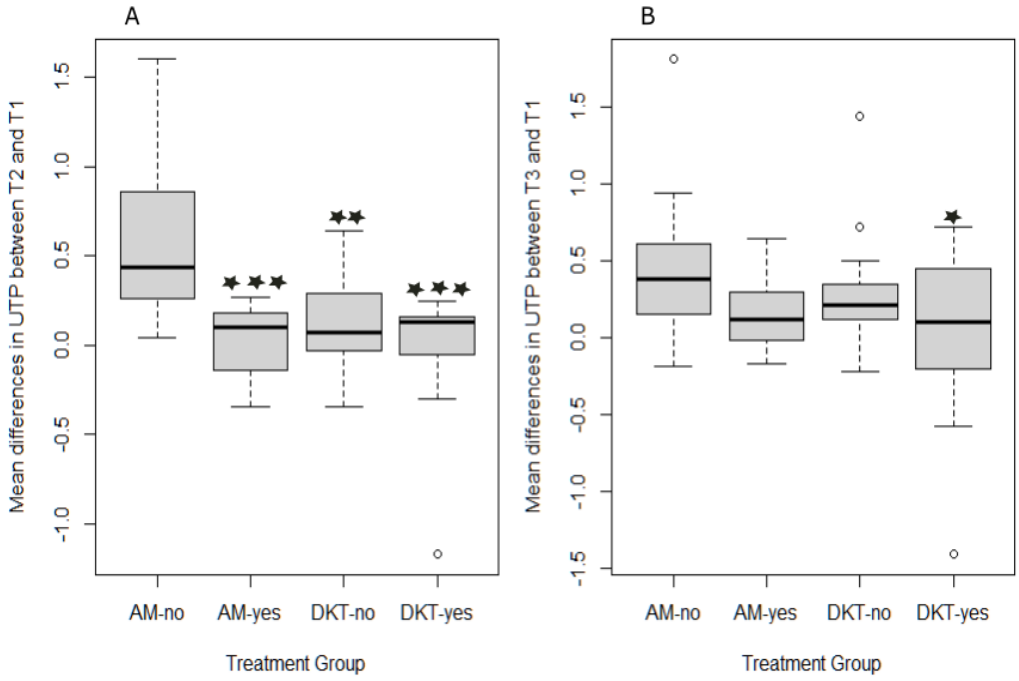


Figure 20 Mean difference in UTP between A) 24 and 0 hrs, B) 48 and 0 hrs.*

These boxplots summarize the association between the mean difference recorded in the renal marker, UTP, across two time points against the treatment groups. Those with a significant association are recognized by the symbols: ★ (p-value < 0.05), ★ ★ (p-value < 0.005) and ★★★ (p-value < 0.001).

There was no significant effect of the treatment group on the difference in mean urinary pH between the time points (p-value > 0.05).

4.2.7 Other factors associated with the change in renal marker values

The intraoperative factors which were found to be associated with the change in mean renal marker values are listed in Table 19 (See Appendix B).

* ★ p-value < 0.05

Table 19 List of factors associated with the change in mean renal marker values between time points.

Renal marker	Factors
BUN 48-0	l-Temp (p-value 0.03), l-SpO_2 (p-value 0.04), $ETCO_2$ (p-value 0.05)
BUN 48-24	fluids (p-value < 0.05)
KIM-1 OD 24-0	age (p-value < 0.05)
KIM-1 OD 48-0	m-MAP (p-value < 0.05), weight (p-value < 0.05), age (p-value < 0.05)
KIM-1 RATIO 24-0	l-SpO_2 (p-value < 0.05)
KIM-1 RATIO 48-0	l-SpO_2 (p-value < 0.05)
NAG ACTIVITY 24-0	l-SAP (p-value < 0.05), $ETCO_2$ (p-value < 0.03), body weight (p-value < 0.05), maximum isoflurane (p-value < 0.03), m-isoflurane (p-value < 0.1)
NAG ACTIVITY 48-0	l-MAP (p < 0.05), l-SAP (p-value < 0.05), body weight (p-value < 0.05), $ETCO_2$ (p-value < 0.03)
NAG INDEX 24-0	l-SAP (p-value < 0.05), anaesthesia time (p-value 0.05), m-isoflurane (p-value < 0.001), body weight (p-value < 0.05)
NAG INDEX 48-0	l-MAP (p < 0.05), l-SAP (p-value < 0.05), body weight (p-value < 0.05), m-isoflurane (p-value < 0.001)
NAG INDEX 48-24	l-MAP (p < 0.05)
NGAL 24-0	l-SAP (p-value < 0.05), l-Temp (p-value < 0.05)
NGAL 48-0	FiO_2 (p-value < 0.05)
RBP 24-0	l-MAP (p-value < 0.05), l-DAP (p-value < 0.05), m-isoflurane (p-value < 0.01)
RBP 48-0	l-MAP (p-value < 0.05), l-DAP (p-value < 0.05), FiO_2 (p-value < 0.05), m-isoflurane (p-value < 0.01), m-MAP (p-value < 0.05)
RBP 48-24	FiO_2 (p-value < 0.05)

(continued)

Table 19 (Continued).

Renal marker	Factors
SCr 24-0	l-Temp (p-value < 0.05)
SCr 48-0	l-SpO_2 (p-value < 0.05), m-Isoflurane (p-value < 0.05)
SCr 48-24	body weight (p-value < 0.05), maximum Isoflurane (p-value < 0.05), m-Isoflurane (p-value < 0.01)
SDMA 24-0	fluids (p-value < 0.05), body weight (p-value < 0.05)
SDMA 48-0	age (p-value < 0.05), l-SpO_2 (p-value < 0.05), m-SpO_2 (p-value < 0.005)
SDMA 48-24	maximum isoflurane % (p-value < 0.05)
UCr 48-0	body weight (p-value < 0.05), age (p-value < 0.001), m-SpO_2 (p-value < 0.01)
UCr 48-24	age (p-value < 0.001), l-SAP (p-value 0.001), m-DAP (p-value < 0.05), m-Temp (p-value < 0.01), m-Isoflurane (p-value < 0.01)
UNCR 24-	m-SpO_2 (p-value < 0.1)
UNCR 48-0	FiO_2 (p-value < 0.005), l-SpO_2 (p-value < 0.05).
UNCR 48-24	l-SpO_2 (p-value < 0.1)
UP:C 24-0	l-SpO_2 (p-value < 0.05), m-SpO_2 (p-value < 0.05), m-MAP (p-value < 0.05).
UP:C 48-0	FiO_2 (p-value < 0.01)
UP:C 48-24	l-DAP p-value (< 0.1)
uRBP:Cr 48-0	m-DAP (p-value < 0.01), FiO_2 (p-value < 0.05), m-MAP (p-value < 0.2).
UTP 24-0	fluids (p-value < 0.01), m-Isoflurane (p-value < 0.01), premedication agent (p-value < 0.05)
UTP 48-0	fluids (p-value < 0.05), m-Isoflurane (p-value < 0.005)

The statistically significant differences between the categories of the variables associated with the renal biomarkers are summarized in Table 20.

Table 20 Factors associated with the change in renal marker values between time points.

Marker	Variable	F-statistic	M.D*	95% C.I.	p-value
BUN 48-0	***l-Temp***	***5.133***			***0.03***
	33.5-36 °C				
	36-39.5 °C		1.96	0.21-3.72	< 0.05
	l-SpO2	***3.545***			***0.04***
	90-95%				
	95-100%		1.94	0.14-1.94	< 0.05
	$ETCO_2$	***4.24***			***0.003***
	55-64 mmHg				
	45-50 mmHg		4.7	0.4-9.01	0.02
BUN 48-24	***Fluids***	***5.11***			***0.03***
	Didn't receive IV fluids				
	Received IV fluids		1.27	0.14-2.4	< 0.05
KIM-1 OD 24-0	***Age***	***5.49***			***< 0.05***
	12-85 m		14.4	2.07-26.74	
	0-12 m				< 0.05
KIM-1 OD 48-0	***Weight***	***3.02***			***< 0.05***
	2-3 kg				
	1-2 kg		26.86	0.4-53.33	< 0.05
	Age	***4.55***			***< 0.05***
	12-85 m				
	0-12 m		14.53	0.87-28.18	0.04

(continued)

* Mean difference

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
	m-MAP	***3.28***			***0.02***
	85-95 mmHg				
	59-65 mmHg		38.02	1.56-74.48	0.04
	85-95 mmHg				
	65-75 mmHg		33.7	2.58-64.82	0.03
	85-95 mmHg				
	75-85 mmHg		35.46	2.2-68.72	0.03
KIM-1 ratio 24-0	***l-SpO_2***	***2.91***			***< 0.05***
	77-82%				
	87-92%		2.84	0.16-5.51	0.03
KIM-1 ratio 48-0	***l-SpO_2***	***2.7***			***< 0.05***
	77-82%				
	87-92%		2.82	0.23-5.42	< 0.03
NAG activity 24-0	***l-SAP***	***6.88***			***< 0.05***
	90-135 mmHg				
	45-90 mmHg		5.13	1.2-9.05	< 0.05
	Weight	***4.13***			***< 0.05***
	0-3 kg				
	3-5 kg		4.8	0.06-9.54	< 0.05
	Mean isoflurane	***3.14***			***< 0.1***
	1-1.5%				
	1.5-2%		13.13	0.35-25.92	< 0.05

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
	$ETCO_2$	***2.76***			***< 0.03***
	35-40 mmHg				
	30-35 mmHg		12.22	0.99-23.44	< 0.03
	40-45 mmHg				
	30-35 mmHg		12.27	0.66-23.88	< 0.04
	Max isoflurane	***3.44***			***< 0.03***
	0.4-1%				
	2-2.6%		15.38	1.18-29.58	< 0.03
	1-1.5%				
	2-2.6%		16.68	2.82-30.54	< 0.02
	1.5-2%				
	2-2.6%		15.29	1.16-29.42	< 0.03
NAG activity 48-0	***l-MAP***	***4.84***			***< 0.05***
	l-SAP	***9.67***			***< 0.05***
	90-135 mmHg				
	45-90 mmHg		7.23	2.55-11.9	< 0.005
	$ETCO_2$	***2.86***			***< 0.03***
	40-45 mmHg				
	30-35 mmHg		14.49	2.41-26.58	< 0.02
	Weight (2 categories)	***8.24***			***< 0.05***
	0-3 kg				
	3-5 kg		7.34	2.21-12.47	< 0.01

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
	Weight (> 2 categories)	***4.55***	15.94	0.72-15.94	***< 0.01***
	1-2 kg				
	4-5 kg				< 0.05
			16.9	3.6-30.21	
	2-3 kg				
	4-5 kg				< 0.01
NAG Index 24-0	***l-SAP***	***4.57***			***< 0.05***
	90-135 mmHg				
	45-90 mmHg		1.81	0.1-3.53	< 0.05
	Duration of anaesthesia	***21.38***			***0.05***
	Mean isoflurane	***21.38***			***< 0.001***
	0.3-1%				
	1.5-2%		15.71	9.82- 21.6	< 0.001
	1-1.5%				
	1.5-2%		15.13	9.26-20.99	< 0.001
	Weight	***6.21***			***< 0.05***
	0-3 kg				
	3-5 kg	3.25		0.62-5.88	< 0.02
	1-2 kg				
	4-5 kg	10.14		3.76-16.52	< 0.001
	2-3 kg				
	4-5 kg	9.49		3.67-15.31	< 0.001
	3-4 kg				
	4-5 kg	7.76		2.18-13.33	< 0.01

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
NAG Index 48-0	***l-MAP***	***7.79***			***< 0.05***
	l-SAP	***9.99***			***< 0.05***
	90-135 mmHg				
	45-90 mmHg		3.46	1.25- 5.67	< 0.005
	Mean isoflurane	***11.4***			***< 0.001***
	0.3-1%				
	1.5-2%		15.18	7.46-22.91	< 0.001
	1-1.5%				
	1.5-2%		14.14	6.46-21.82	< 0.001
	Weight (2 categories)	***9.17***			***< 0.05***
	0-3 kg				
	3-5 kg		4.23	1.42-4.23	
	Weight (> 2 categories)	***8.03***			***< 0.001***
	1-2 kg				
	4-5 kg		11.25	3.85-18.66	< 0.001
	2-3 kg				
	4-5 kg		11.25	4.83-17.66	< 0.001
	3-4 kg				
	4-5 kg		8.36	2.1-14.61	0.005
NAG Index 48-24	***l-MAP***	***5.1***			***< 0.05***
	60-110 mmHg				
	35-60 mmHg		1.35	0.14- 2.57	0.03

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
NGAL 24-0	***l-SAP***	***3.39***			***< 0.05***
	80-100 mmHg				
	100-140 mmHg		4.95	0.56-9.34	0.02
	l-Temp	***3.17***			***< 0.05***
	37.5-39.5 °C				
	36.5-37.5 °C		7.54	0.46- 14.61	< 0.05
NGAL 48-0	***FiO_2***	***10***			***< 0.05***
	96-100%				
	94-96%		7.15	2.11-12.19	< 0.02
UNCR 24-0	***m-SpO_2***	***3.022***			***0.06***
	97-100%				
	88-95%		0.78	0.008-1.56	0.05
UNCR 48-24	***l-SpO_2***	***2.52***			***< 0.1***
	75-90%				
	90-95%		0.55	0.05-1.15	0.08
UNCR 48-0	***FiO_2***	***13.74***			***< 0.005***
	96-100%				
	94-96%		0.35	0.15-0.54	< 0.005
	l-SpO_2	***4.93***			***< 0.05***
	75-90%				
	90-95%		0.7	0.08-1.33	< 0.05

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
RBP 24-0	***l-MAP (2 categories)***	***5.83***			***< 0.05***
	60-110 mmHg				
	35-60 mmHg		3.68	0.61-6.76	< 0.05
	l-MAP (> 2 categories)	***2.85***			< 0.05
	60- 70 mmHg				
	55-60 mmHg		6.59	0.25-12.93	< 0.05
	l-DAP (2 categories)	***4.96***			***< 0.05***
	45-90 mmHg				
	20-45 mmHg		3.27	0.32-6.22	
	l-DAP (> 2 categories)	***4.74***			< 0.05
	40-60 mmHg				
	20-40 mmHg		4.36	0.79-7.93	0.01
	Mean isoflurane	***6.65***			**< 0.01**
	1.5-2%				
	0.3-1%		7.57	0.46-14.68	< 0.05
	1.5-2%				
	1-1.5%		10.2	3.08-17.32	< 0.005
RBP 48-0	***l-MAP (2 categories)***	***9.2***			***< 0.05***
	60-110 mmHg				
	35-60 mmHg		5.49	1.85-9.13	< 0.05
	l-MAP (> 2 categories)	***5.8***			***< 0.002***
	60-70 mmHg				
	55-60 mmHg		11.07	3.81-18.33	< 0.002

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
	l-DAP (2 categories)	***5.97***			***< 0.05***
	45-90 mmHg				
	20-45 mmHg		4.4	0.78-8.03	< 0.05
	l-DAP (> 2 categories)	***4.7***			***< 0.05***
	40-60 mmHg				
	20-40 mmHg		4.77	0.37-9.17	< 0.05
	Minimum FiO_2	***17.63***			***< 0.05***
	96-100%				
	94-96%		7.63	3.58-11.68	< 0.005
	m-MAP	***5.8***			***< 0.05***
	Mean isoflurane	***5.2***			***< 0.01***
	1.5-2%				
	0.3-1%		9.89	0.7-19.05	< 0.05
	1.5-2%				
	1-1.5%		12.07	2.89-21.24	< 0.01
RBP 48-24	***Minimum FiO_2***	***7.1***			***< 0.05***
	96-100%				
	94-96%		6.16	1-11.32	< 0.05
uRBP:Cr 48-0	***m-DAP***	***2.45***			***< 0.01***
	m-MAP				***< 0.2***

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
	FiO_2	***8.2***			***< 0.05***
	96-100%				
	94-96%		0.005	0.001-0.009	< 0.05
SCr 24-0	***l-Temp***	***3.16***			**< 0.05**
SCr 48-0	***l-SpO2***	***3.19***			***< 0.05***
	77-82%				
	97-100%		58.3	0.56-116.04	< 0.05
	Mean Isoflurane	***4.7***			***< 0.05***
	0.3-1%				
	1-1.5%		26.22	5.5-46.93	< 0.01
SCr 48-24	***Weight***	***4.5***			***< 0.05***
	0-3 kg				
	3-5 kg		13.71	0.7-26.71	< 0.05
	Max. isoflurane	***3.65***			***< 0.05***
	0.4-1%				
	1.5-2%		25.09	3.8-46.37	< 0.02
	Mean isoflurane	***5.14***			***< 0.01***
	0.3-1%				
	1-1.5%		20.15	4.93-35.37	0.007
SDMA 24-0	***Fluids***	***4.55***			***0.04***
	Received IV fluids				
	Did not receive IV fluids		2.41	0.14-4.68	< 0.005

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
	Weight	***4.49***			***< 0.05***
	3-5 kg				
	0-3 kg		2.4	0.13-4.68	< 0.05
SDMA 48-0	***Age***	***6.11***			***< 0.05***
	12-85 m				
	0-12 m		2.69	0.51-4.88	<0.05
	l-SpO₂	***3.51***			***< 0.05***
	87-92%				
	92-97%		5.05	0.98-9.13	<0.05
	87-92%				
	97-100%		6.5	0.11-12.89	<0.05
	m-SpO₂	***6.81***			***< 0.005***
	88-95%				
	97-100%		4.69	1.62-7.76	< 0.005
SDMA 48-24	***Maximum isoflurane***	***2.93***			***< 0.05***
	2-2.6%				
	1-1.5%		4.82	0.11-9.53	< 0.05
	2-2.6%				
	1.5-2%		4.88	0.06-9.7	< 0.05
UCr 48-0	***Weight (2 categories)***	***6.53***			***< 0.05***
	3-5 kg				
	0-3 kg		0.1	0.02-0.19	<0.05
	Weight (> 2 categories)	***4.36***			***< 0.01***
	3-4 kg				
	1-2 kg		0.27	0.06-0.47	0.007

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
	m-SpO$_2$	***5.17***			***< 0.01***
	88-95%				
	97-100%		0.16	0.04-0.29	< 0.01
	Age (2 categories)	***82.12***			***< 0.001***
	12-85 m				
	0-12 m		0.25	0.19-0.3	0.001
	Age (> 2 categories)	***674.4***			***< 0.001***
	7-12 m				
	2-7 m		0.006	0.04-0.08	< 0.001
	12-25 m				
	2-7 m		0.19	0.16-0.21	< 0.001
	25-85 m				
	2-7 m		0.48	0.45-0.51	< 0.001
	12-25 m				
	7-12 m		0.12	0.1-0.15	< 0.001
	25-85 m				
	2-7 m		0.42	0.38-0.44	< 0.001
	25-85 m				
	12-25 m		0.29	0.26-0.32	< 0.001
UCr 48-24	***m-DAP***	***3.48***			***< 0.05***
	75-110 mmHg				
	35-55 mmHg		0.2	0.01-0.39	< 0.05
	m-Temp	***5.46***			***< 0.01***
	37.5-38.5 °C				
	36.5-37.5 °C		0.26	0.07-0.44	< 0.01

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
	Age (2 categories)	***63.17***			***< 0.001***
	12-85 m				
	0-12 m		0.31	0.23-0.38	
	Age (> 2 categories)	***148.5***			***< 0.001***
	7-12 m				
	2-7 m		0.06	0.002-0.12	< 0.05
	12-25 m				
	2-7 m		0.18	0.11-0.25	< 0.001
	25-85 m				
	2-7 m		0.57	0.49-0.65	< 0.001
	12-25 m				
	7-12 m		0.12	0.06-0.19	< 0.001
	25-85 m				
	7-12 m		0.51	0.44-0.58	< 0.001
	25-85 m				
	12-25 m		0.38	0.3-0.47	< 0.001
	l-SAP	***6.44***			***0.001***
	45-60 mmHg				
	60-80 mmHg		0.55	0.17-0.93	0.002
	45-60 mmHg				
	80-100 mmHg		0.55	0.21-0.89	< 0.001
	45-60 mmHg				
	100-140 mmHg		0.56	0.2-0.91	< 0.001
	Mean isoflurane	***5.4***			***< 0.01***
	1.5-2%				
	1-1.5%		0.31	0.05-0.59	< 0.05

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
UP:C 24-0	***l-SpO_2***	***3.38***			***< 0.05***
	95-100%				
	75-90%		0.14	0.01-0.27	< 0.05
	m-SpO_2	***3.58***			***< 0.05***
	97-100%				
	88-95%		0.14	0.01-0.27	0.03
	m-MAP	***2.83***			***< 0.05***
	59-65 mmHg				
	85-95 mmHg		0.3	0.03-0.58	0.02
UP:C 48-0	***FiO_2***	***11.19***			***< 0.01***
	96-100%				
	94-96%		0.15	0.05-0.25	< 0.01
UP:C 48-24	***l-DAP***	***3.19***			***< 0.1***
	40-60 mmHg				
	20-40 mmHg		0.07	0.002-0.134	< 0.05
UTP 24-0	***Fluids***	***11.58***			***< 0.01***
	Did not receive IV fluids				
	Received IV fluids		0.37	0.15-0.58	< 0.005
	Mean isoflurane	***7.53***			***< 0.01***
	0.3-1%				
	1.5-2%		0.81	0.16-1.46	0.01
	1-1.5%				
	1.5-2%		1	0.35-1.65	0.001

(continued)

Table 20 (Continued).

Marker	Variable	F-statistic	M.D	95% C.I.	p-value
	Premedication agent	***5.59***			***< 0.05***
	AM-no IV				
	DKT-no IV		0.27	0.04-0.5	< 0.05
UTP 48-0	***Fluids***	***5.28***			***< 0.05***
	Did not receive IV fluids				
	Received IV fluids		0.29	0.04-0.54	
	Mean isoflurane (%)	***6.58***			***< 0.005***
	0.3-1%				
	1.5-2%		1.03	0.28-1.78	< 0.005
	1-1.5%				
	1.5-2%		1.13	0.38-1.88	< 0.005

Univariate logistic regression

Logistic regression analysis was performed to investigate the effects of the various intraoperative variables on renal markers, as summarized in Table 21.

Table 21 Univariable analysis of factors associated with a change in the renal biomarkers between different study time points.

Renal marker across time points	Variables	Category	OR	p-value
BUN 24-0	Weight (kg)	0 to 3	**Ref**	
		3 to 5	3.26	< 0.05
	Weight (kg)	1 to 2	**Ref**	
		2 to 3	0.12	0.04
		3 to 4	1.00	1.00
		4 to 5	0.67	0.72
BUN 48-0	l-Temp (°C)	33.5-36	**Ref**	
		36-39.5	0.028	< 0.05
	l-SpO_2 (%)	75-90	**Ref**	
		90-95	0.18	
		95-100	0.76	0.04
BUN 48-24	l-MAP (mmHg)	35 to 60	**Ref**	
		60 to 110	0.18	0.01
	l-DAP (mmHg)	20 to 45	**Ref**	
		45 to 90	0.25	0.04
	l-SpO_2 (%)	75 to 90	**Ref**	
		90 to 95	6.36	0.03
		95 to 100	4.28	0.13
	l-MAP (mmHg)	35 to 55	**Ref**	
		55 to 60	0.55	0.55

(continued)

Table 21 (Continued).

Renal marker across time points	Variables	Category	OR	p-value
		60 to 70	0.22	0.06
		70 to 110	0.07	0.03
	m-MAP (mmHg)	59 to 75	**Ref**	
		75 to 95	0.138	0.03
		95 to 129	0.19	0.07
KIM-1 RATIO 48-0	$ETCO_2$ (mmHg)	30 to 45	**Ref**	
		45 to 64	3.3.	0.04
	l-SpO_2 (%)	75 to 90	**Ref**	
		90 to 95	0.174	0.02
	Age (m)	2 to 7	**Ref**	
		7 to 12	0.250	0.05
		12 to 25	0.19	0.05
		25 to 85	0.07	0.03
	Weight (kg)	1 to 2	**Ref**	
		2 to 3	0.067	0.02
		3 to 4	0.08	0.03
		4 to 5	0.04	0.04
	m-SpO_2 (%)	77 to 82	**Ref**	
		82 to 87	0.5	0.68
		87 to 92	0.15	0.15

(continued)

Table 21 (Continued).

Renal marker across time points	Variables	Category	OR	p-value
		92 to 97	0.11	0.06
		97 to 100	0.083	0.12
NAG ACTIVITY 24-0	l-SAP (mmHg)	50 to 90	**Ref**	
		90 to 135	3.89	0.02
	$ETCO_2$ (mmHg)	30 to 35	**Ref**	
		35 to 40	13.2	0.03
		40 to 45	5.14	0.18
		45 to 50	8	0.12
		50 to 55	2	0.61
		55 to 64	2	0.66
NAG ACTIVITY 48-0	l-MAP (mmHg)	35 to 60	**Ref**	
		60 to 110	3.75	0.03
	l-Temp (°C)	33.5 to 36	**Ref**	
		36 to 36.5	0.125	0.03
		36.5 to 37.5	0.375	0.24
		37.5 to 39.5	0.56	0.61
	$ETCO_2$ (mmHg)	30 to 35	**Ref**	
		35 to 40	6.3	0.06
		40 to 45	10.5	0.02
		45 to 50	2.62	0.38

(continued)

Table 21 (Continued).

Renal marker across time points	Variables	Category	OR	p-value
		50 to 55	1.17	0.89
		55 to 64	3.5	0.33
	m-MAP (mmHg)	59 to 75	**Ref**	
		75 to 95	4.125	0.04
		95 to 129	3.125	0.18
NAG INDEX 48-0	l-MAP (mmHg)	35 to 60	**Ref**	
		60 to 110	7.916	0.01
	l-SAP (mmHg)	50 to 90	**Ref**	
		90 to 135	4.95	0.02
	l-MAP (mmHg)	35 to 55	**Ref**	
		55 to 60	1.08	0.95
		60 to 70	6.5	0.05
		70 to 110	13	0.03
	m-MAP (mmHg)	59 to 75	**Ref**	
		75 to 95	4.125	0.04
		95 to 129	3.125	0.18
NGAL 24-0	m-MAP (mmHg)	59 to 65	**Ref**	
		65 to 75	0.19	0.18
		75 to 85	0.05	0.03
		85 to 95	0.25	0.32
		95 to 129	0.15	0.15

(continued)

Table 21 (Continued).

Renal marker across time points	Variables	Category	OR	p-value
NGAL 48-0	Age (m)	0 to 12	**Ref**	
		12 to 85	4.36	< 0.05
NGAL 48-24	Age (m)	0 to 12	**Ref**	
		12 to 85	3.8	0.03
RBP 24-0	l-MAP (mmHg)	35 to 60	**Ref**	
		60 to 110	3.80	0.03
	l-DAP (mmHg)	20 to 40	**Ref**	
		40 to 60	4.07	0.03
		60 to 90	1.22	0.85
	m-SpO_2 (%)	88 to 95	**Ref**	
		95 to 97	0.24	0.10
		97 to 100	0.17	0.05
RBP 48-0	l-MAP (mmHg)	35 to 60	**Ref**	
		60 to 110	7.12	0.005
	l-DAP (mmHg)	20 to 45	**Ref**	
		45 to 90	5.50	0.006
	l-SpO_2 (%)	75 to 90	**Ref**	
		90 to 95	0.437	0.25
		95 to 100	0.15	0.03

(continued)

Table 21 (Continued).

Renal marker across time points	Variables	Category	OR	p-value
	l-DAP (mmHg)	20 to 40	**Ref**	
		40 to 60	3.41	0.05
		60 to 90	0.83	0.88
	l-Temp (°C)	33.5 to 36	**Ref**	
		36 to 36.5	0.08	0.02
		36.5 to 37.5	0.34	0.20
		37.5 to 39.5	0.64	0.7
	Weight (kg)	1 to 2	**Ref**	
		2 to 3	10.29	< 0.05
		3 to 4	5.08	0.16
		4 to 5	1.5	0.79
	m-SpO_2 (%)	88 to 95	**Ref**	
		95 to 97	0.37	0.17
		97 to 100	0.11	0.008
	m-MAP (mmHg)	59 to 75	**Ref**	
		75 to 95	1.86	0.36
		95 to 129	5.33	0.05
RBP 48-24	l-MAP (mmHg)	35 to 60	**Ref**	
		60 to 110	3.68	0.04
	FiO_2 (%)	94 to 96	**Ref**	
		96 to 100	24.0	0.04

(continued)

Table 21 (Continued).

Renal marker across time points	Variables	Category	OR	p-value
SCr 24-0	Fluids	yes	**Ref**	
		no	0.28	0.03
	Duration of anaesthesia	20 to 35	**Ref**	
		35 to 50	12.6	0.04
		50 to 80	4.09	0.25
SCr 48-0	m-MAP (mmHg)	59 to 75	**Ref**	
		75 to 95	5.5	0.05
		95 to 129	1.83	0.46
SDMA 24-0	Age (m): 2 categories	0 to 12	**Ref**	
		12 to 85	4.15	0.02
	Fluids	yes	**Ref**	
		no	0.21	0.008
	Age (m): > 2 categories	2 to 7	**Ref**	
		7 to 12	1.28	0.73
		12 to 25	5.87	0.04
		25 to 85	3.67	0.15
SDMA 48-0	l-SpO_2 (%)	75 to 90	**Ref**	
		90 to 95	0.21	0.04
		95 to 100	0.15	0.04

(continued)

Table 21 (Continued).

Renal marker across time points	Variables	Category	OR	p-value
	m-SpO_2 (%)	88 to 95	**Ref**	
		95 to 97	0.11	0.004
		97 to 100	0.04	0.001
UNCR 48-0	l-SpO_2 (%)	75 to 90	**Ref**	
		90 to 95	0.06	0.02
		95 to 100	0.67	0.65
UNCR 48-24	m-SpO_2 (%)	88 to 95	**Ref**	
		95 to 97	0.20	0.04
		97 to 100	0.389	0.22
UP:C 24-0	Age (m): 2 categories	0 to 12	**Ref**	
		12 to 85	0.169	0.01
	Fluids	yes	**Ref**	
		no	6.666	0.007
	Age (m): > 2 categories	2 to 7	**Ref**	
		7 to 12	0.97	0.98
		12 to 25	0.15	0.05
		25 to 85	0.2	0.15
	m-SpO_2 (%)	88 to 95	**Ref**	
		95 to 97	7	0.02
		97 to 100	6.42	0.04

(continued)

Table 21 (Continued).

Renal marker across time points	Variables	Category	OR	p-value
UP:C 48-24	$ETCO_2$ (mmHg)	30 to 35	**Ref**	
		35 to 40	5.25	0.17
		40 to 45	12	< 0.05
		45 to 50	6	0.16
		50 to 55	4.5	0.26
		55 to 64	3	0.50
	Max. Isoflurane (%)	0.4 to 1	**Ref**	
		1 to 1.5	2.75	0.17
		1.5 to 2	4.95	< 0.05
		2 to 2.6	1.375	0.81
	m-Temp (°C)	35.5 to 36.5	**Ref**	
		36.5 to 37.5	1	1
		37.5 to 38.5	10	0.05
uRBP:Cr 48-24	l-DAP (mmHg)	20 to 40	**Ref**	
		40 to 60	3.61	< 0.05
		60 to 90	1.08	0.95
	m-DAP (mmHg)	35 to 55	**Ref**	
		55 to 75	5.2	0.02
		75 - 110	1.44	0.65
UTP 24-0	Fluids	yes	**Ref**	
		no	4.50	0.04

4.2.8 Summarizing the intraoperative effects of fluid and treatment protocols

Fluid administration

The effect of administrating IV fluids perioperatively was found to be associated with HR (p-value < 0.05), as shown in Figure 21. Those given fluids had a mean HR of 160.17 ± 24.58 bpm and those without had a mean HR of 147.32. ± 23.55 bpm. Cats who received fluids had an HR that was higher by 0.4-25.29 units than those who did not receive fluids during general anaesthesia and surgery.

IV fluid administration had no significant effect on $ETCO_2$, RR, End-tidal agent %, m-MAP, m-SAP, m-DAP, m-temp, l-temp, isoflurane %, l-MAP, l-SAP, or l-DAP (p-value < 0.05) in this study.

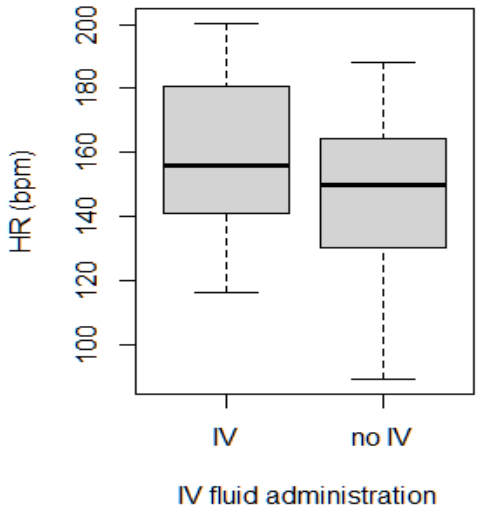


Figure 21 Association of IV fluid administration with HR (bpm).

The boxplot summarizes the positive association between heart rate and fluid administration as observed in this study.

Inhalant anaesthetic agent

The inhalant anaesthetic agent, isoflurane, was found to be associated with the following variables in this study: $ETCO_2$ (p-value < 0.05), ET isoflurane % (p-value < 0.001), m-MAP

(p-value < 0.001), m-SAP (p-value < 0.001), m-DAP (p-value < 0.001), and duration of anaesthesia (p-value 0.04).

Treatment protocols

The following variables were found to differ for the two treatment protocols, i.e. AM and DKT: $ETCO_2$, RR, ET isoflurane %, m-MAP, m-SAP, m-DAP, isoflurane %, l-MAP, l-SAP, l-DAP, HR, m-Temp, and l-temp (p-value < 0.05), as shown in Figure 22 and Figure 23. The variables, m-SpO_2 or l-SpO_2 (p-value > 0.05), were not associated with the effect of treatment protocols.

The HR, RR, m-isoflurane, and ET-isoflurane % was higher for those who received the AM protocol than the DKT protocol (p-value < 0.05). The m-MAP, l-MAP, m-SAP, m-DAP, l-DAP, m-Temp, l-Temp and $ETCO_2$ was higher for those who received the DKT protocol than the AM protocol. For more information on the differences in variable values, see Table 9.

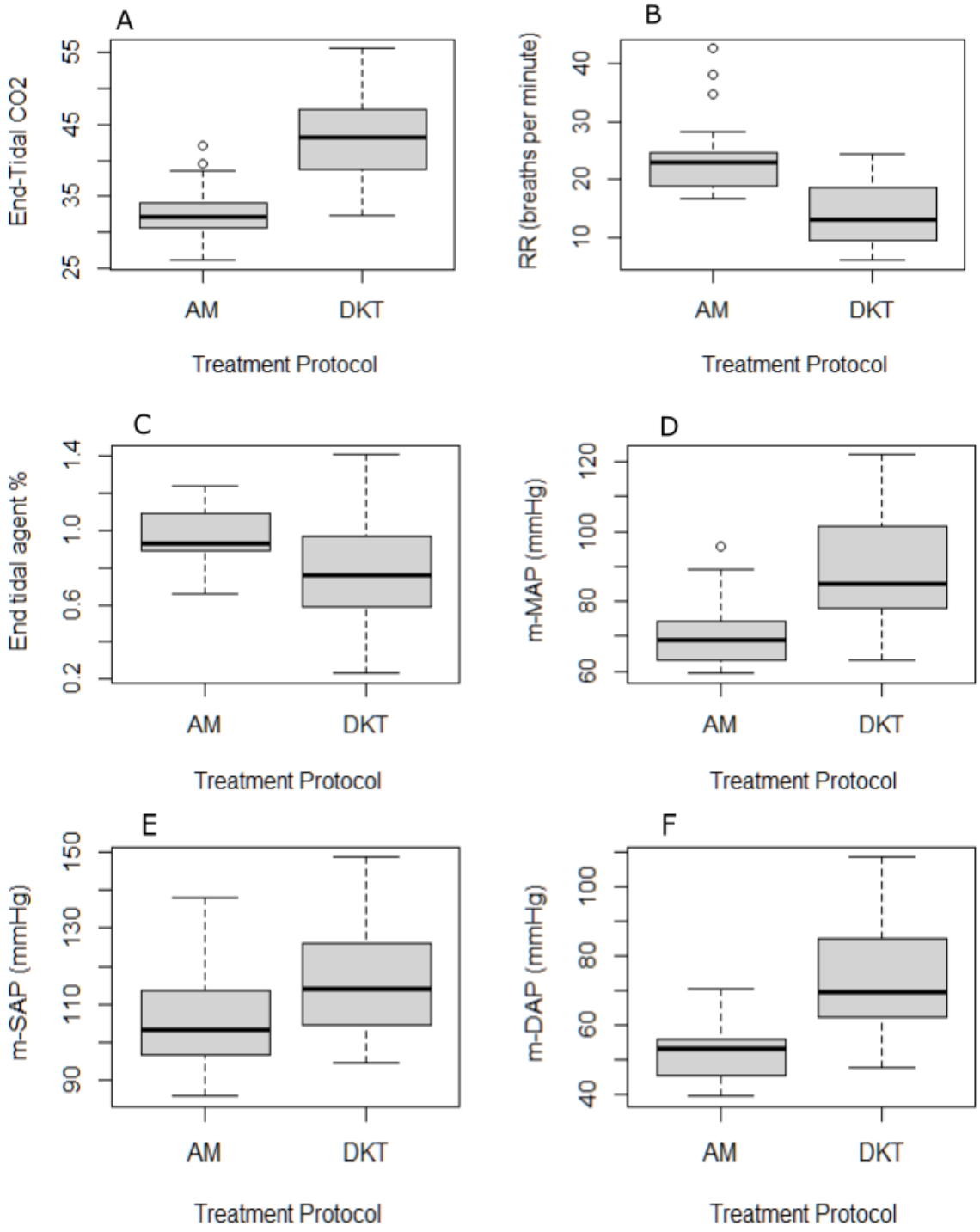


Figure 22 Intraoperative variables (mean values) such as A) $ETCO_2$, B) RR, C) ET isoflurane (%), D) m-MAP (mmHg), E) m-SAP (mmHg), and F) m-DAP (mmHg) with different anaesthetic protocols.

These boxplots summarize the differences in the variables recorded between the two treatment protocols.

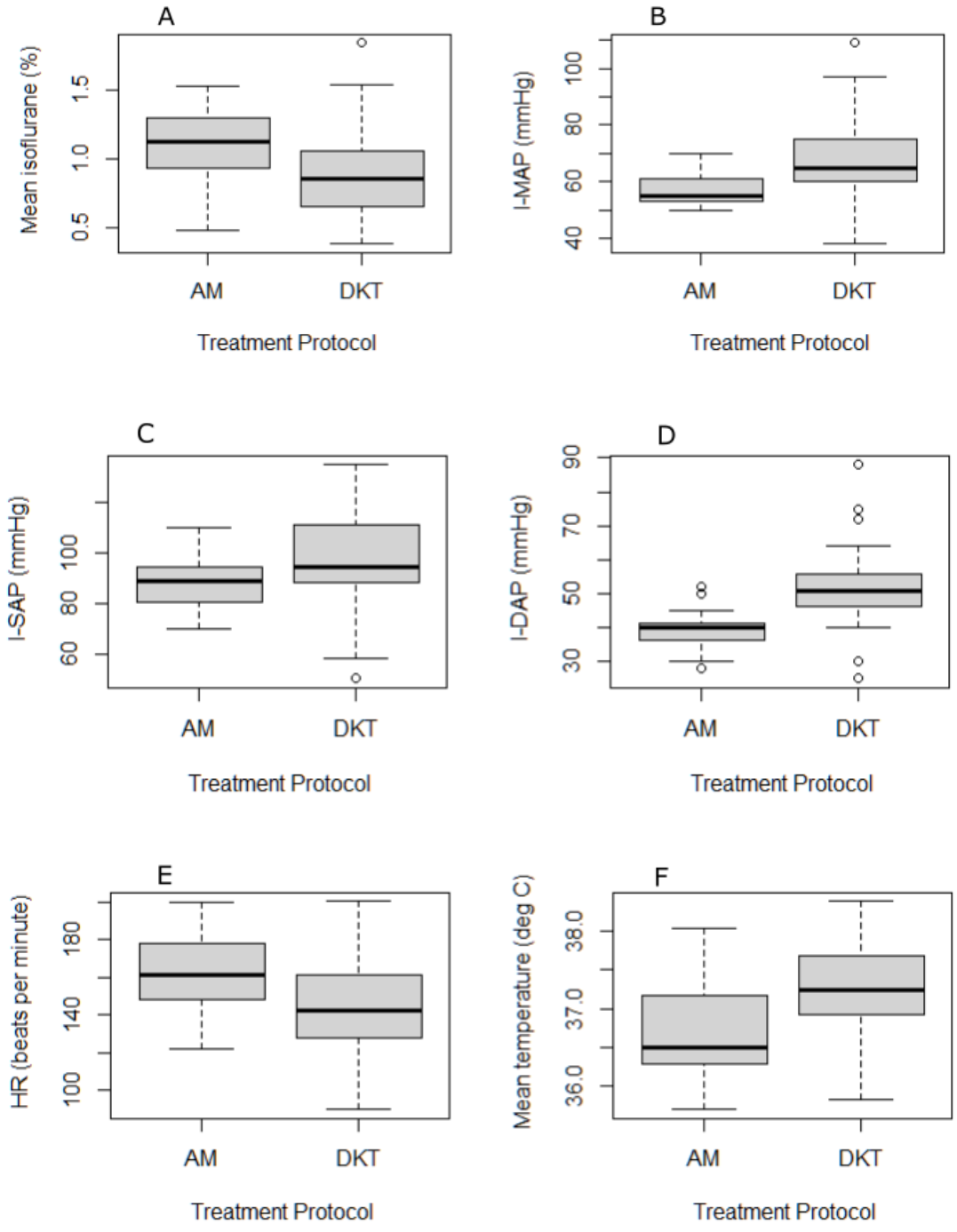


Figure 23 Intraoperative variables (lowest values) such as A) m-Isoflurane (%), B) l-MAP (mmHg), C) l-SAP (mmHg), D) l-DAP (mmHg), E) HR (bpm), and F) m-Temp (°C) with different anaesthetic protocols.

These boxplots summarize the differences in the variables recorded between the two treatment protocols.

DISCUSSION

This study was conducted to investigate the effects of general anaesthesia and intravenous fluid therapy perioperatively on both traditional and novel renal markers in cats undergoing routine ovariohysterectomy. The traditional markers were used to provide information on function, while the novel markers were used to provide information on structure, mainly the integrity of the glomerular and tubular cells (primarily PCT cells). The findings of this study did not support our hypothesis that the use of anaesthesia would increase the novel renal marker values postoperatively as we were unable to find any clinically relevant changes in the renal marker values over 48 hrs post-exposure to anaesthesia and surgery.

There are two main possibilities which explain the absence of any major change in the renal marker concentrations in this study. Firstly, there might have been a complete absence or mild grade injury resulting in the lack of major elevation in renal marker values. Considering that the physiological parameters such as HR, arterial BP and oxygenation were mostly maintained above the minimum threshold during surgery, it is not surprising that the risk factors studied here may have been unable to create a harmful environment in the renal tissue of the study cats. In support of this, a study by Gibbs et al. (2019) on general anaesthesia in mice reported that controlled-general anaesthesia did not cause any significant changes in renal markers. Furthermore, a retrospective human study testing varying durations and degrees of hypotension in non-cardiac patients found an increased risk of postoperative AKI-association (identified through SCr elevation) in patients exposed to sustained hypotension (< 55 mmHg) for more than 10 mins (Sun et al., 2015). The quick recovery (< 5 mins) from any transient dips in MAP readings (> 60 mmHg for our study population) in the present study, thus, appears less likely to induce measurable AKI.

Another reason for the apparent lack of renal marker elevation in our study could be that these markers were not sensitive enough to detect the presence of mild injury induced by exposure to the anaesthetic agents. To determine the absence or presence of renal injury on exposure to the conditions provided in this study, more sensitive or elaborative experimental trials would need to be performed to determine the subsequent changes in the renal cortical and medullary environment.

The cats in our study were all apparently healthy adults. There were no significant differences in the signalment, physical examination, and biochemical findings between the cats in the different treatment groups prior to undergoing surgeries. This makes it less likely for these factors to be confounding our study results. The overall increase in HR and RR for the study population before the surgery was attributed to the stress induced on the cats due to their unfamiliarity with the environment and people. The magnitude of changes we observed in the baseline values of RR, body temperature and HR were similar to the findings reported by Nibblett et al. (2015) for stressed cats. The latter study compared the effect of handling on cats at home versus clinic to report the changes in parameters such as HR and RR in response to induced stress. In another study, the BP of cats was observed to increase during various activities such as when they were being fed or when their cages were cleaned (Mishina et al., 2006). This stimulatory effect on cardiovascular and respiratory function in response to an unfamiliar environment and strangers is commonly known as the "white-coat effect" (Cremer & Ricco, 2018). In a study on white coat effects in cats, the SAP was shown to be elevated by 17.6 mmHg over the control; however, it was shown to reduce with time (Belew et al., 1999). In our study, the procedures and environment would have ensured elevated cardio-respiratory function whenever the cats were conscious and introduced to new people/activities.

4.3 Intraoperative Findings

The surgical duration (time of the first incision to time of its closure) for the spay procedures in our study had an average of 40 mins, which is shorter than the mean time of 70 mins taken by veterinary students to perform OHE in another study (Vicente & Bergstrom, 2018). The effect of remaining under general anaesthesia for a longer duration than in our study on the renal structure is unknown and would require further investigation. However, the time taken for spays in high caseload clinics and shelters by experienced veterinarians would be similar to or shorter than in this study. This would suggest that the risk of renal injury in cats undergoing spays, which are swiftly performed and well-monitored would be minimal.

There was a drop in the parameters such as HR, arterial blood pressure (MAP, SAP, DAP), and body temperature over the course of the surgery compared to the baseline measurements

as an expected result of the depressor effects of the premedication agents/inhalant anaesthetic. All the major macrosystemic parameters (HR, SpO_2, RR, MAP) were maintained within the physiological ranges. The intraoperative SAP values observed in our study were corroborated by the BP readings reported (oscillometry) in studies involving anaesthetized cats (Branson et al., 1997; Goodnight et al., 2015).

The mean temperature recorded for the study population in the current study was similar to the observations of another study on cats after OHE (Bosch et al., 2012). One possible mechanism for the loss of body heat is the suppression of the hypothalamic response and sympathetic tone, along with peripheral vasodilation leading to heat loss (Diaz & Becker, 2010). This is further potentiated by fluid evaporation from the exposed mucosal surface during surgery (Hall, 1976). Our study population's intraoperative body temperature and arterial blood pressure readings appeared to fall within the normal range for anaesthetized cats.

Some of the present study cats were in various stages of pregnancy, which may have led to varied arterial BP post-surgery and, in turn, affected the renal markers by 24 and 49 hrs. In another study evaluating the effects of laparoscopic OHE in cats, pregnant cats had a higher MAP and abdominal pressure-perfusion than other female cats (Bosch et al., 2012). Unfortunately, this variable (stage of pregnancy) was not considered for our study, and the magnitude of any potential effect it may have had on changes in the renal biomarker values is unknown. It would be interesting to look further into the association between pregnancy and renal function. Further research must be conducted to determine the difference in renal function in pregnant and non-pregnant cats under a stress-induced renal environment.

4.4 Effect Of The Anaesthetic Protocols On Physiological Parameters

Two different anaesthetic protocols were adopted for this study, i.e., AM and DKT and the differences in the intraoperative physiological parameters between the two protocols are described below.

4.4.1 Heart Rate and Respiratory Rate

The current study found stable but decreased cardio-respiratory parameters (HR and RR) in the groups that received the DKT protocol compared to AM protocol. Dexmedetomidine

(α-2 agonist) can cause peripheral hypertension initially, which is followed by central depressor effects such as decreased cardiac output (Thompson, 2005) and arterial BP (DAP and SAP), especially when combined with butorphanol (Varkoulis et al., 2020). A similar decrease in RR (Khenissi et al., 2017; Thompson, 2005) was observed with butorphanol (Varkoulis et al., 2020), as seen in our study in those premedicated with the DKT protocol. Ketamine may further worsen the depressor effect on RR (Khenissi et al., 2017) and increase the duration of sedation up to 43 mins (Volpato et al., 2015). In contrast to its action on respiration, ketamine prevents cardio-depressant effects of other agents by stimulating the release of catecholamines such as epinephrine (Goodnight et al., 2015). However, we still observed a greater depressor effect on HR and RR in those medicated with DKT than with AM protocol.

4.4.2 Arterial Blood Pressure

While the HR was lower in the DKT protocol groups than in the AM protocol groups, the AM group had a lower m-MAP, l-MAP, m-SAP, and m-DAP than DKT. The mean MAP observed for the DKT groups in our study was similar to the MAP recorded in a study by Bosch et al. (2012) in sedated (buprenorphine, ketamine, midazolam) cats right before OHE. Alfaxalone combination, unlike DKT, is reported to gradually reduce HR in a dose-dependent manner (Taboada & Murison, 2010), and acepromazine potentiates the cardio-depressant effects when combined with other agents in contrast to ketamine (Goodnight et al., 2015). Thus, in our study, AM protocol had a more substantial depressor effect on arterial pressure (MAP, SAP, and DAP) than the DKT protocol.

A study investigating the effects of acepromazine, alfaxalone and isoflurane in cats observed that the arterial BP (MAP) initially dropped and then stabilized (77 mmHg) during ovariectomy (Taboada & Murison, 2010), which is consistent with our AM group m-MAP values. A similar decrease in BP was reported by Beths et al. (2014), where alfaxalone (IV) in combination (medetomidine and morphine) was observed to cause hypotension (DAP < 80 mmHg) in 59% of the cats undergoing the spay procedure, corrected using fluids and reducing the rate of alfaxalone infusion. This suggests that there might be a higher risk of

renal damage with AM protocol if the pressures, primarily MAP, fall below the threshold of 60 mmHg where renal damage would be expected to occur.

Interestingly, it was reported that the RBF remained stable even though the dogs in a study experienced an acepromazine-induced drop in MAP to 66 mmHg (Bostrom et al., 2003). However, the increased susceptibility of cats to surgical complications and kidney damage, along with the risk of reduced renal perfusion and hypotension during surgery, demands more precaution for cat patients. Species-specific focused studies on renal perfusion and oxygenation during surgery are required to better understand the effect of MAP maintained at 60-66 mmHg, and below, on renal tissue.

4.4.3 Body Temperature

In this study, the body temperature was lower for AM than for DKT groups intraoperatively. The mean temperature observed in the AM groups in this study was similar to that observed in another study using the same class of drugs (Cremer & Ricco, 2018). Alfaxalone was shown to reduce rectal temperature, further hastened by the use of supplementary oxygen, steadily during the period of anaesthesia (Whittem et al., 2008). Reduced body temperature leads to reduced cell metabolism, thereby decreasing the oxygen demand of various organs. This might prove beneficial to renal tubular cells (high metabolic activity) by potentially reducing the rate of cell turnover by lowering the rate of metabolism. This is supported by the findings of a human study by Funahashi et al. (2014) in subjects undergoing partial nephrectomy, wherein cold ischemia (safe up to 58 mins) was less likely to cause renal damage than warm ischemia (up to 25 mins). In fact, the former was shown to prevent renal injury. However, we are unaware of the core body temperature in our study at this stage as the rectal temperature is not an accurate reflection of core temperature.

4.4.4 Oxygenation Saturation

There was no evidence of an association between oxygen saturation and the treatment protocols. There was no significant difference in SpO_2 levels between the groups. The peripheral circulation had a normal mean oxygen saturation for all the groups. However, we are unsure of the oxygenation status at the level of renal structures and whether it was

affected by the procedures in this study. Even though Goodnight et al. (2015) reported that premedication-induced hypotension had no significant effect on microvasculature in cats, unlike in other species. The authors surmised that this might be due to the peripheral vasodilatory effects of the anaesthetic agents counteracting the effects of surgical manipulation, which generally causes vasoconstriction, that would have reduced RBF. Goodnight et al. (2015) found that renal microcirculation remains unaffected by intraoperative procedures, irrespective of the changes in HR, MAP, SAP, and temperature, with the exception of surgical manipulation. This suggests that the macro-circulatory parameters are not a good reflection of micro-circulation and, thus, may not be adequate tools for determining changes in renal circulation.

4.4.5 End-Tidal Carbon Dioxide

The $ETCO_2$ measured in our study for the whole population was borderline normal, i.e., just above 35 mmHg (Reuss-Lamky, 2016). We observed a lower mean with the AM groups than with DKT. It is unclear whether the supine position during surgery may have exacerbated certain cardiovascular and respiratory depressor effects that any anaesthetics might have had. $ETCO_2$ is known to decrease with hypotension (involving reduced blood volume)/hypoperfusion (Aminiahidashti et al., 2018) and reduced body temperature in humans. We found AM groups (more than DKT) to have reduced arterial BP and body temperature with increased HR and RR, along with normal oxygen saturation and these parameters, when put together, are suggestive of drug-induced/deep sedation hypopneic-hypoventilation based on the report by Krauss and Hess (2007). Similar findings were noted by Beths et al. (2014) with the use of alfaxalone in combination with an opioid and α-$_2$ agonist in cats wherein they observed a mean $ETCO_2$ of 21 mmHg and reduced body temperature (36.3 °C). The AM protocol, therefore, appears to have a stronger influence on cardio-respiratory variables, with an increased risk of hypoventilation and hypotension than DKT.

Neither one of the anaesthetic protocols used in this study was shown to have placed the renal tissue in an environment that could induce renal injury. However, AM did substantially influence the systemic parameters, suggesting an increased risk of anaesthetic complications

with this combination of drugs. Interestingly, the AM protocol had a more significant effect on UTP than DKT by 48 hrs. This may be due to the reduced arterial BP in those who received the AM protocol than DKT, which may place the renal cortical structures at a higher risk of transient injury, if any, with the former. However, this would require further investigation to observe the risk factors associated with glomerular damage.

4.4.6 Isoflurane

The cats receiving the AM protocol typically required a higher percentage and longer duration of isoflurane to maintain the depth of anaesthesia compared to those receiving the DKT protocol. The DKT combination placed the animals in a medium plane of anaesthesia, whereas alfaxalone (AM protocol) was given to effect, as the immediate dose-dependent anaesthetic effects of Alfaxalone are related to its plasma concentration (Whittem et al., 2008). DKT protocol provided deeper and longer sedation than the AM combination.

4.5 Renal Markers At Baseline

The animals included in our study were found to be apparently healthy on physical examination with no clinical signs indicating potential renal damage at the time of recruitment. Their health status was further supported by baseline blood biochemistry and urine analysis results with no significant difference in the renal biomarker values between the treatment groups. However, many cats in the study population were low-grade proteinuric (> 0.2) that could be either physiological or pathological in origin, with the latter being induced by renal or extrarenal factors. Stress-induced renal vasoconstriction (Grauer, 2007), is one of the many causes of physiological proteinuria (Grauer, 2011). Since a white-coat effect was observed in this study, it might be plausible that the borderline proteinuria recorded here was transient in nature. There is a need for further investigation to identify possible pathological causes for low-grade proteinuria if it lasts for more than two months. Our study could not sample the cats beyond the 48-hour post-surgery mark to determine its persistence.

To the best of our knowledge, there were no standardized or reference range values for novel markers for cats at the time of this study, and so we compared our results against those

from other published studies. As mentioned in the review, the true urinary KIM-1 and RBP concentration in healthy cats have not been formally reported (Hokamp & Nabity, 2016). We used an assay developed by Bland et al. (2019) that helped analyse the KIM-1 ratio which was theorized to reflect the true KIM-1 concentrations in urine. The KIM-1 ratio of our study cats (1.24-9.76) was similar to that reported by Bland et al. (2019), i.e., 4.8-8.8 in healthy male cats. Their study used this ratio to reflect the KIM-1 values across kidney conditions of varying severity. Based on this study, the KIM-1 ratio of our study cats appears to be normal, i.e., a normal cell turnover with KIM-1 tubular exoprotein excretion.

This study's mean NAG activity and NAG Index are higher than the values reported in recent studies (Jepson et al., 2008; Kongara et al., 2020; Panboon et al., 2017). However, it was similar to the values observed by Uechi et al. (1998), where the mean NAG activity was 22.1 (10.5- 46.1 U/L), and the NAG index varied from 2.1-13 U/g for healthy cats. Hokamp and Nabity (2016) reviewed that the NAG index is valued at < 11 U/g in healthy cats and dogs. Based on these sources, the mean NAG values found in our study fall within the normal range.

The cats in our study had a mean NGAL higher than the values reported in other studies on cats (Wang et al., 2017; Wu et al., 2019). However, the mean NGAL of our study cats was within the range found for dogs (Steinbach et al., 2014). Similarly, the mean UNCR (0.83 ± 0.75 µg/mmol) of our study cats was similar to the mean UNCR value (0.3 µg/mmol) reported in a dog study by Cobrin et al. (2016). One possible explanation for the discrepancy with other feline studies on NGAL is that we used an assay with cat-specific antibodies. In contrast, the other studies performed Western blot using anti-canine antibodies. It is probable that our methodology was more sensitive in detecting elevations in NGAL concentration.

4.6 Renal Markers Across Timepoints

Previous studies in humans have concluded that there needs to be a two or three-fold increase in the novel marker activity and/or normalized values to classify an individual as having experienced renal damage or AKI (Waikar et al., 2010). While we did observe

changes in specific indicator values such as UP:C (increased), UTP (increased), BUN (decreased), UCr (increased), NAG activity (decreased), NAG Index (decreased), SCr (increased), SDMA (decreased), and KIM-1 ratio (decreased) across the time points, these changes did not appear to be clinically significant. The change in mean SCr did not exceed 0.3 mg/dl (26.5 μmol/L) or increase by 1.5 times over 48 hrs. The absence of such an increase in SCr in our study reveals that functional AKI was absent in our feline models in accordance with both the AKIN (Acute Kidney Injury Network) and the RIFLE (Risk, Injury, Failure, Loss, and End-Stage) criteria.

Furthermore, Mishra et al. (2003) found that milder forms of ischemia (min. period of 5 mins) did not cause apoptotic changes in the renal tubular cells. This indicates that a prolonged state of reduced perfusion is required to induce tubular injury, supporting the theory that there was insufficient cause for renal injury and elevated marker concentrations (particularly the novel tubular markers) as most of our cats had normal physiological parameters throughout the procedure. The slight dips in the recorded MAP values below or near the lower threshold in some of our study cats were quickly managed, and thus, these occurrences lasted for less than five minutes which does not appear to be long enough nor severe enough to induce renal injury.

The slight decrease observed in the novel renal marker values, not all statistically significant, contradicted our initial hypothesis of observing an increase in these markers in response to the presence of renal damage induced by anaesthesia. These surprising findings may be attributed to an increased flow of blood to the kidneys postoperatively (increased abdominal perfusion along with improved hydration) resulting in diluted concentration or due to specific reno-protective effects of the anaesthetic agents (such as isoflurane) along with lowered metabolism leading to reduced turnover of renal tubular cells. This supposed increase in perfusion and lowered metabolism may have ensured improved oxygen supply to the tubular cells, thus ensuring continued tubular re-absorption of RBP, along with the absence of upregulation and turnover of specific proteins/enzymes (KIM-1, NAG and NGAL).

Lobetti and Lambrechts (2000) observed a significant increase in urine GGT values post-OHE in dogs. An increase of that nature was not observed in any of the novel renal markers

studied here. However, it must be noted that the surgical (148.46 ± 55.81 mins) and anaesthetic (184.43 ± 59.55 mins) duration was longer in the former study than what was observed in our study. Similarly, Davis (2015) found that a MAP of less than 40 mmHg sustained for at least 60 mins, followed by reperfusion for 2 hrs, was required to produce an elevation in NGAL values, indicative of AKI. Thus, we can assume that the level of injury, if any, was not detected by the novel markers used here.

There was a gradual increase in SCr levels up to 48 hrs post-exposure; however, the trend fell short of being considered significant enough to indicate AKI. But due to SCr individuality, even a minor change could indicate a monumental shift in renal function. However, there are other potential confounding factors in the current study, such as the protein-rich diet provided during their stay at the hospital since ingredients like fish is a rich source of creatine (Backlund et al., 2011). Fish has been shown to increase urine creatinine outflow in 2-5 days when introduced to the human diet (Kesteloot & Joossens, 1993). The increased UCr and SCr in our study, along with reducing urine pH (Passlack et al., 2018), are more likely to be associated with a high protein diet, further supported by the use of unfasted serum samples to analyze the traditional renal markers.

To provide further evidence in our study, the other traditional markers such as BUN and SDMA reduced slightly over the 48 hrs, making it unlikely that the primary cause for the increase in SCr and UCr concentrations was a result of renal dysfunction. A decrease in BUN and an increase in SCr are indicative of non-renal causation linked to diet or liver function (Cornell French et al., 2013). Increasing protein has been shown to increase urea excretion (Passlack et al., 2018), which, along with improved hydration, may have resulted in the reduced BUN concentration observed here.

SDMA has been reported to have low reliability when sampled sequentially, compared to SCr, in dogs (Kopke et al., 2018) with possible false results (lowered values) encumbered with hemolytic samples (Elliott & White, 2019). SDMA was reasoned to be subject to biological variability in the study as they observed a sequential decrease in SDMA concentration with a concurrent increase in SCr, a finding that is similar to what was observed in our current study. The 0 hr baseline values for the serum markers in our study cats may have been higher than usual due to other factors such as dehydration and/or stress.

It appears that the traditional markers in our study were influenced by diet, hydration, stress, and biological variability, which highlights the importance of having markers that are not affected by extra-renal factors to study and monitor the kidneys.

4.7 RAAS Involvement

According to our study, the traditional renal indicators such as UTP and UP:C increased slightly over 48 hrs. According to a canine and porcine study by Chiu et al. (1994), increased IAP (intra-abdominal pressure) during laparoscopy resulted in reduced renal cortical blood flow. Interestingly, a minimal biphasic pattern, i.e., an initial increase in blood flow followed by a decrease, was observed in the cortical blood flow in the same study. In another study, control cats that were anaesthetized with butorphanol/propofol/isoflurane and given crystalloid infusion were compared to treatment cats that were given angiotensin II, using contrast-ultrasonography and scintigraphy to explore the changes in renal parenchymal microvasculature (Stock et al., 2016). The treatment group acted as a simulation model for RAAS action, wherein they observed a dose-dependent decrease in renal cortical perfusion while the renal medullary vasculature remained unchanged. While their findings were not statistically significant, it raises the question of whether the cortex, containing the glomeruli, was subjected to hyperfiltration because of RAAS system activation due to the drop in arterial BP induced by sedatives, thereby causing an increased leakage of proteins into the tubular lumen. In contrast, the tubular segments remained unaffected by the changes in renal pressure. This might be a potential cause for the observed increase in UP:C, and UTP in our study.

4.8 Fluid And Isoflurane Administration

Our study observed an increased HR was associated with those receiving IV fluids at 3ml/kg during anaesthesia and surgery. Interestingly, fluid administration was not linked to the changes in BP (MAP, SAP) in our study. This is in contrast to the findings of a mouse study which observed an association between fluid administration with MAP and none with HR (Zuurbier et al., 2002). This difference in the latter study from the current study can be attributed to the low rate of fluid infusion (3 ml/kg for cats), whose consequent effects may

have been overlapped by that of the anaesthetics. We did observe an association of fluids with UTP that might suggest that increased blood flow/volume, along with the intact renal autoregulatory mechanism (renal vasoconstriction), may have resulted in increased filtration pressure across the GFB. The administration of IV fluids for OHE in healthy cats does not appear to be imperative for maintaining renal function based on the findings of this study.

In our current study, the animals that required a higher percentage of isoflurane had lower arterial BP, i.e., MAP and SAP. Poterack et al. (1991) observed reduced heart rate, SAP, MAP (decreased by 70%) and abdominal aortic blood flow (decreased by 71%) in cats who received isoflurane at 3%. However, in our study, the isoflurane concentrations required to maintain anaesthesia were generally lower than 3% due to prior premedication with the treatment protocols. It is difficult to determine any effect that administration of isoflurane may have had in this study, as they may have been overshadowed by the effects of the premedication agents in this study.

4.9 Protective Function Of Anaesthesia

In an interesting study which evaluated the effects of laparoscopic OHE on cats, non-pregnant cats were observed to have elevated intra-abdominal pressure (from 2.08 to 7.5 mmHg), abdominal perfusion pressure (from 85.8 to 124.2 mmHg), along with MAP (from 87.9 to 128 mmHg) immediately after OHE (Bosch et al., 2012). This increase, which remained elevated for a few hours before returning to baseline, was believed to be a response to either pain associated with the surgery along with hypothermia, fluid overload or increased sympathetic stimulation during recovery post-anaesthesia. We are concerned with the latter since this might indicate that the observed decrease in cardiovascular parameters (MAP, SAP) during surgery, when within the safe threshold, may not affect kidney function, especially if it correlates to improved RBF post-surgery, provided that the increase in pressure does not lead to intra-abdominal hypertension.

It has been noted that an acute increase (Villa et al., 2016) or extremely high intra-abdominal pressure (> 11.5 mmHg) can result in the activation of the RAAS, leading to vasoconstriction and reduced renal blood supply in humans, which, along with reduced

abdominal perfusion pressure (< 72 mmHg), increases the risk of renal impairment and AKI (Gül, 2019). However, the normal IAP in adult cats ranges from 0-7.7 mmHg in sedated animals and up to 13.7 mmHg in those awake (Smith & Sande, 2012). Therefore, a significant transient elevation in IAP, without leading to hypertension, may rather improve renal perfusion. However, more studies should be performed to observe the renal microcirculation immediately after elective surgeries such as OHE in cats to better understand the after-effects of such intra-abdominal pressure on renal circulation.

Volatile anaesthetics, such as isoflurane, are proposed to have a reno-protective function by preventing renal tubular cell inflammation (CD73 action), adenosine synthesis and reducing calcium entry into the sarcolemma (Motayagheni et al., 2017). These changes that restrict cell apoptosis may prevent the release of specific tubular proteins, such as KIM-1 and NAG enzyme, which are shed into the tubular lumen after cell death. However, this can only be determined after observing the histopathological sections of renal tissue by comparing isoflurane anaesthetized cats and control animals. The above-provided evidence supports the idea that a controlled-anaesthetic environment leading to transient but safe variations in systemic function (HR, BP), subsequently followed-up by a safe-range stimulated post-operative response (intra-abdominal pressure, RBF), may stabilize, and reduce the risk of injury to the kidneys.

4.10 Limitations Of The Study

The following limitations apply to this study. The animals were not allowed to acclimate to their new environment before subjecting them to sampling and treatment (handling stress). The effect of constant stress induced by being housed in the same room where they were handled for sampling is unknown. The BP was not recorded at the other two time points. i.e., 24 and 48 hrs, to observe whether they were higher than usual due to stress, potentially related to the white coat effect as previously discussed. Elevated cardio-pulmonary parameters before and after surgery (up to 48 hrs) may overlap the effects of the peri-operative factors on renal circulation.

There were some missing data, such as the values recorded intraoperatively, i.e., surgical duration and DAP, for a few cats, however, it is unlikely to have varied the study findings. It must be conceded that per-minute intraoperative recordings, when compared to the five-minute intraoperative recordings of BP performed in this study, would have provided more accurate data on the exact duration of the transient dips in BP during surgery. Furthermore, there is a need for focused research to determine the effects of sustained hypotension (longer than 5 minutes) in cats, as has been performed in humans. This information would provide a window of opportunity for veterinarians to reduce the risk of renal damage in cats during surgeries by applying interventions (vasopressors/inotropes etc.) to elevate BP before an ischemic injury can set in (> 5 mins).

Ideally, the systemic parameters would have been recorded immediately after premedication administration, rather than after being prepared for surgery. This would have provided further information into the initial changes in BP with the various anaesthetic protocols and any effect that it might have had on the renal markers.

There is always a possibility of biological errors during sample analysis in the laboratory. Any effect that storage (-80 °C for a few months) may have had on the various novel marker concentrations is unknown. The use of a dipstick instead of a refractometer to record the USG for all the samples thus, made USG an unreliable marker in this study. Fasted blood samples were not used while measuring SCr levels, which may have led to inaccurate readings. Lastly, the urine sediment was not examined for active signs of damage before storage.

5 CONCLUSION

To summarize, our study found no evidence of subclinical kidney injury in cats subjected to general anaesthesia, with or without fluid administration, and spay procedure in an environment where all vital parameters were constantly monitored and controlled. We observed a slight negative shift in the novel renal marker values (NAG activity), which may be linked to the lowered metabolism, anti-apoptotic action, and reduced oxidative damage through the combined action of reduced core temperature, anaesthesia, RAAS

autoregulation or post-procedural increase in renal perfusion. However, as we have no evidence to support this, further studies are required to evaluate its repeatability and determine the exact cause by studying renal cortical and medullary perfusion (and oxygenation) intra- and post-operatively.

It would be interesting to note the effect of general anaesthesia and surgery on diseased animals to compare the changes in renal marker values to better understand them. Studies evaluating sequential changes in these biomarkers in unhealthy and control animals may help in improved differentiation of the biological changes from variations that may validate the concern of tissue injury.

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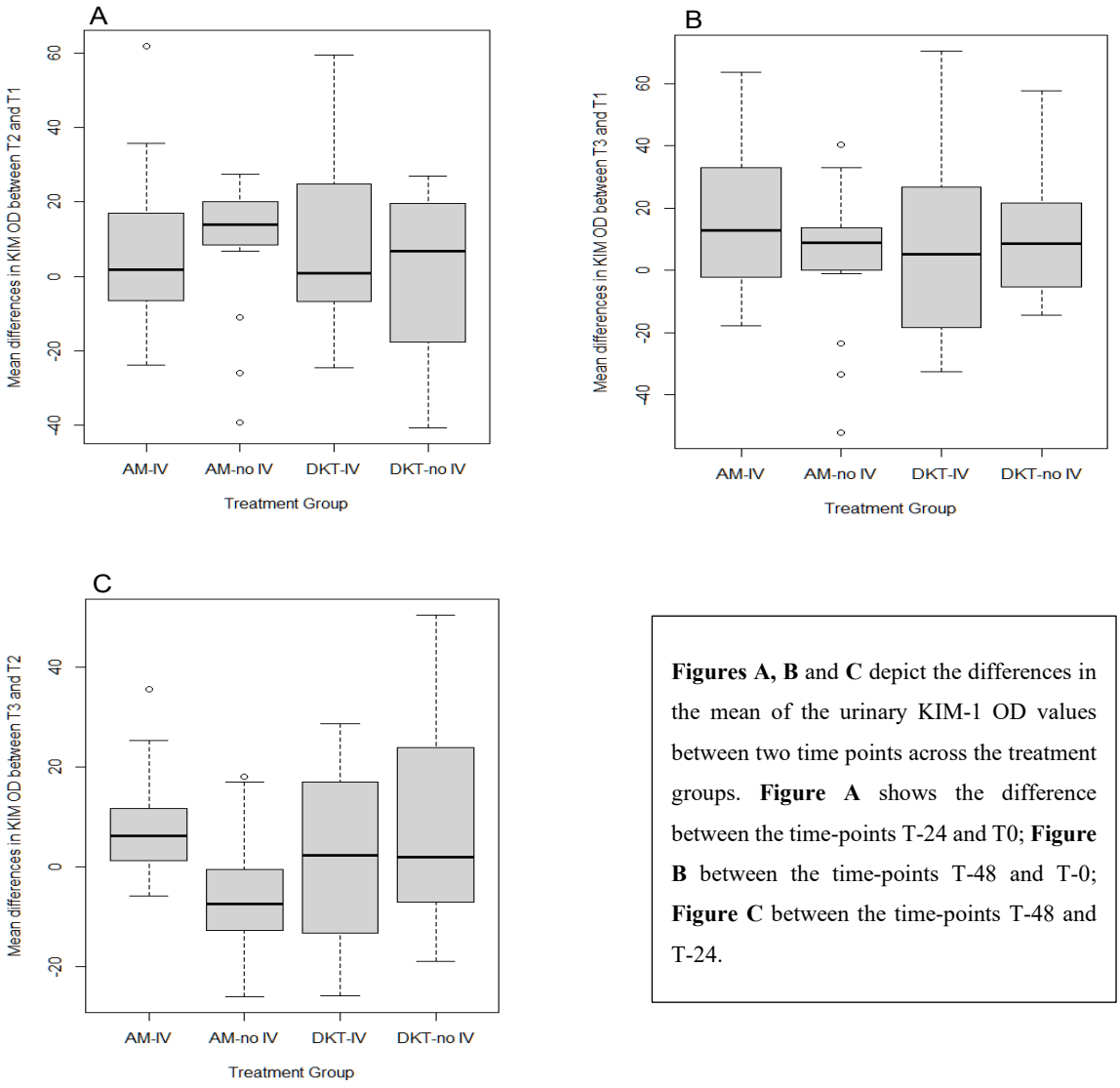
7 Appendix A

Comparing changes in the renal marker values for the sample units in different treatment groups between the time-points using ANOVA.

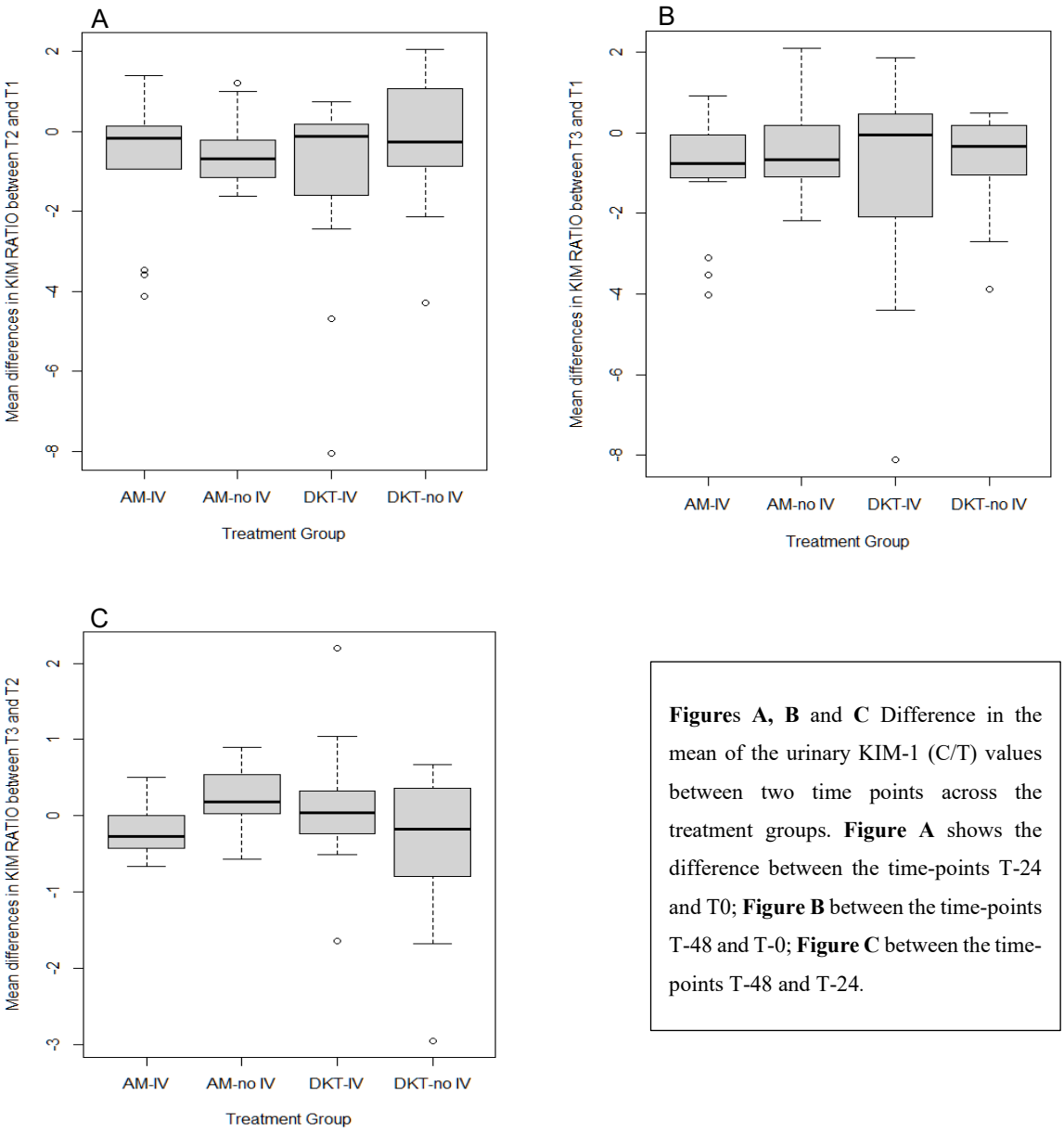
1) Kidney Injury Molecule-1 (KIM-1) OD Values



Figures A, B and **C** depict the differences in the mean of the urinary KIM-1 OD values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

No significant difference was observed in the mean KIM-1 OD between T24-0 and T48-0 (p-value > 0.2). However, a weakly significant mean difference in KIM-1 OD was observed across T48-24, especially for AM-IV. No significant pair difference (< 0.05) was observed between T48-24.

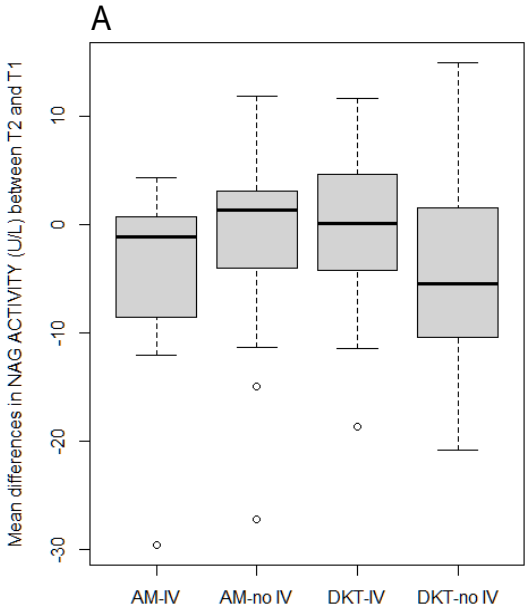
2) KIM-1 RATIO

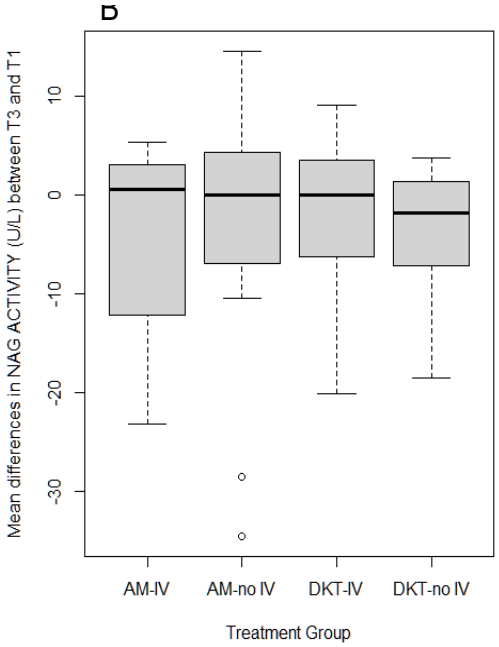


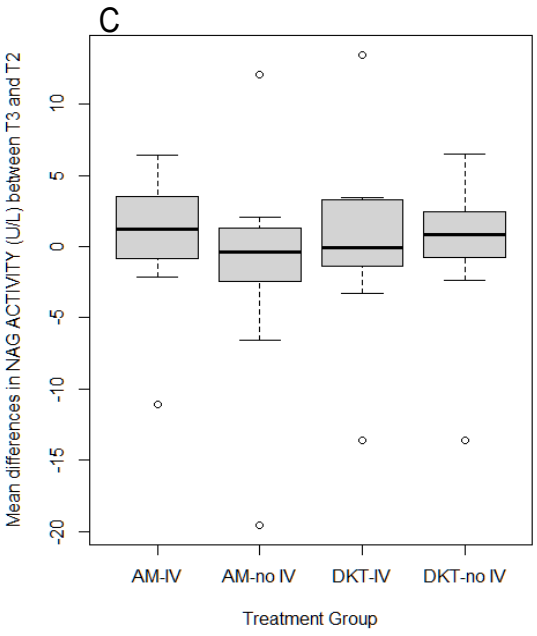
Figures A, B and **C** Difference in the mean of the urinary KIM-1 (C/T) values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

No significant difference was observed in the mean KIM-1 ratio across T24-0 and T48-0 (p-value > 0.2). However, a significant mean difference in the KIM-1 ratio was observed across T48-24 (p-value < 0.2), albeit no significant difference between paired treatment groups (p-value < 0.05) was observed.

3) N-Acetyl β-D-Glucosaminidase (NAG) Activity



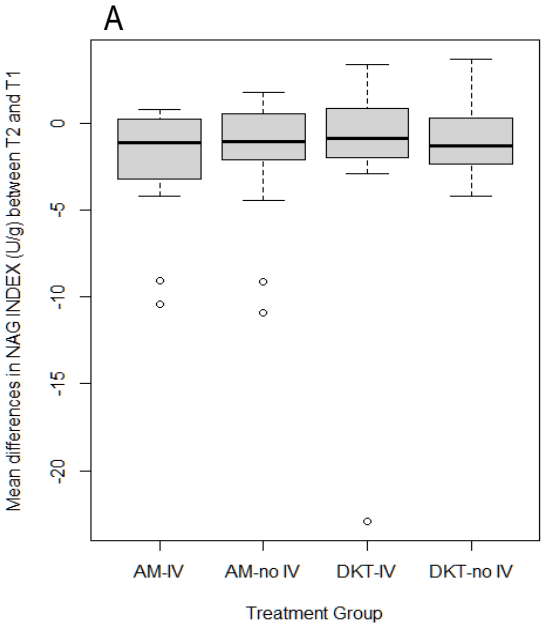


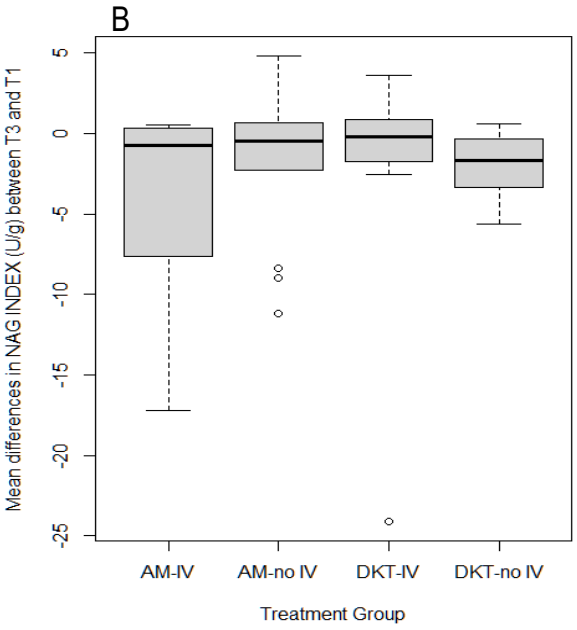


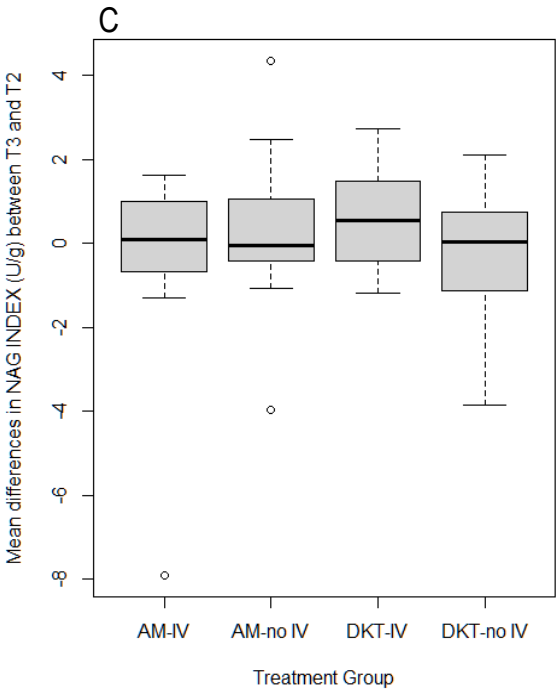
Figures A, B and **C** Difference in the mean of the urinary NAG values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

No significant mean difference was observed in the NAG activity between T24-0, T48-0, and T48-24 (p-value > 0.2).

4) NAG Index



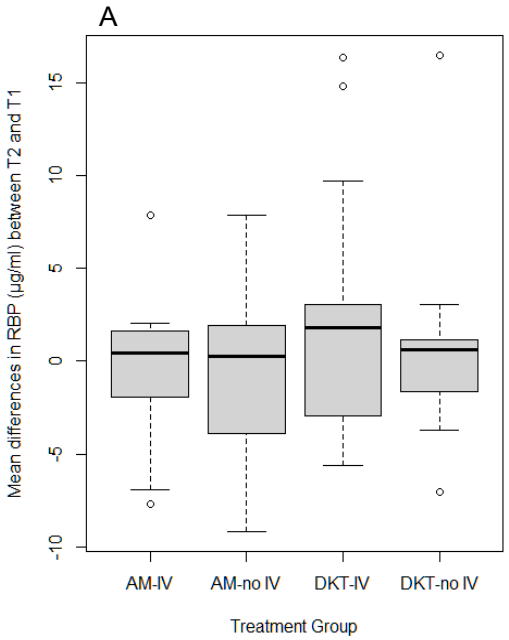


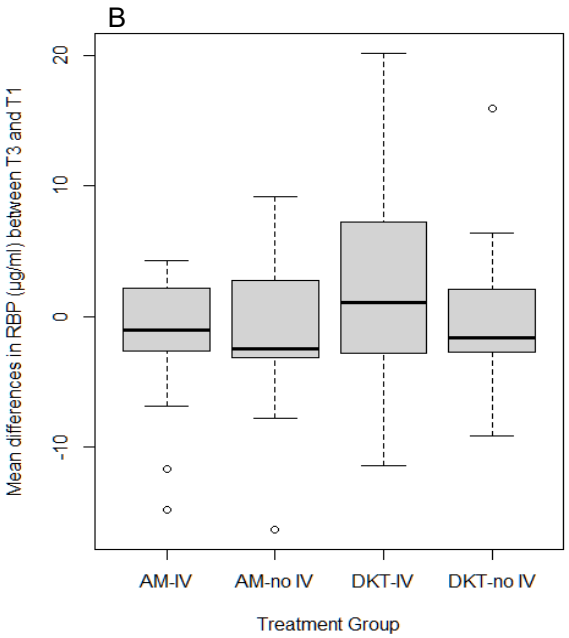


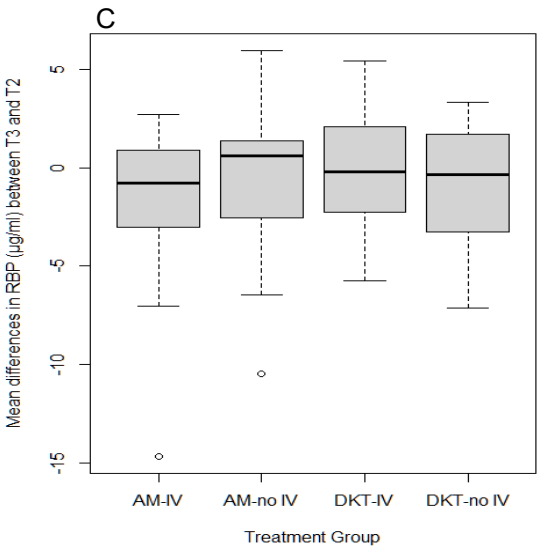
Figures A, B and **C** depict the differences in the mean of the urinary NAG Index values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

No significant difference was observed in the mean NAG index between T24-0, T48-0and T48-24 (p-value > 0.2).

5) Retinol Binding Protein (RBP)



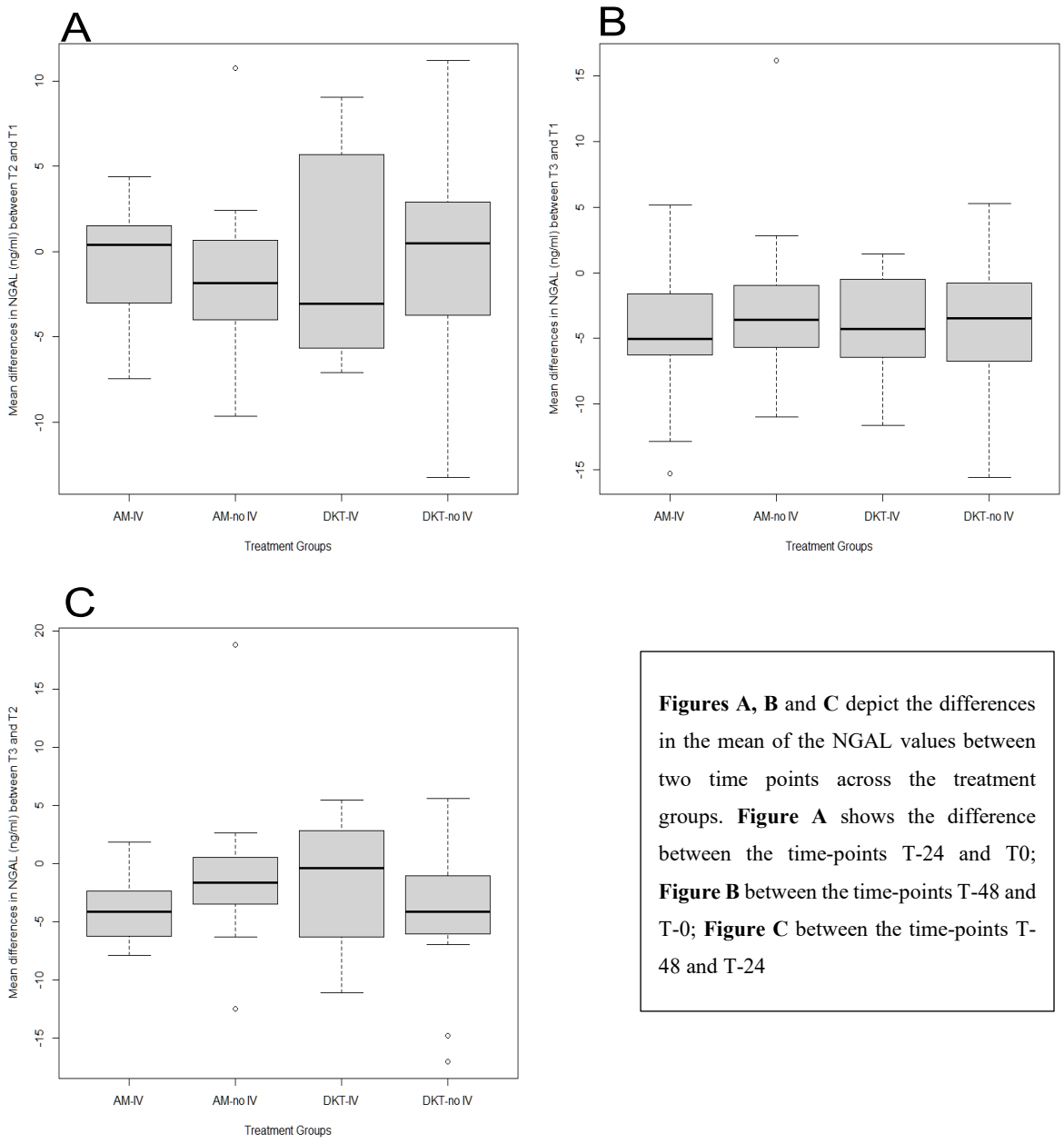




Figures A, B and **C** depict the differences in the mean of the RBP values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

No significant mean difference was observed in the RBP values across T24-0, T48-0and T48-24 (p-value > 0.2).

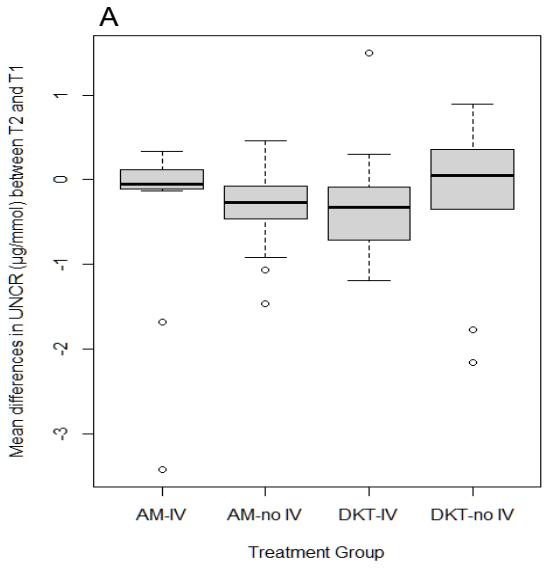
6) Neutrophil Gelatinase-associated Lipocalin (NGAL)

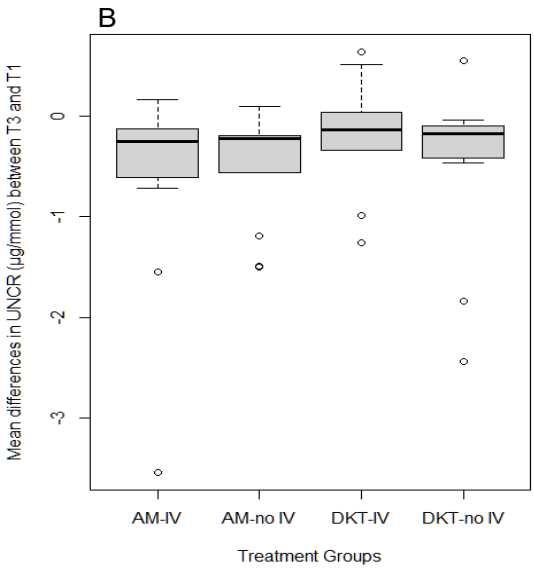


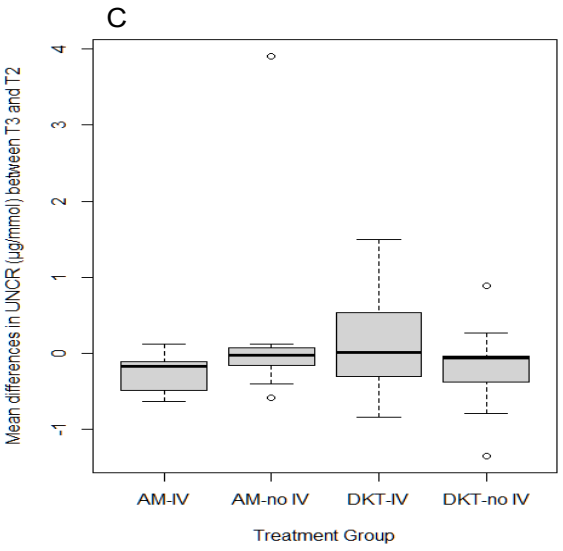
Figures A, B and **C** depict the differences in the mean of the NGAL values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24

No significant mean difference was found in the NGAL values across T24-0, T48-0and T48-24 (p-value > 0.2).

7) Urine NGAL: creatinine (UNCR)



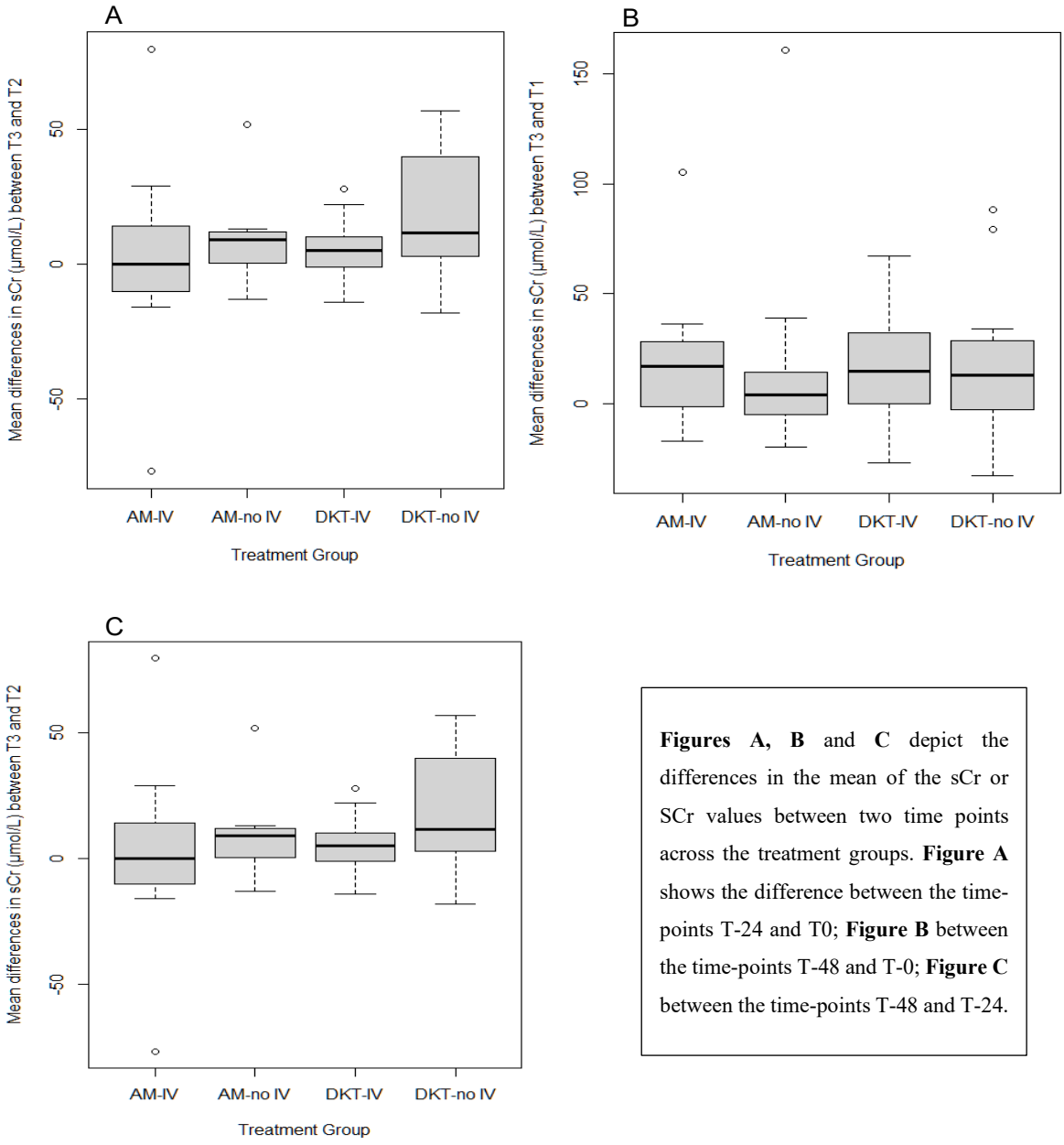




Figures A, B and **C** depict the differences in the mean of the UNCR values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24

No significant mean difference was observed for UNCR across T24-0, T48-0and T48-24 (p-value > 0.2).

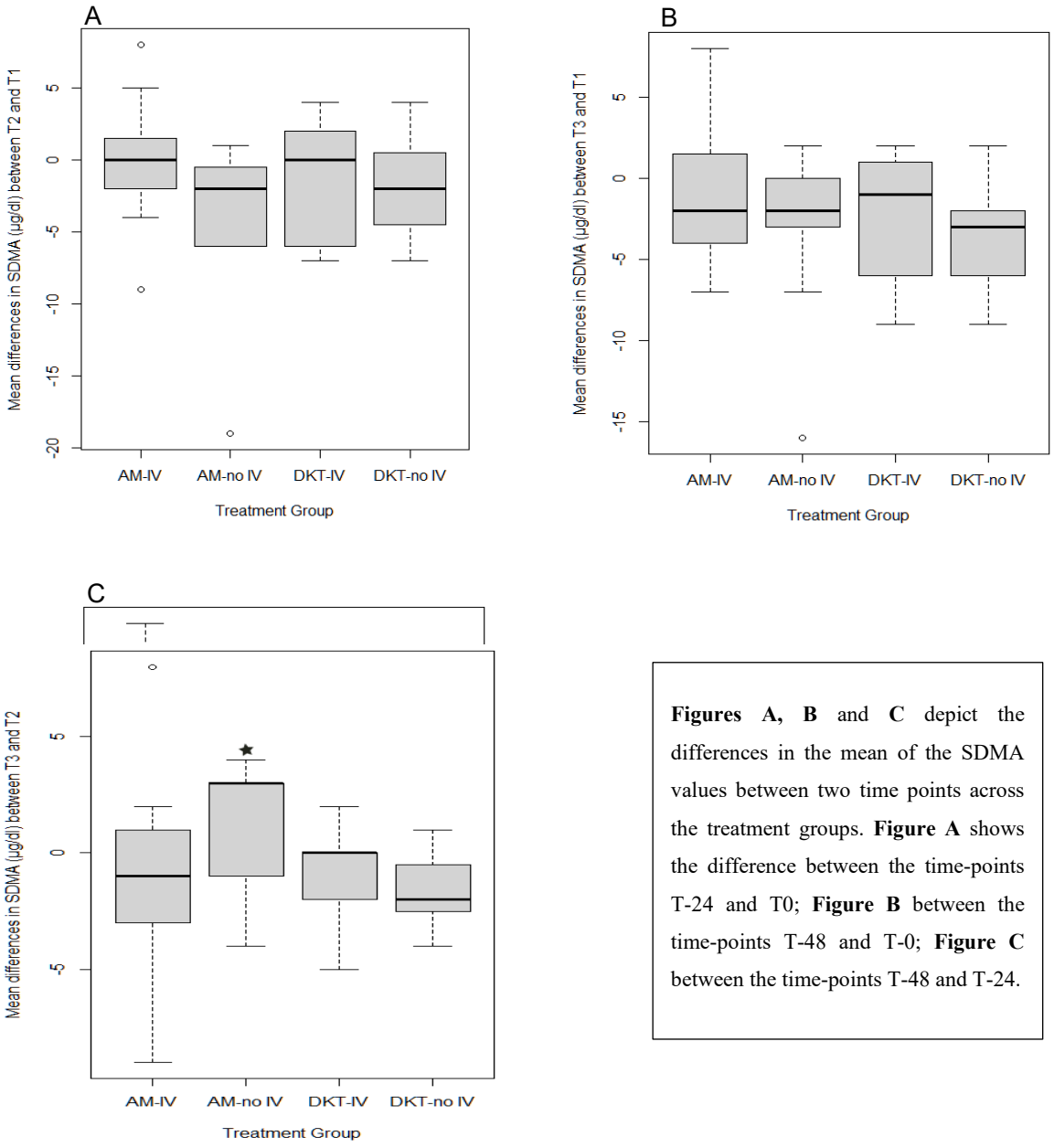
8) Serum creatinine (SCr)



Figures A, B and **C** depict the differences in the mean of the sCr or SCr values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

No significant mean difference was observed in the mean SCr values across T24-0, T48-0and T48-24 (p-value > 0.2).

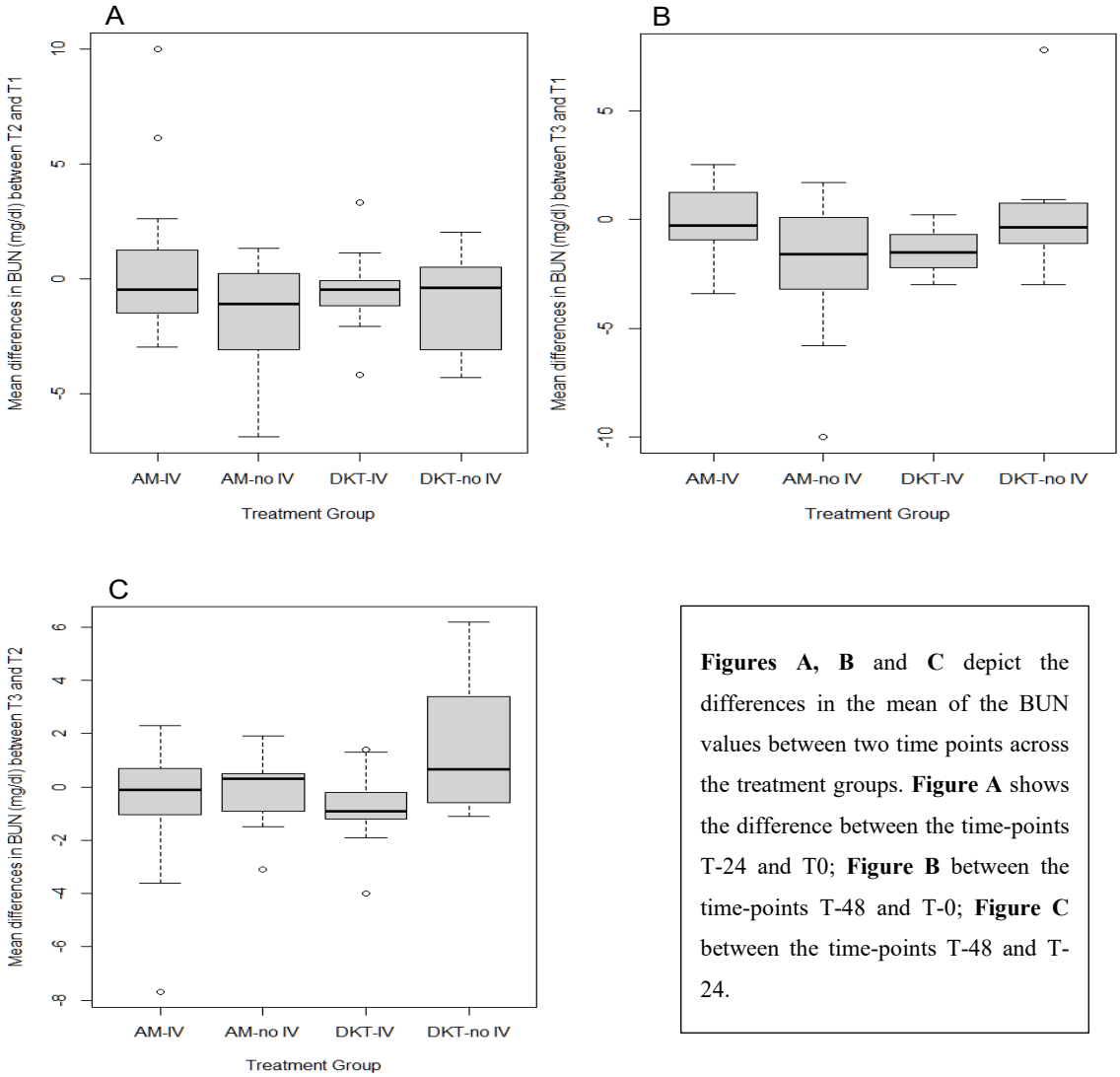
9) SDMA



Figures A, B and **C** depict the differences in the mean of the SDMA values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

A significant difference was observed in the mean SDMA between T24-0 and T48-24 (p-value < 0.2). No significant difference was observed for the same between T48-0. A significant change was observed in AM-no IV between T24-0 and T48-24. No significant pairwise difference was observed (p-value > 0.05).

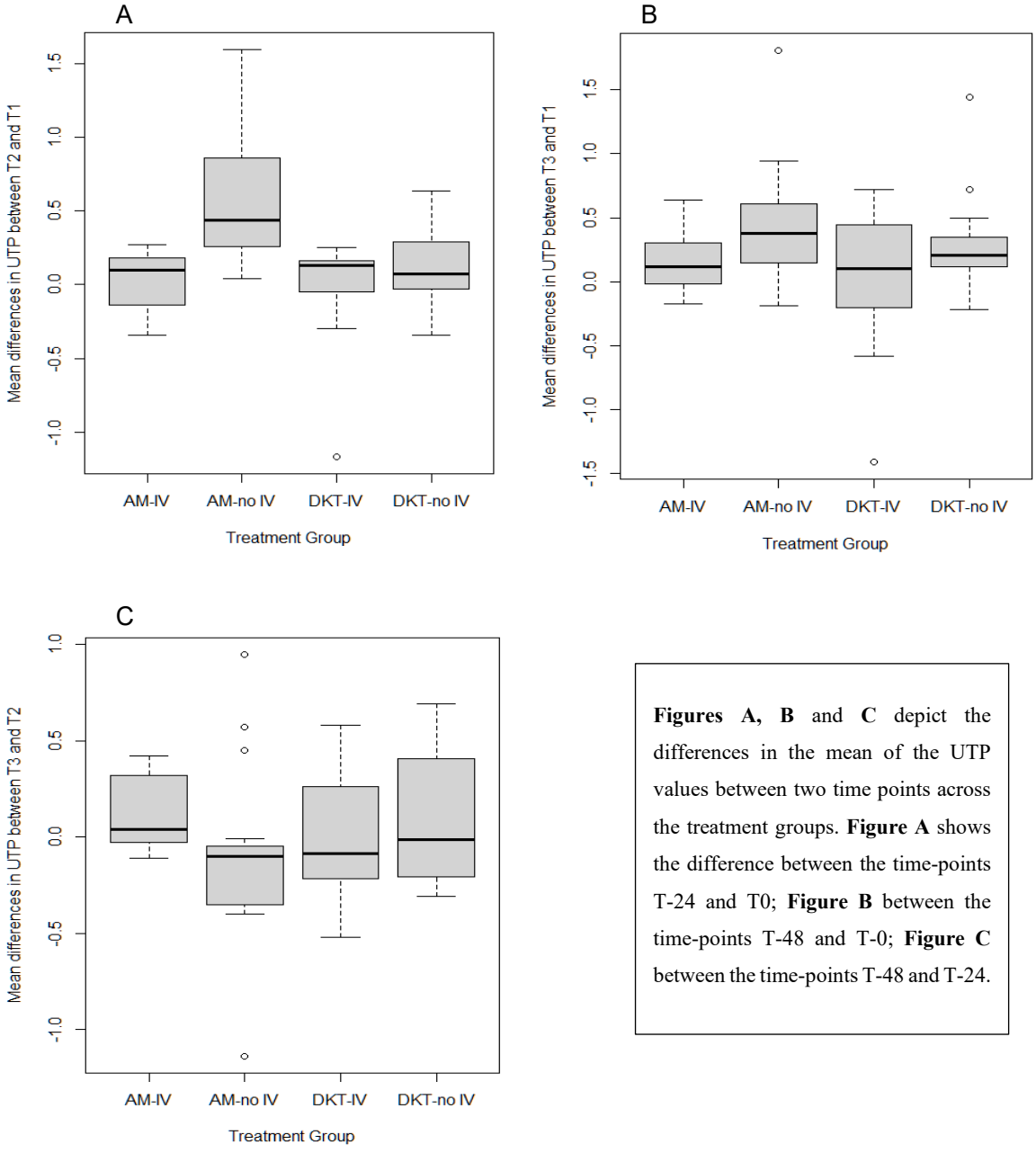
10) BUN



Figures A, B and **C** depict the differences in the mean of the BUN values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

A significant difference was observed in the mean BUN between T24-0. T48-0 and T48-24 (p-value < 0.2). Strong evidence of a statistical difference in AM-no IV between T24-0, AM-no IV and DKT-no IV between T48-0 and DKT between T48-24. No significant pairwise difference was observed (p-value > 0.05) across T24-0 and T48-0. DKT-no IV was found to have a higher BUN value ranging from 0.005-4.37 than DKT-IV between T48-24.

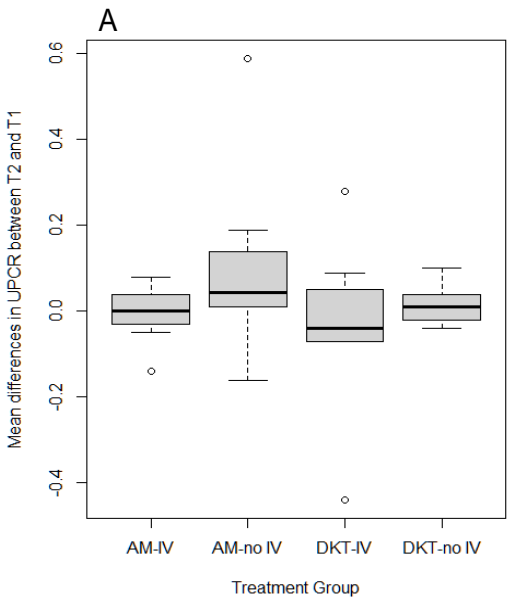
11) Urine Total Protein (UTP)

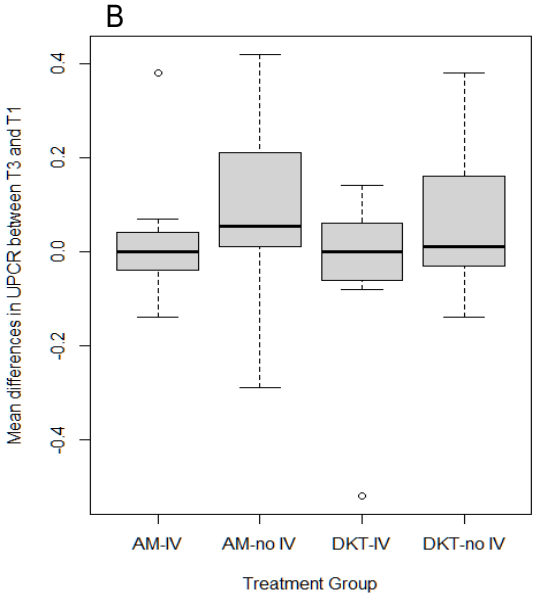


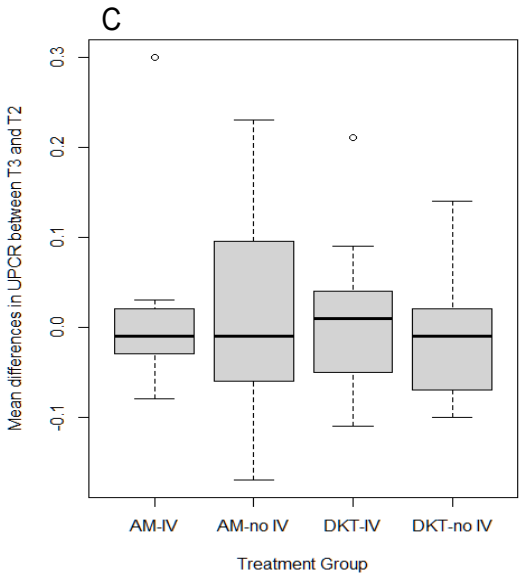
Figures A, B and **C** depict the differences in the mean of the UTP values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

A significant difference in mean UTP was observed between T24-0 and T-48-0, but not T48-24. There was strong evidence for a significant difference in AM-no IV between T24-0. No significant pairwise difference was observed (p-value > 0.05).

12) Urine protein creatinine ratio (UP:C)



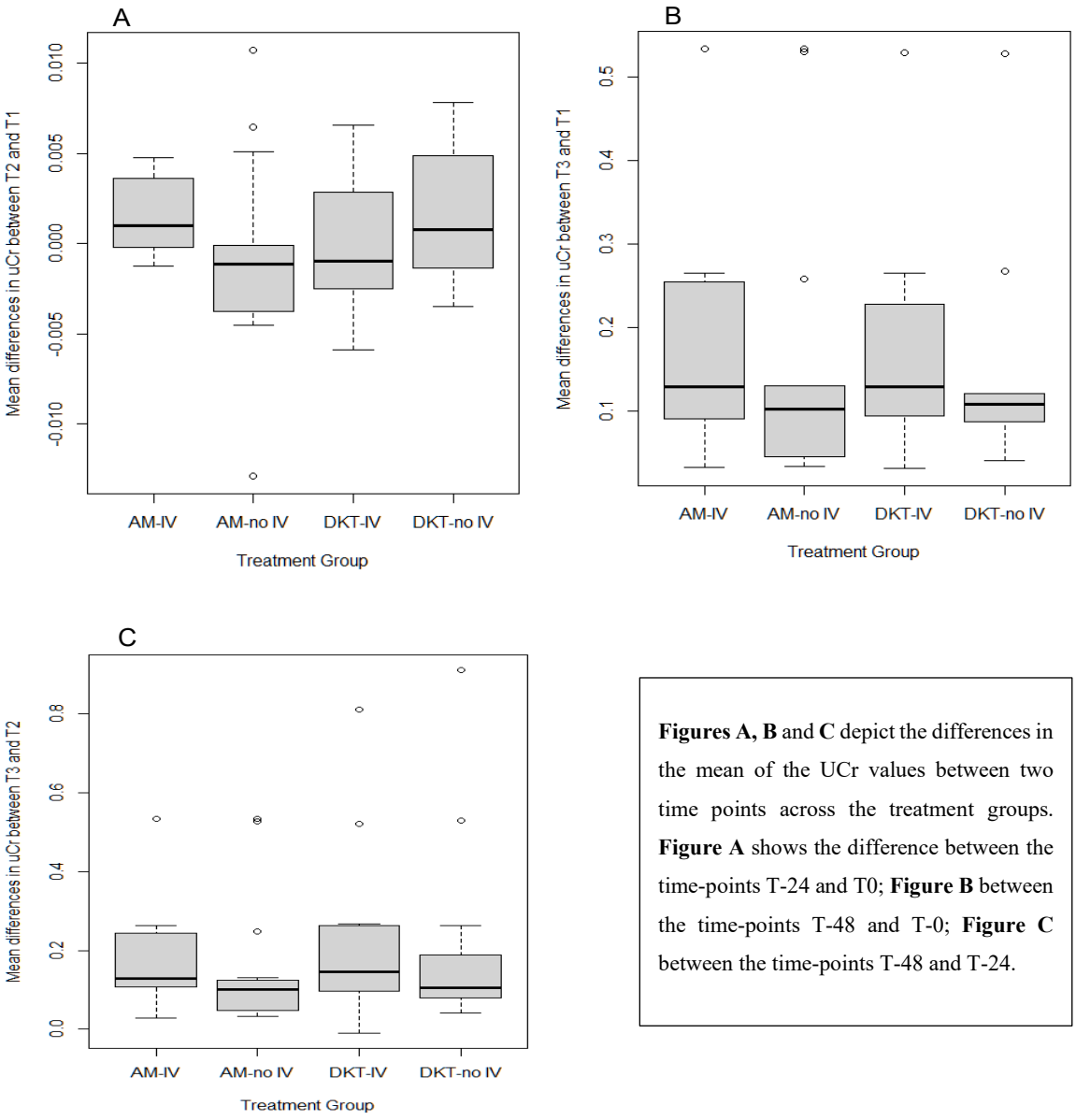




Figures A, B and **C** depict the differences in the mean of the UPCR values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

A significant difference in mean UP:C was observed between T24-0, but not T48-0 and T48-24. No significant pairwise difference was observed (p-value > 0.05).

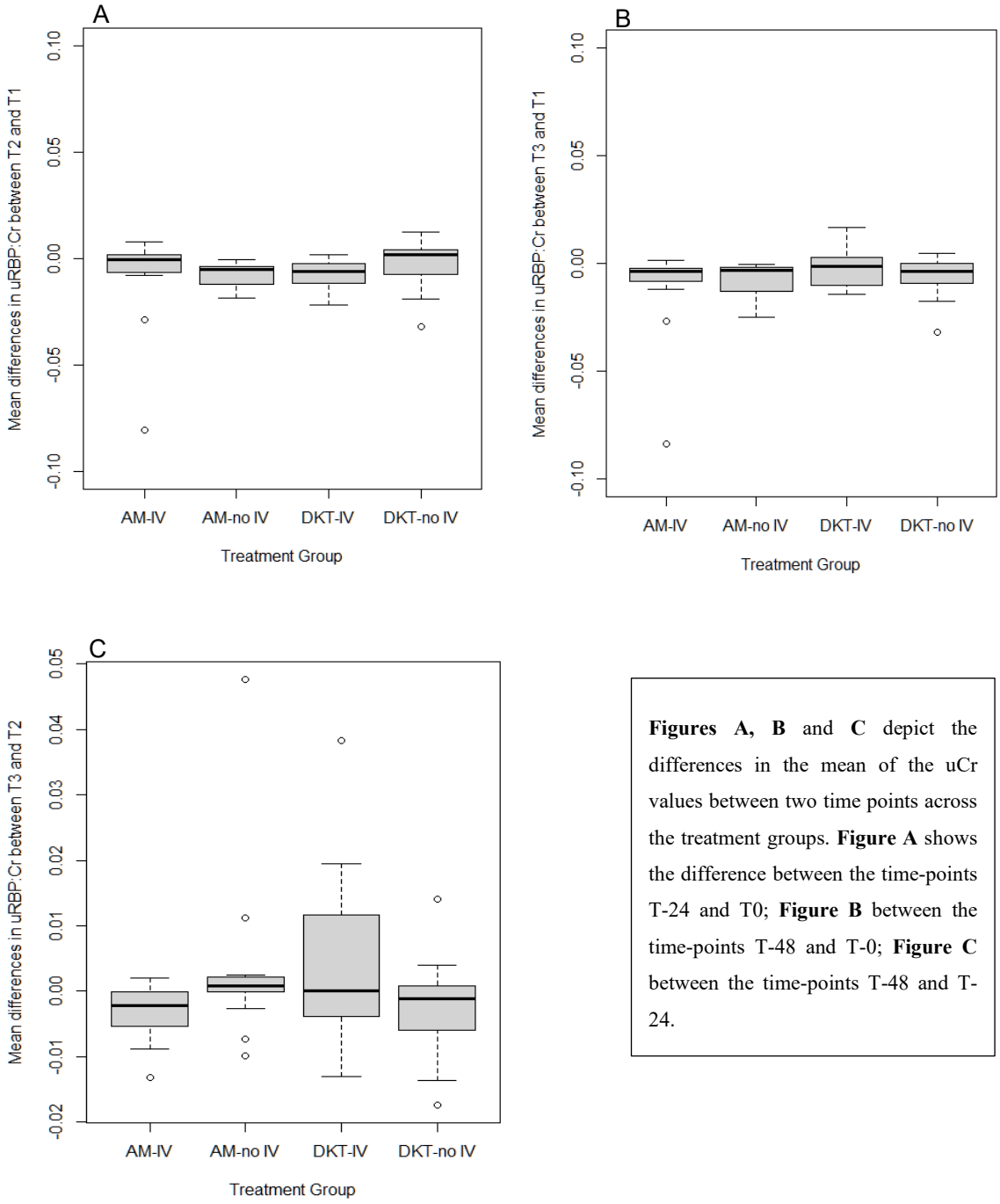
13) Urine creatinine (UCr)



Figures A, B and **C** depict the differences in the mean of the UCr values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

No significant difference was observed while analyzing the means of UCr between T24-0., T48-0 and T48-24.

14) Urine RBP creatinine ratio (uRBP:Cr)



Figures A, B and **C** depict the differences in the mean of the uCr values between two time points across the treatment groups. **Figure A** shows the difference between the time-points T-24 and T0; **Figure B** between the time-points T-48 and T-0; **Figure C** between the time-points T-48 and T-24.

No significant mean difference was observed across T24-0 or T48-0. However, a significant mean difference in uRBP:Cr was observed, with strong evidence, across T48-24; however, no significant pair difference (<0.05) was found.

8 Appendix B

Boxplots for the mean difference in renal markers between the time points for various factors.*

1) BUN 24-0

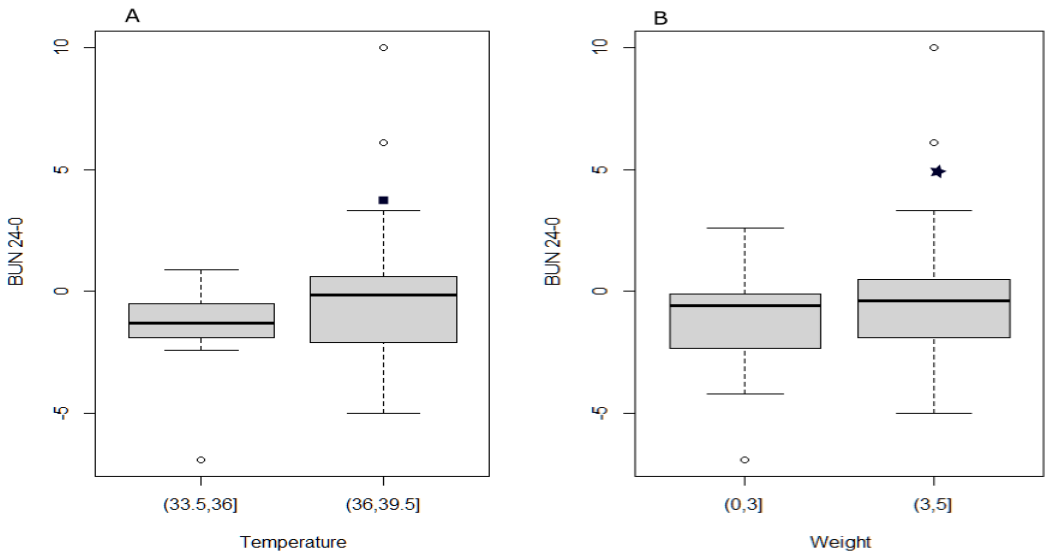


Figure A and **B** Difference in BUN 24-0 with A) l-Temp (°C), and B) weight (kg).

* ★: p-value < 0.05
■: p-value < 0.2

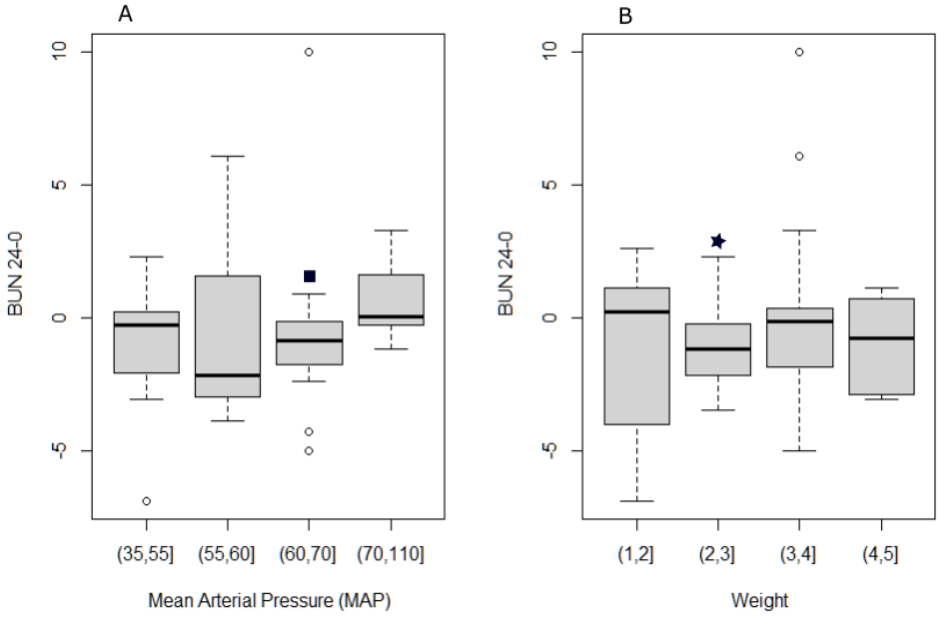


Figure A and **B** Difference in BUN 24-0 with A) l-MAP (mmHg), and B) weight (kg).

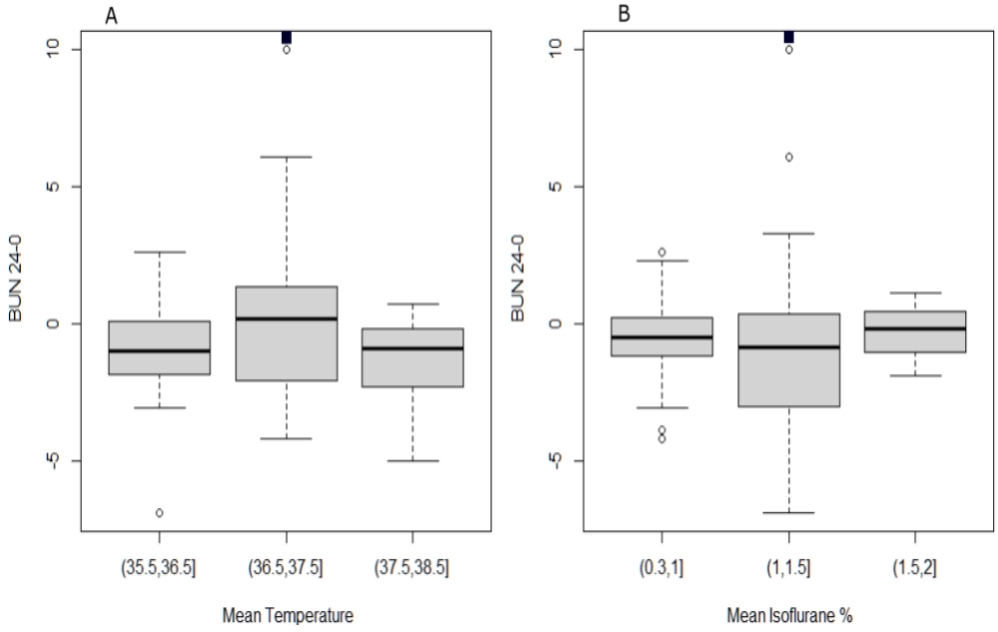
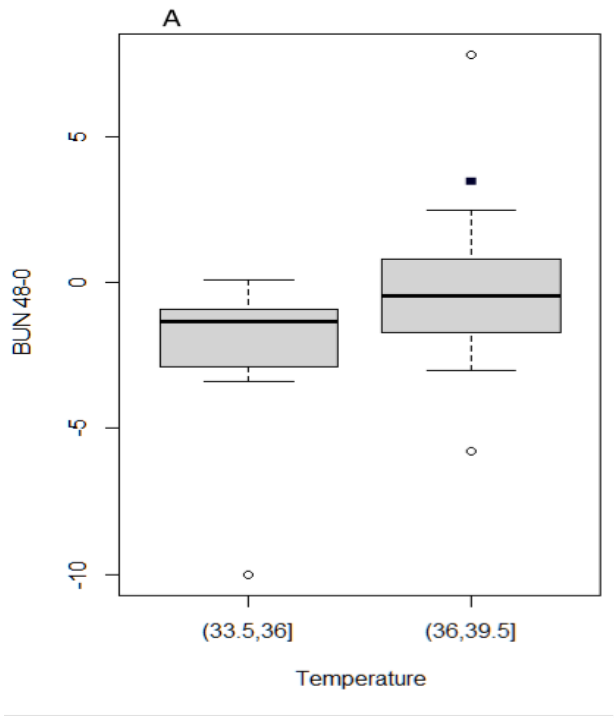


Figure A and **B** Difference in BUN 24-0 with A) m-temp (°C), and B) weight (kg).

BUN 48-0



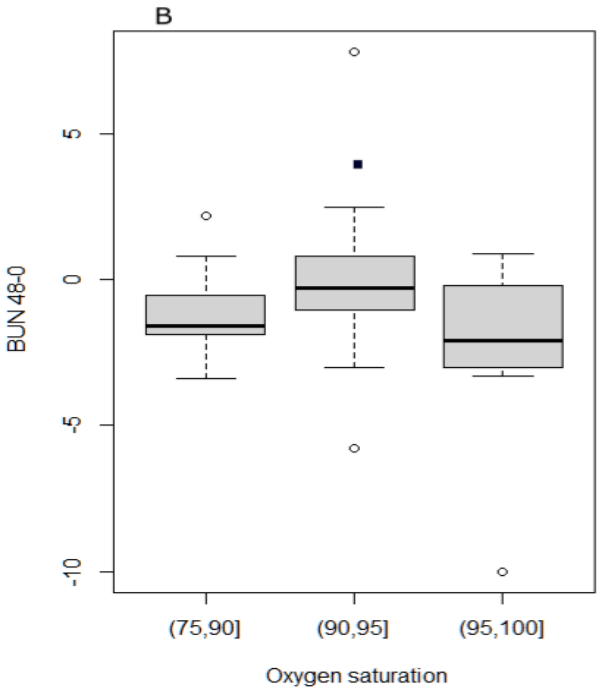


Figure A and **B** Difference in BUN 48-0 with A) l-temp (°C), and B) SpO_2 (%).

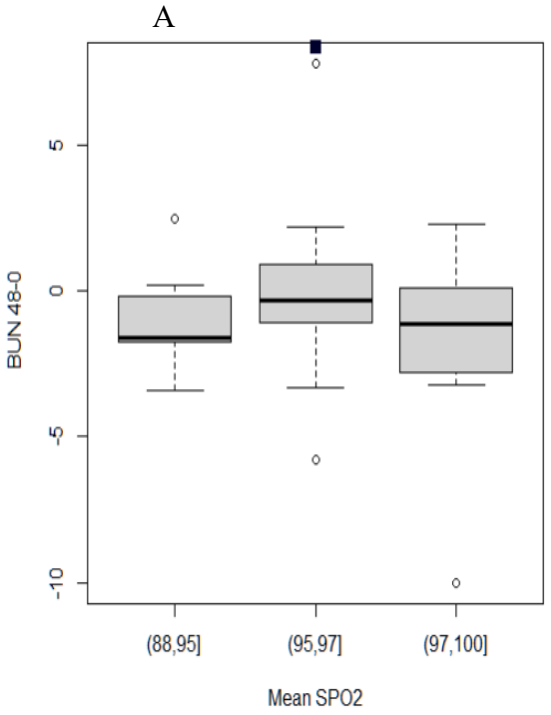


Figure A Mean difference in BUN 48-0 with m-SpO_2 (%).

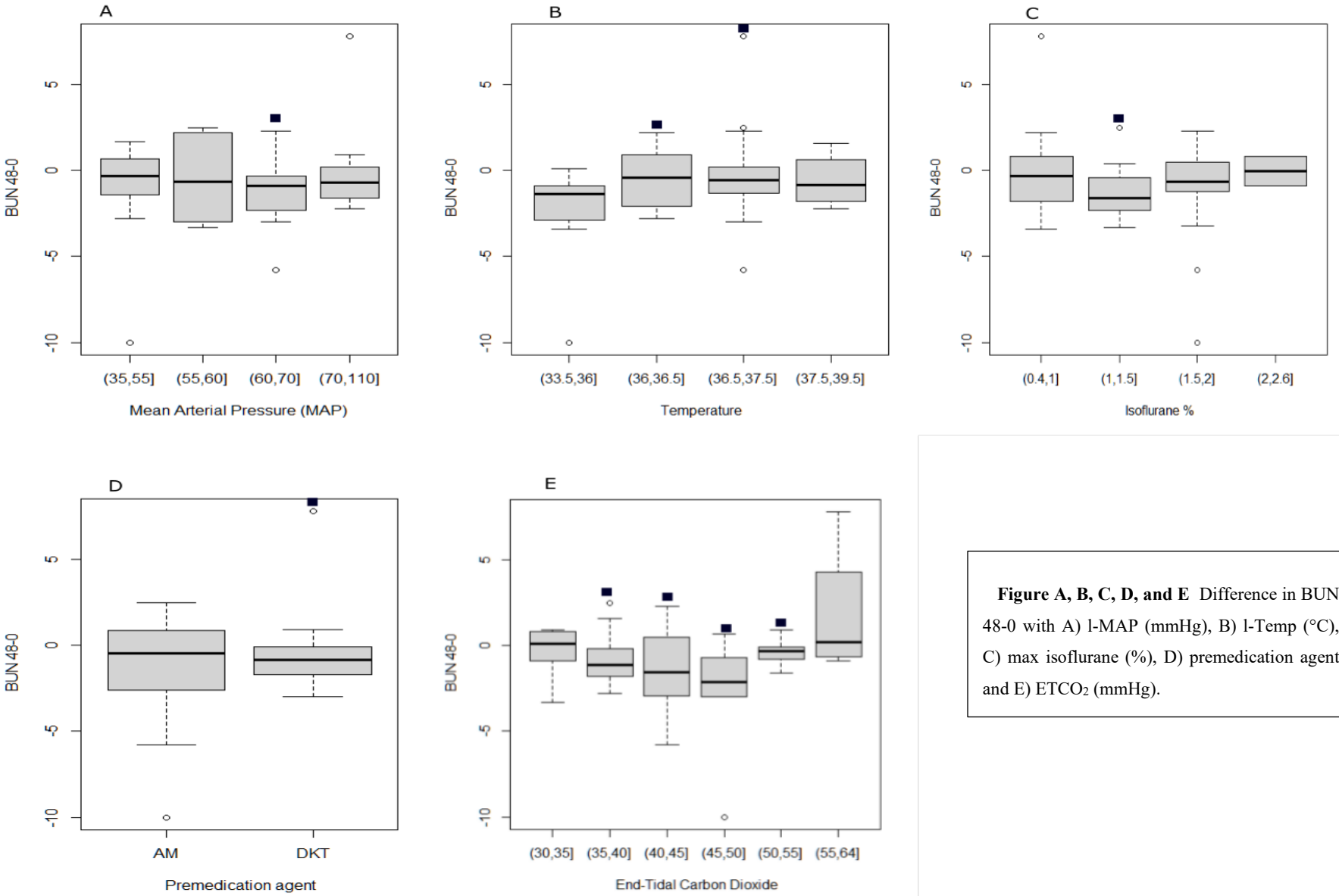


Figure A, B, C, D, and E Difference in BUN 48-0 with A) l-MAP (mmHg), B) l-Temp (°C), C) max isoflurane (%), D) premedication agent and E) $ETCO_2$ (mmHg).

BUN 48-24

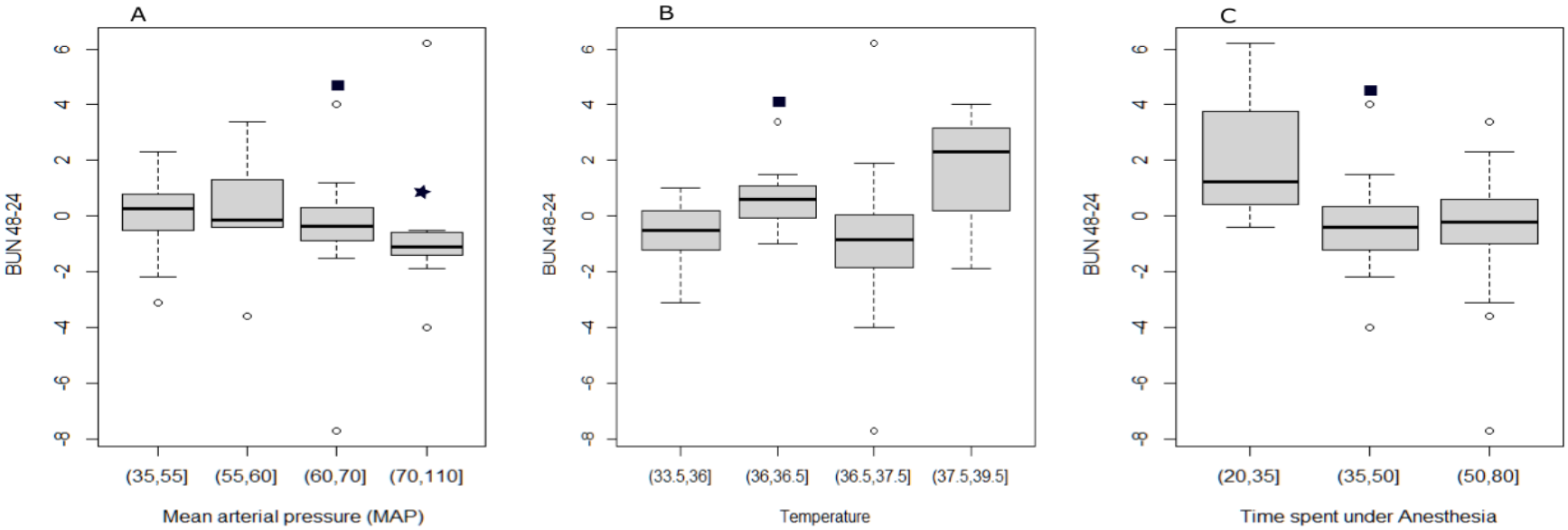


Figure A, B, and C Mean difference in BUN 48-24 with A) l-MAP (mmHg), B) l-Temp (°C) and C) anaesthetic duration (mins).

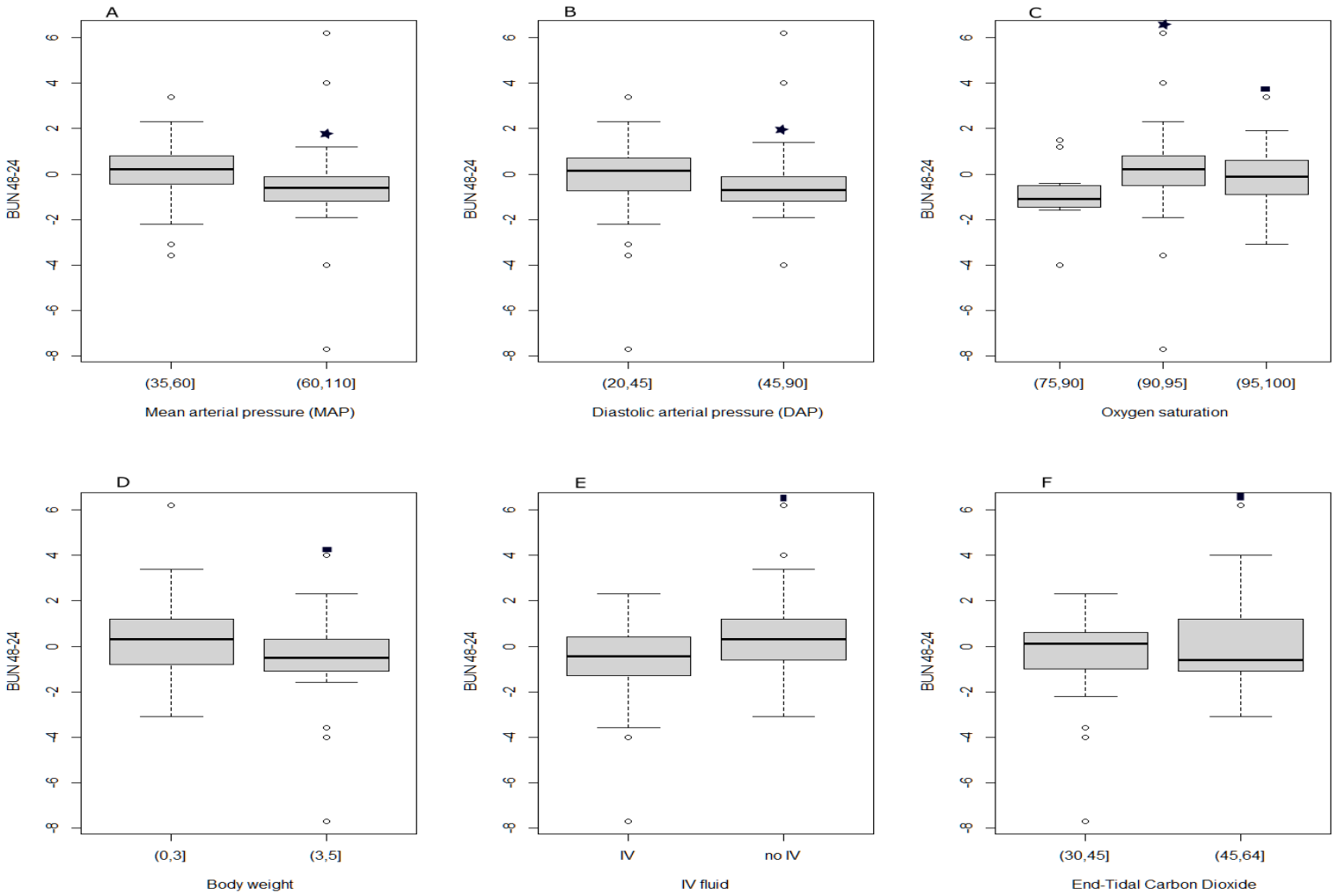


Figure A, B, C, D, E, and F Mean difference in BUN 48-24 with A) l-MAP (mmHg), B) l-DAP (mmHg), C) l-SpO_2 (%), D) body weight (kg), E) fluid administration and F) $ETCO_2$ (mmHg).

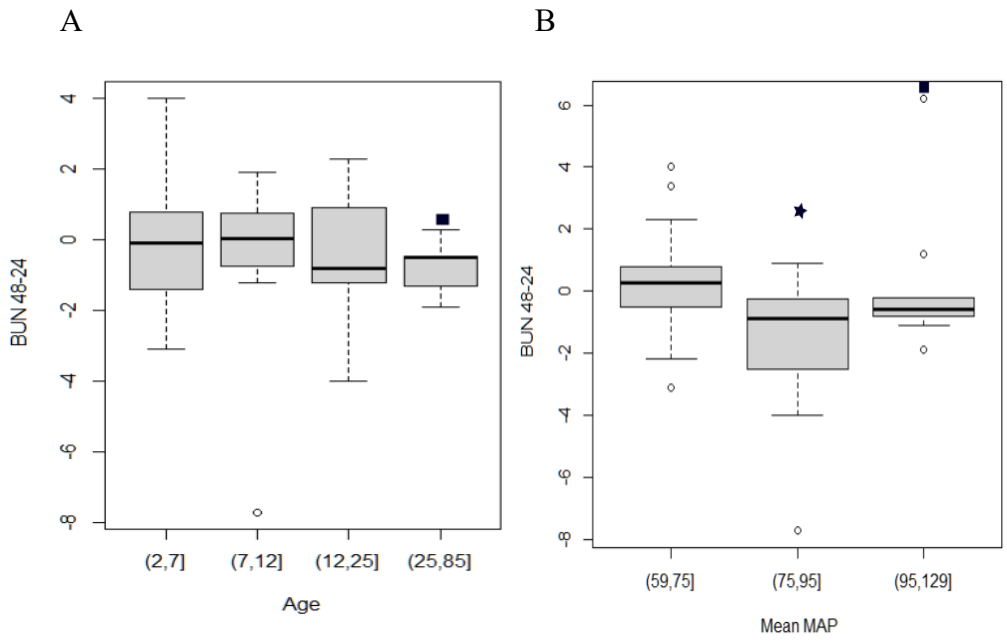


Figure A and B Mean difference in BUN 48-24 with A) age and B) m-MAP (mmHg).

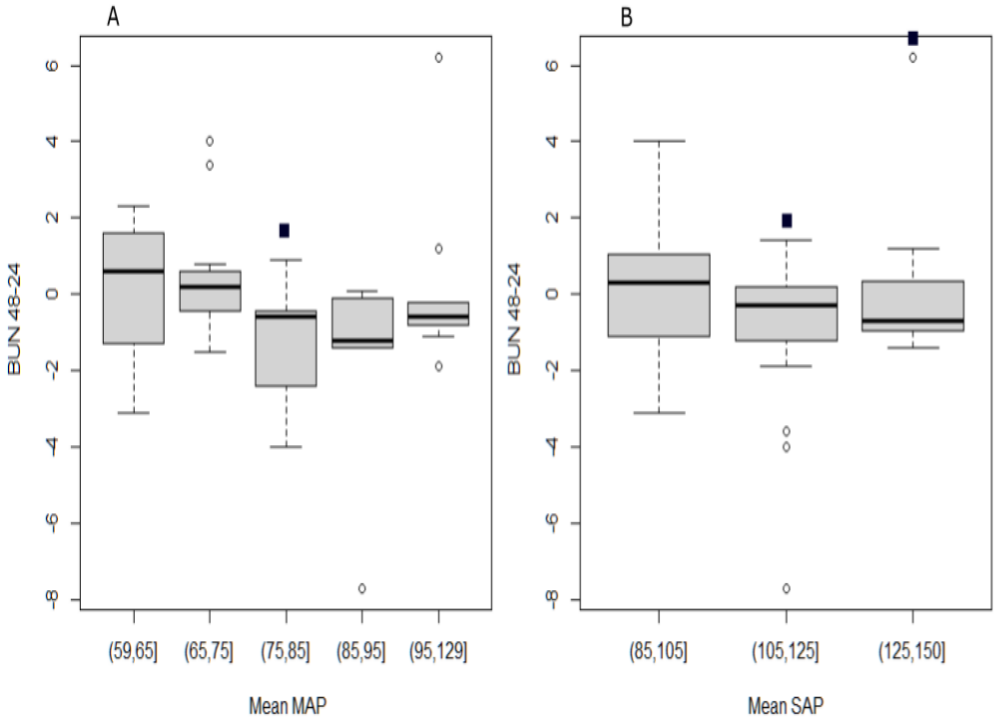


Figure A and B Mean difference in BUN 48-24 with A) m-MAP (mmHg) and B) m-SAP (mmHg).

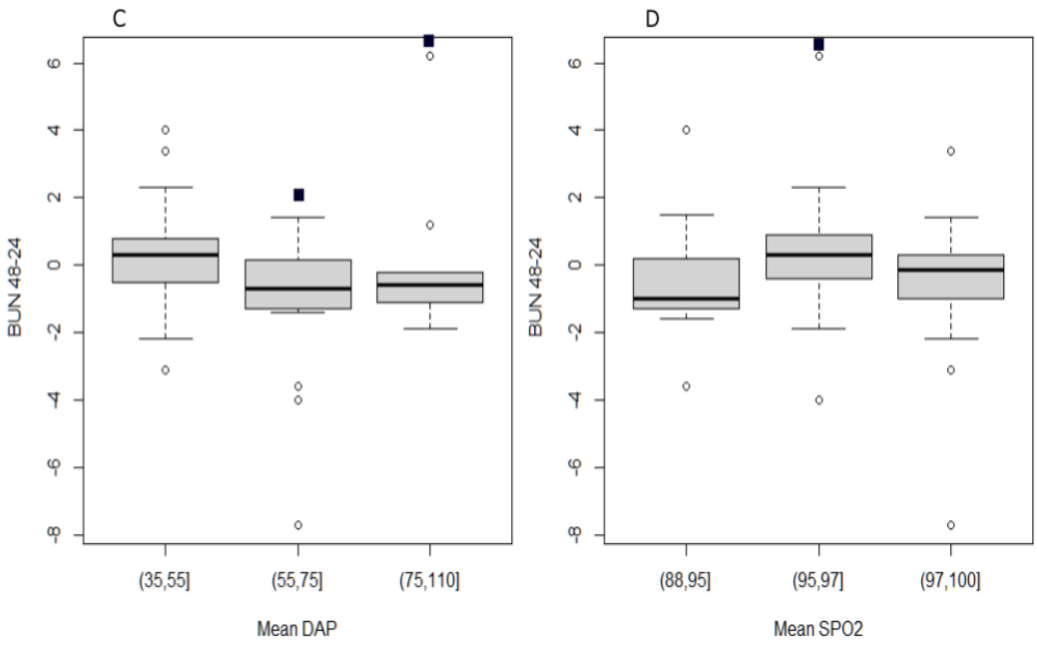


Figure C and D Mean difference in BUN 48-24 with C) m-DAP (mmHg) and D) m-SPO_2 (%).

KIM-1 OD 24-0

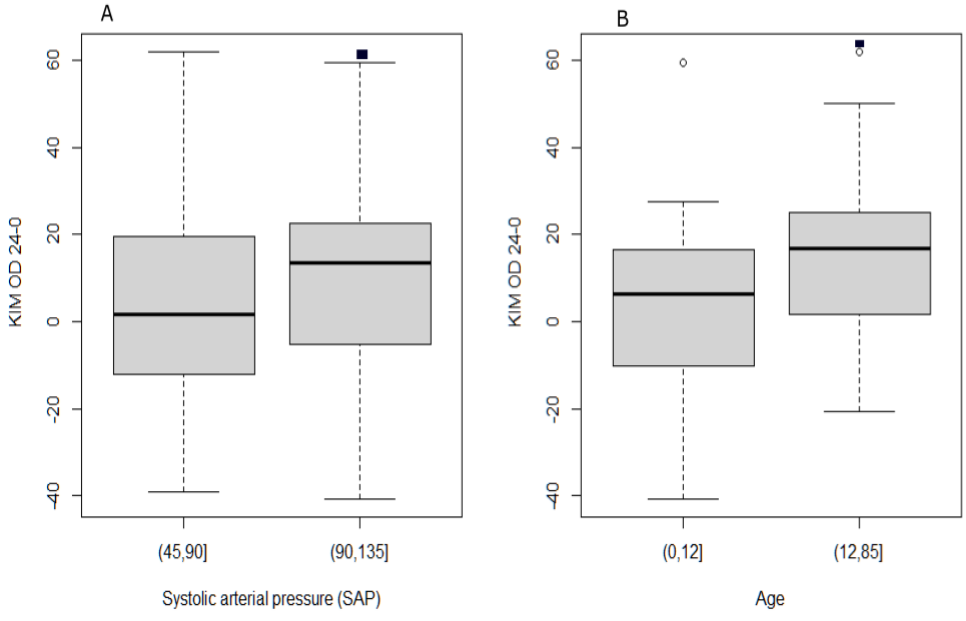


Figure A and B Mean difference in KIM-1 OD 24-0 with A) l-SAP (mmHg) and B) age (m).

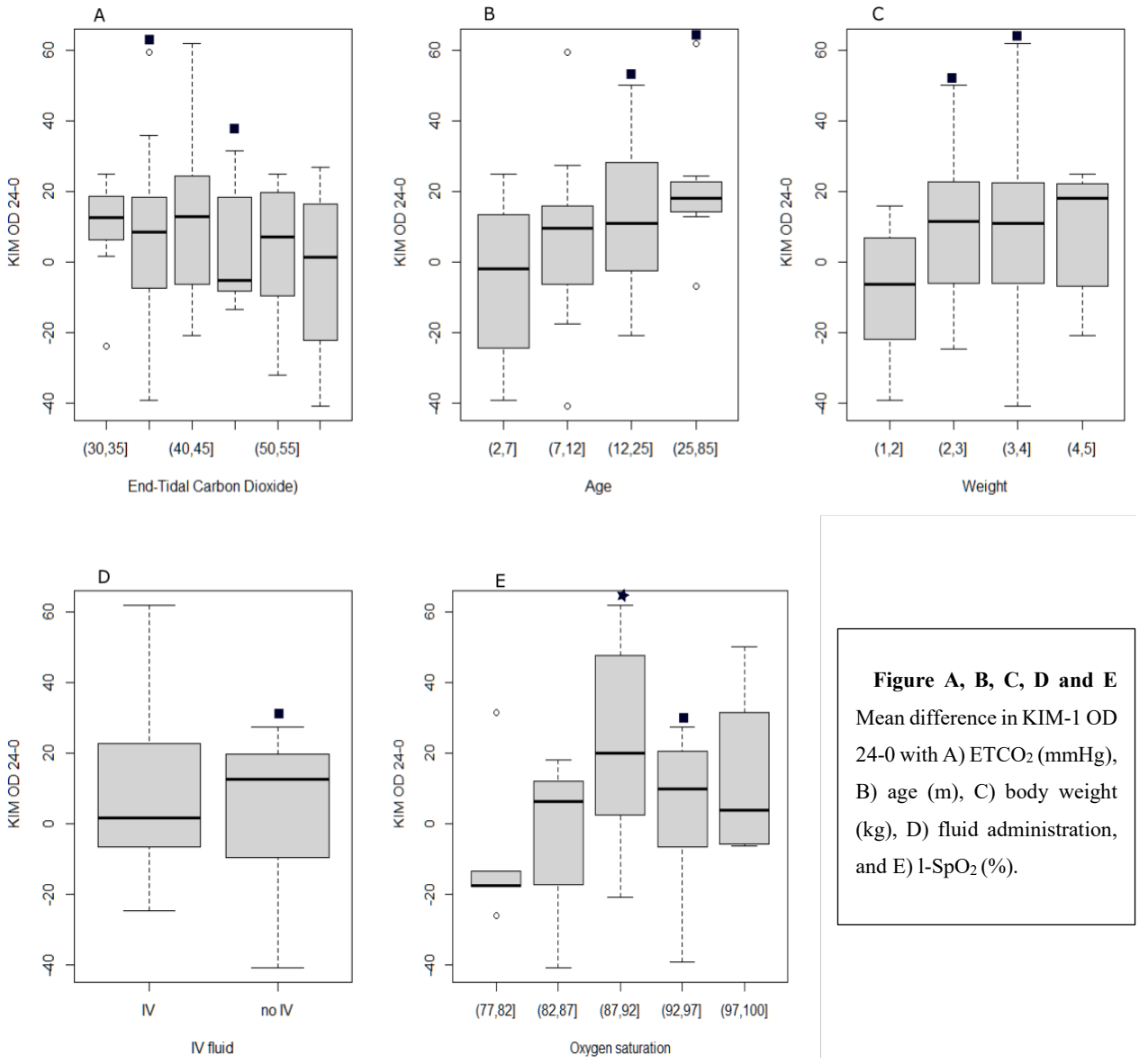


Figure A, B, C, D and E Mean difference in KIM-1 OD 24-0 with A) $ETCO_2$ (mmHg), B) age (m), C) body weight (kg), D) fluid administration, and E) l-SpO_2 (%).

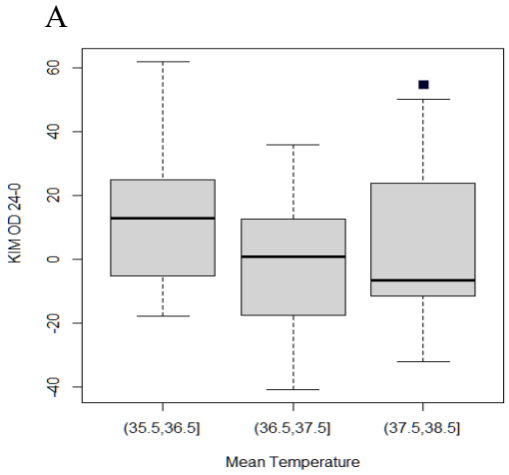
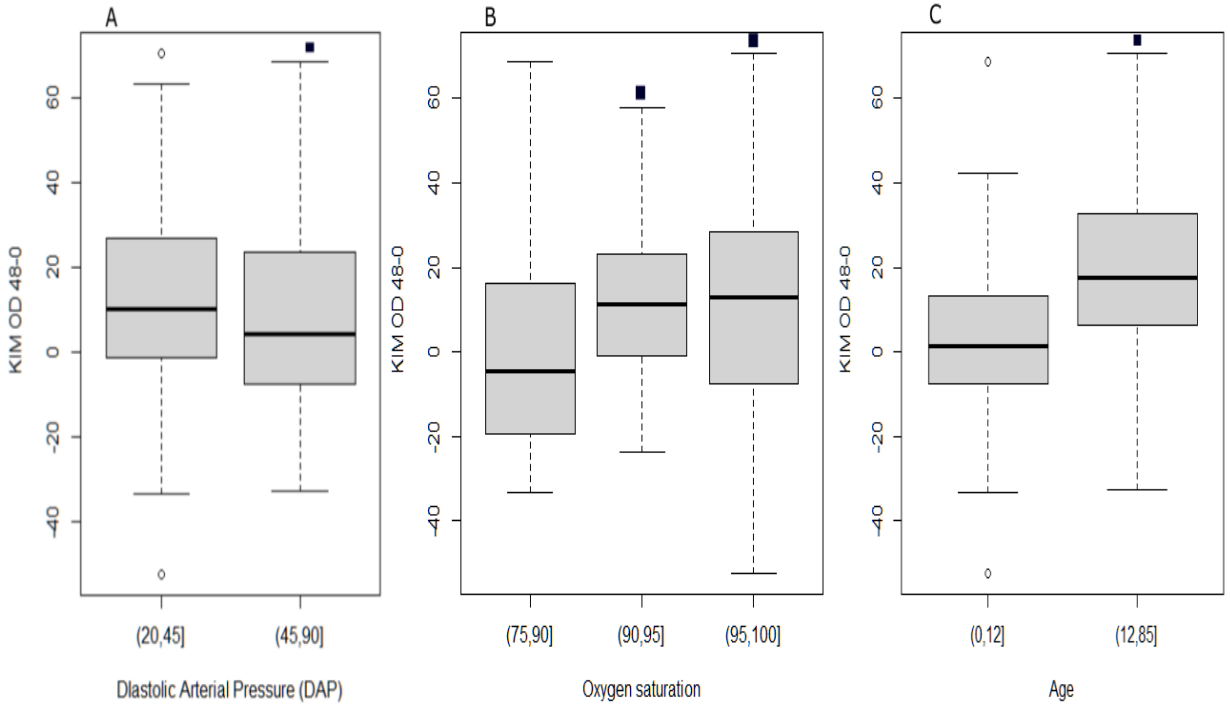


Figure A Mean difference in KIM-1 OD 24-0 with m-Temp (°C).

KIM OD 48-0



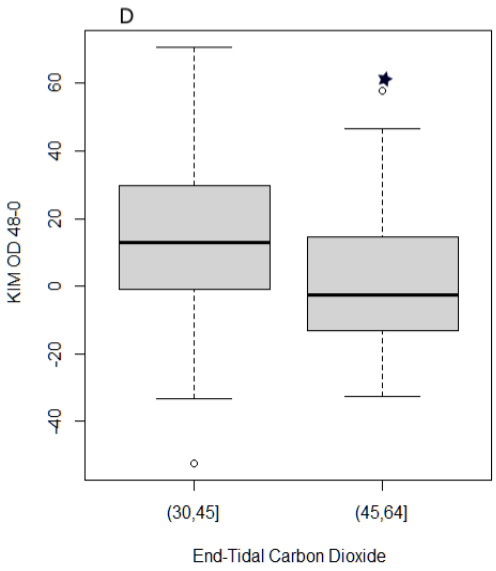


Figure A, B, C and D Mean difference in KIM-1 OD 48-0 with A) l-DAP (mmHg), B) l-SpO_2 (%), C) age (m), and D) $ETCO_2$ (mmHg).

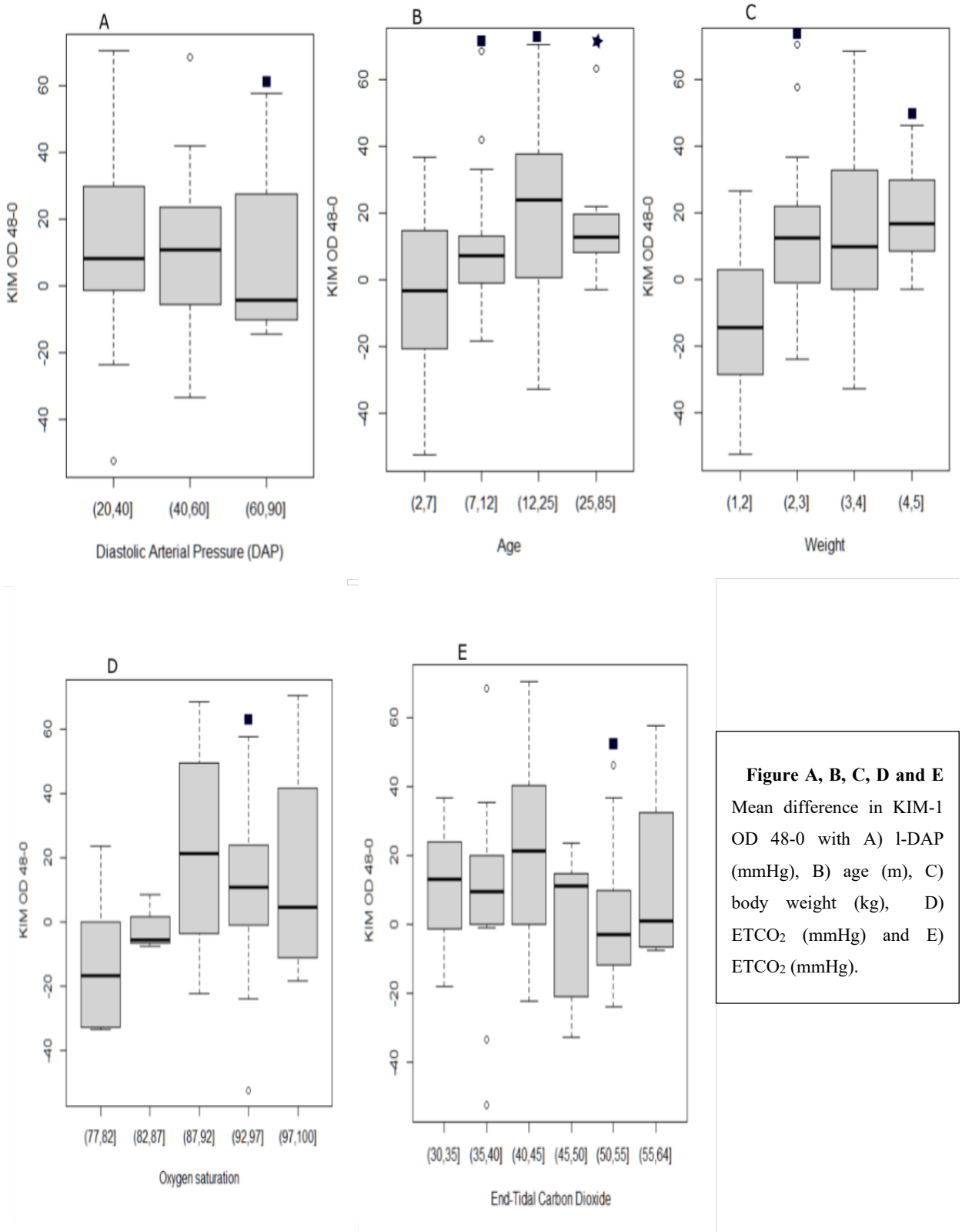


Figure A, B, C, D and E Mean difference in KIM-1 OD 48-0 with A) l-DAP (mmHg), B) age (m), C) body weight (kg), D) $ETCO_2$ (mmHg) and E) $ETCO_2$ (mmHg).

KIM OD 48-24

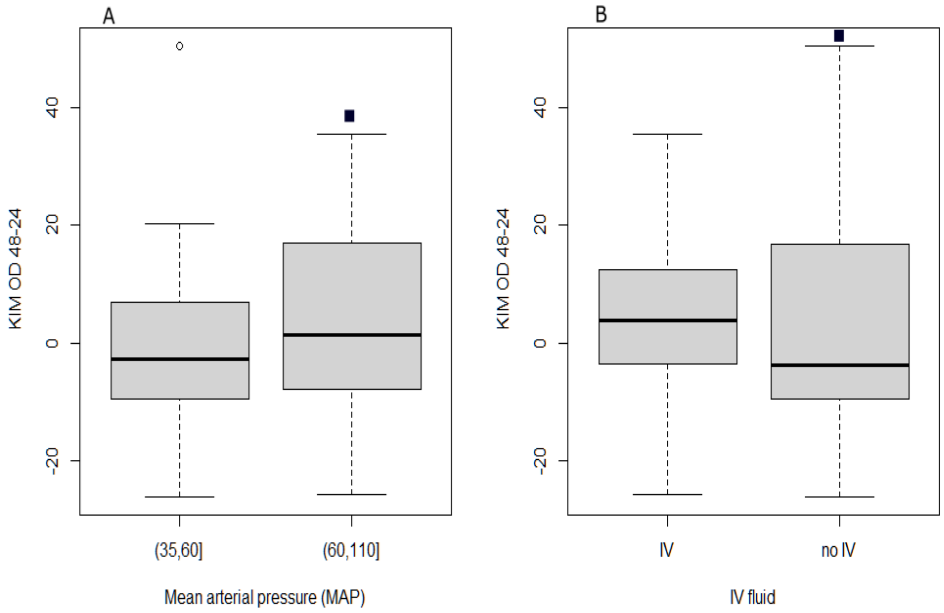


Figure A, and B Mean difference in KIM-1 OD 48-24 with A) l-MAP (mmHg), and B) fluid administration.

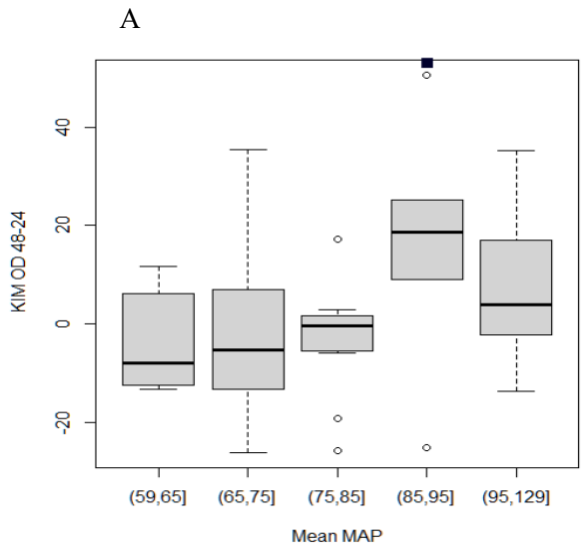


Figure A Mean difference in KIM-1 OD 48-24 with m-MAP (mmHg).

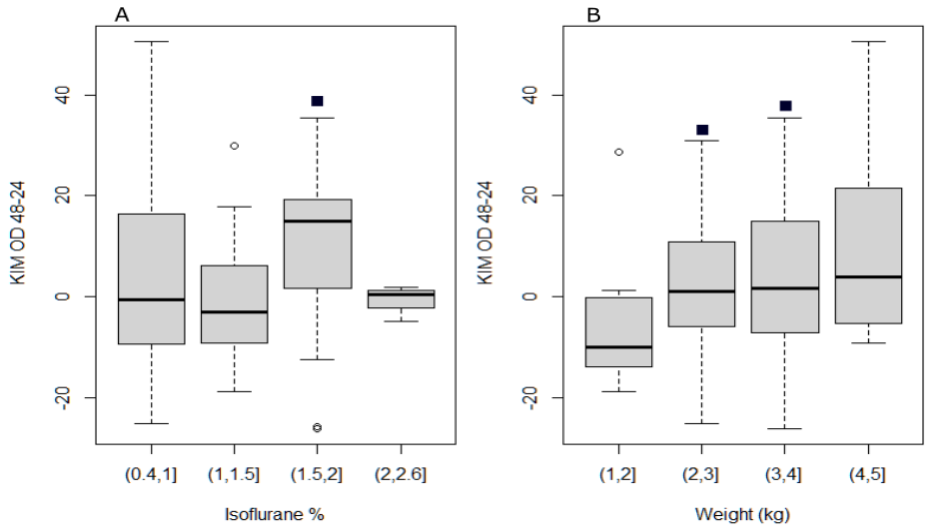


Figure A, and B Mean difference in KIM-1 OD 48-24 with A) maximum isoflurane (%), and B) body weight (kg).

KIM RATIO 24-0

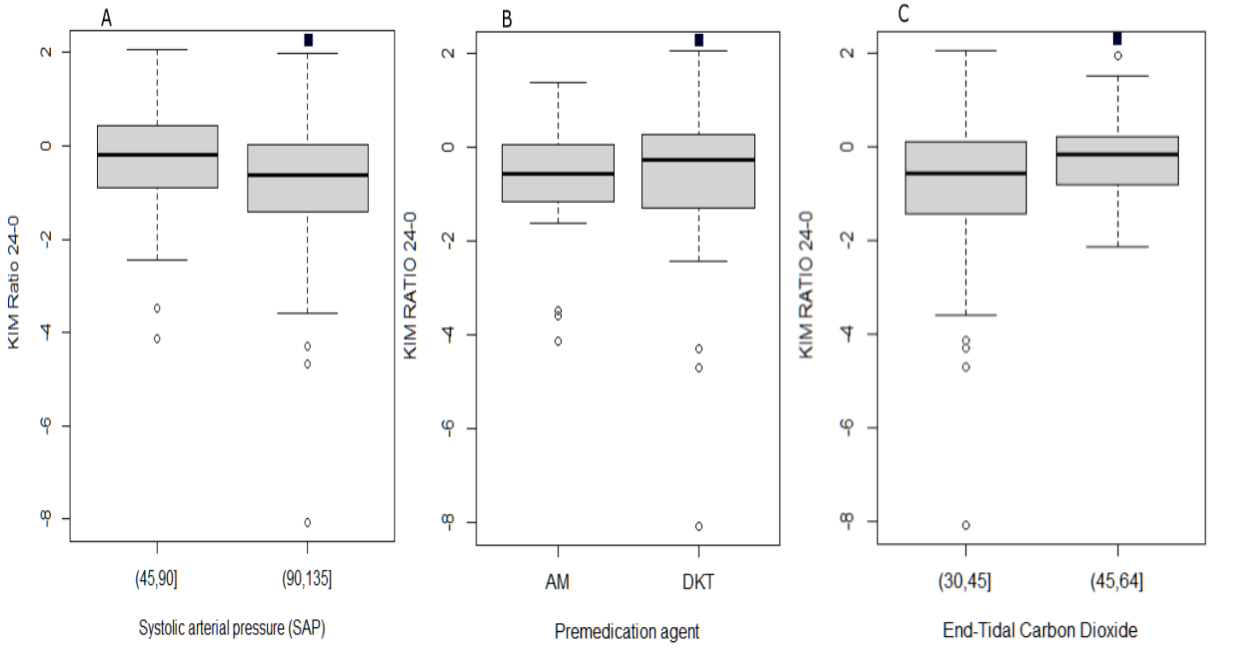


Figure A, B and C Mean difference in KIM-1 ratio 24-0 with A) l-SAP (mmHg), B) premedication agent and C) $ETCO_2$.

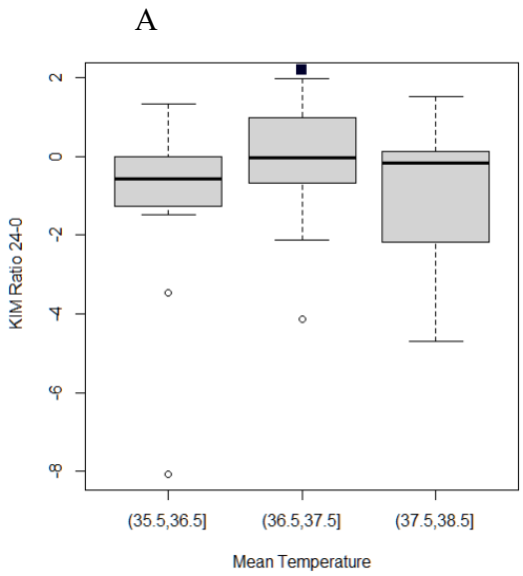
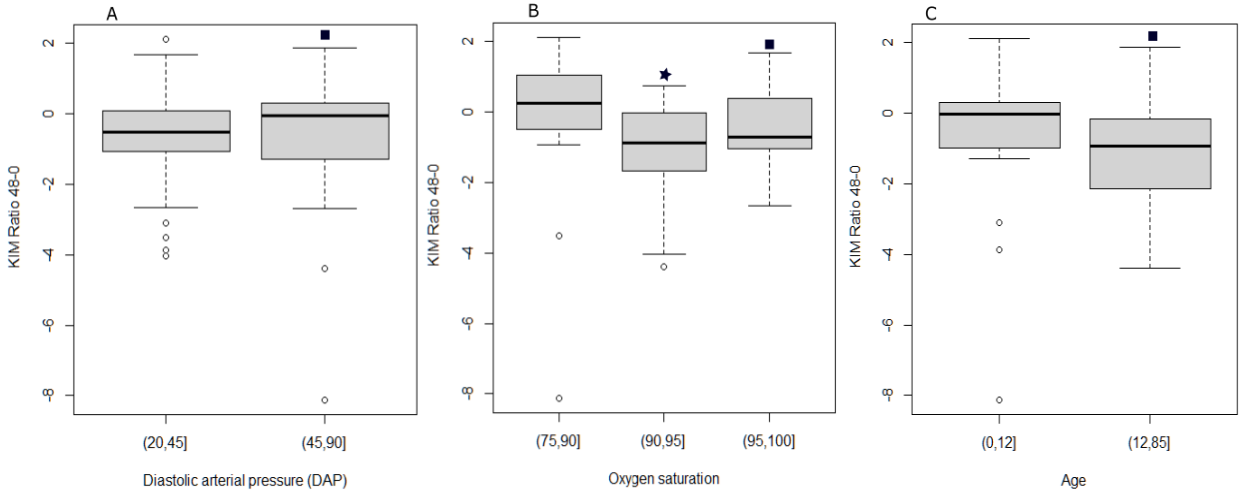


Figure A Mean difference in KIM-1 ratio 24-0 with m-temp (°C).

KIM RATIO 48-0



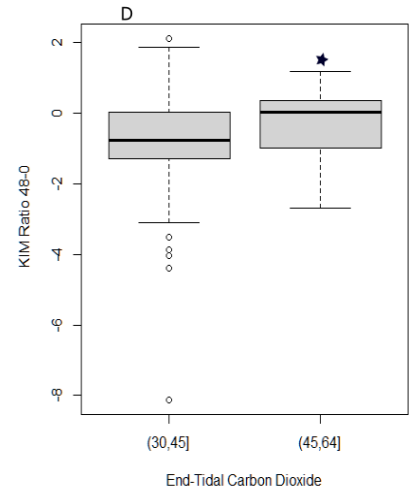


Figure A, B, C, and D Mean difference in KIM-1 ratio 48-0 with A) l-DAP (mmHg), B) SpO_2 (%), C) age (m), and D) $ETCO_2$ (mmHg).

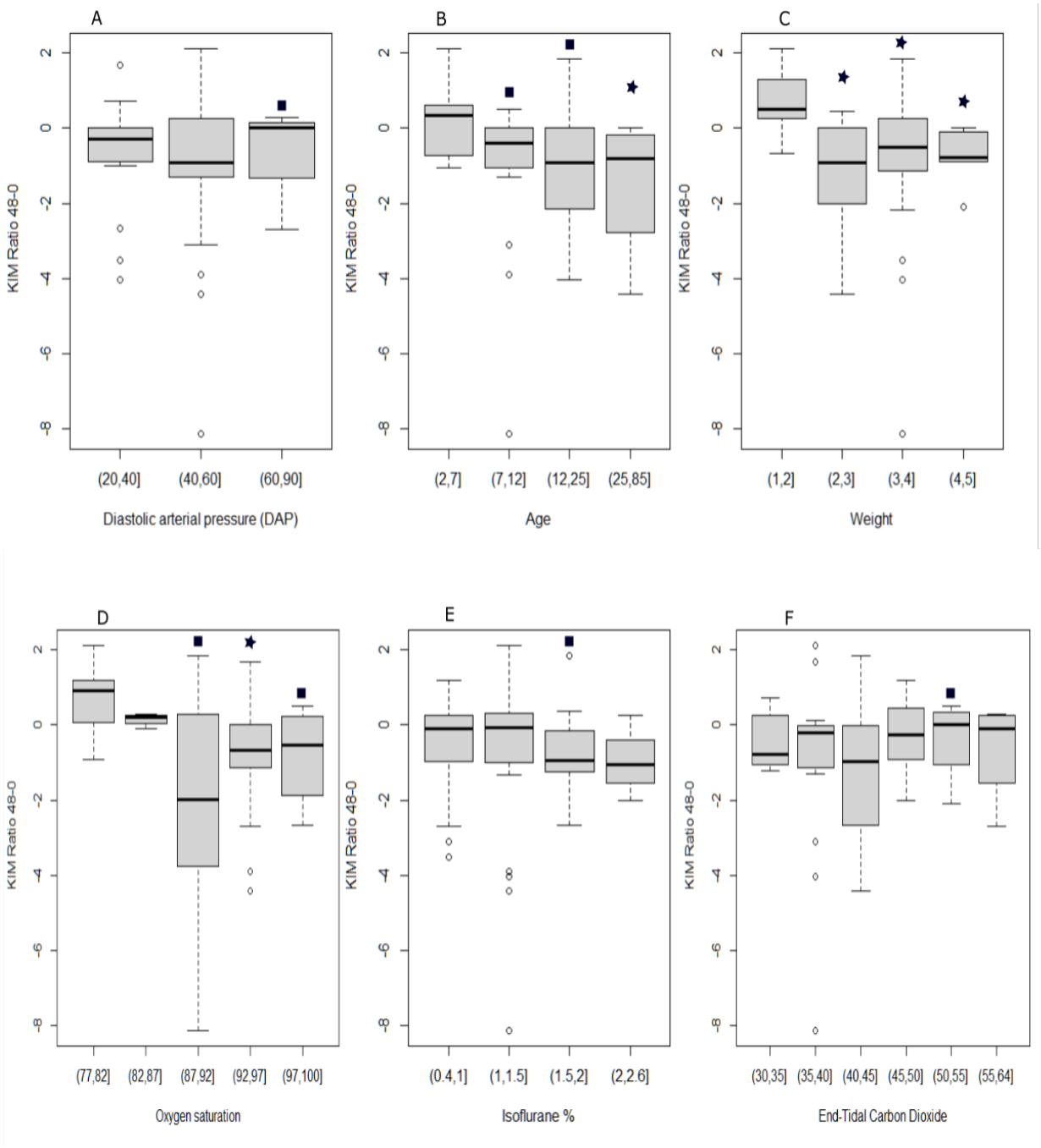


Figure A, B, C, D, E and F Mean difference in KIM-1 ratio 48-0 with A) l-DAP (mmHg), B) age (m), C) body weight (kg), D) l-SpO_2 (%), E) isoflurane (%) and ETCO2 (mmHg).

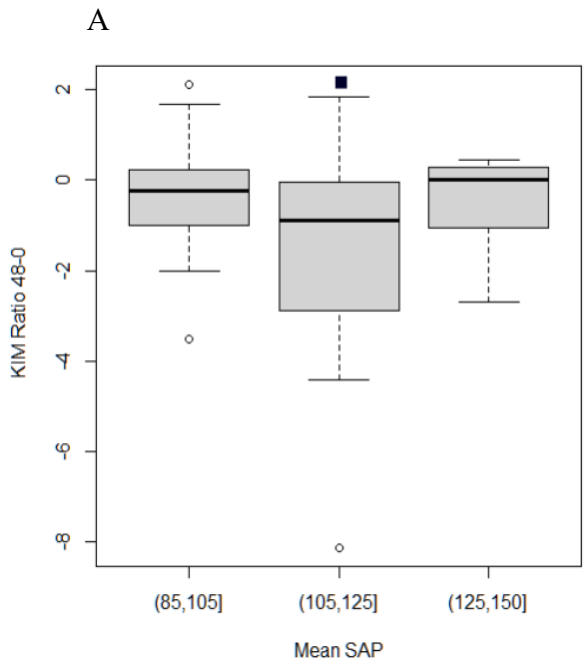


Figure A Mean difference in KIM-1 ratio 48-0 with m-SAP (mmHg).

KIM Ratio 48-24

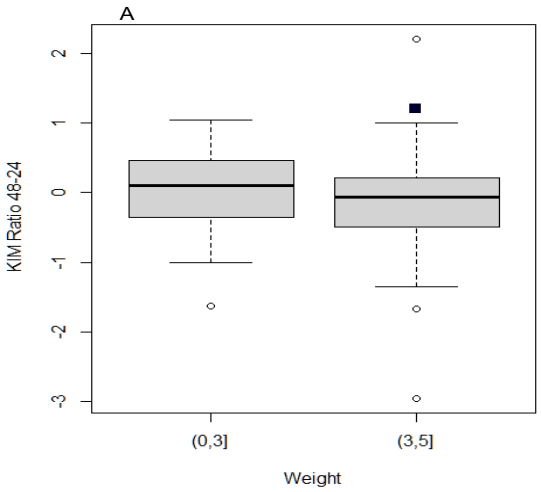


Figure A Mean difference in KIM-1 ratio 48-24 with body weight (kg).

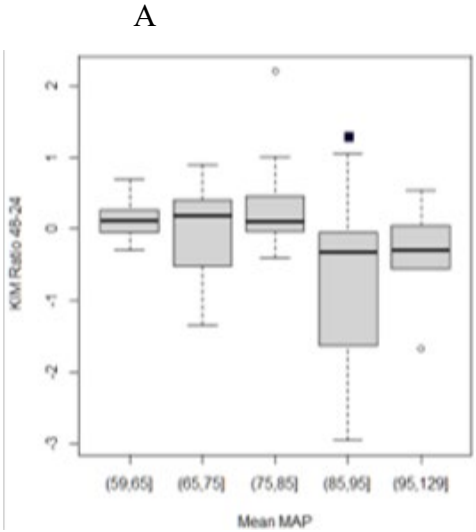


Figure A Mean difference in KIM-1 ratio 48-24 with m-MAP (mmHg).

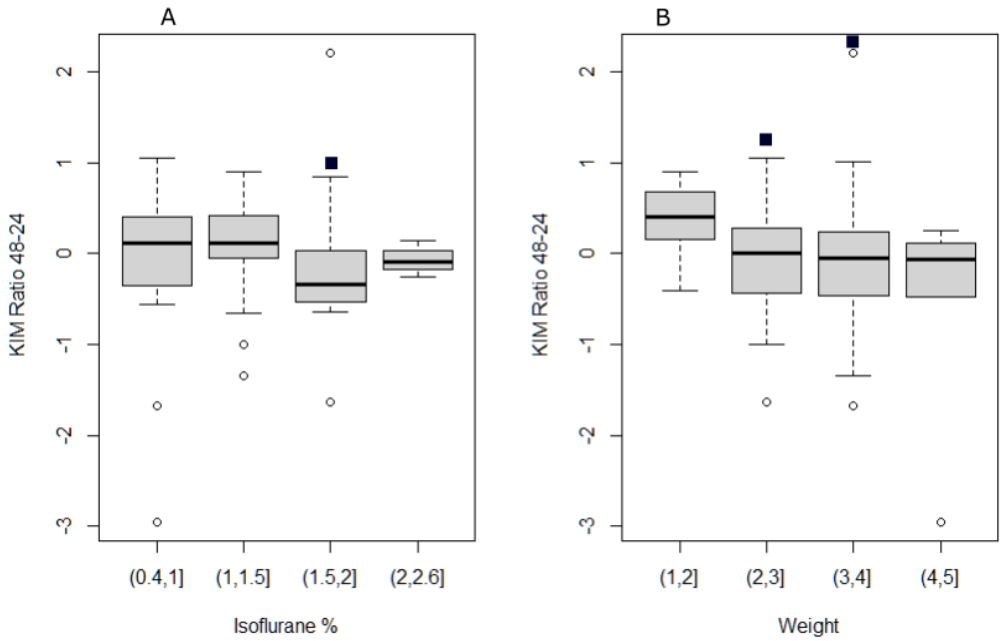


Figure A and B Mean difference in KIM-1 ratio 48-24 with A) isoflurane (%) and B) body weight (kg).

NAG activity 24-0

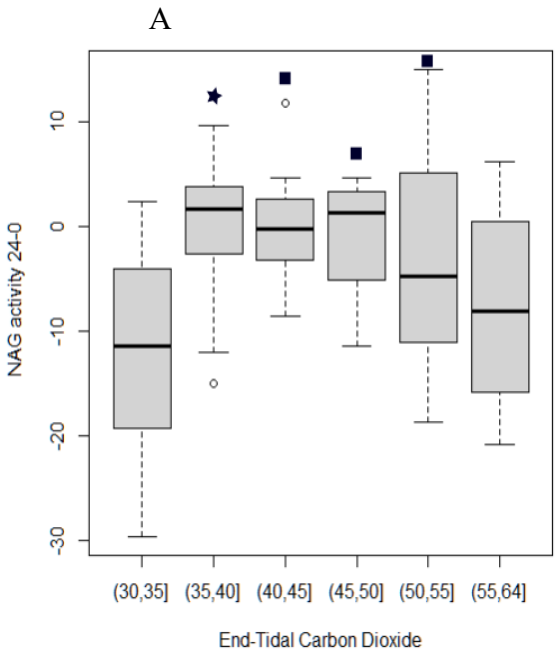


Figure A Mean difference in NAG activity 24-0 with $ETCO_2$ (mmHg).

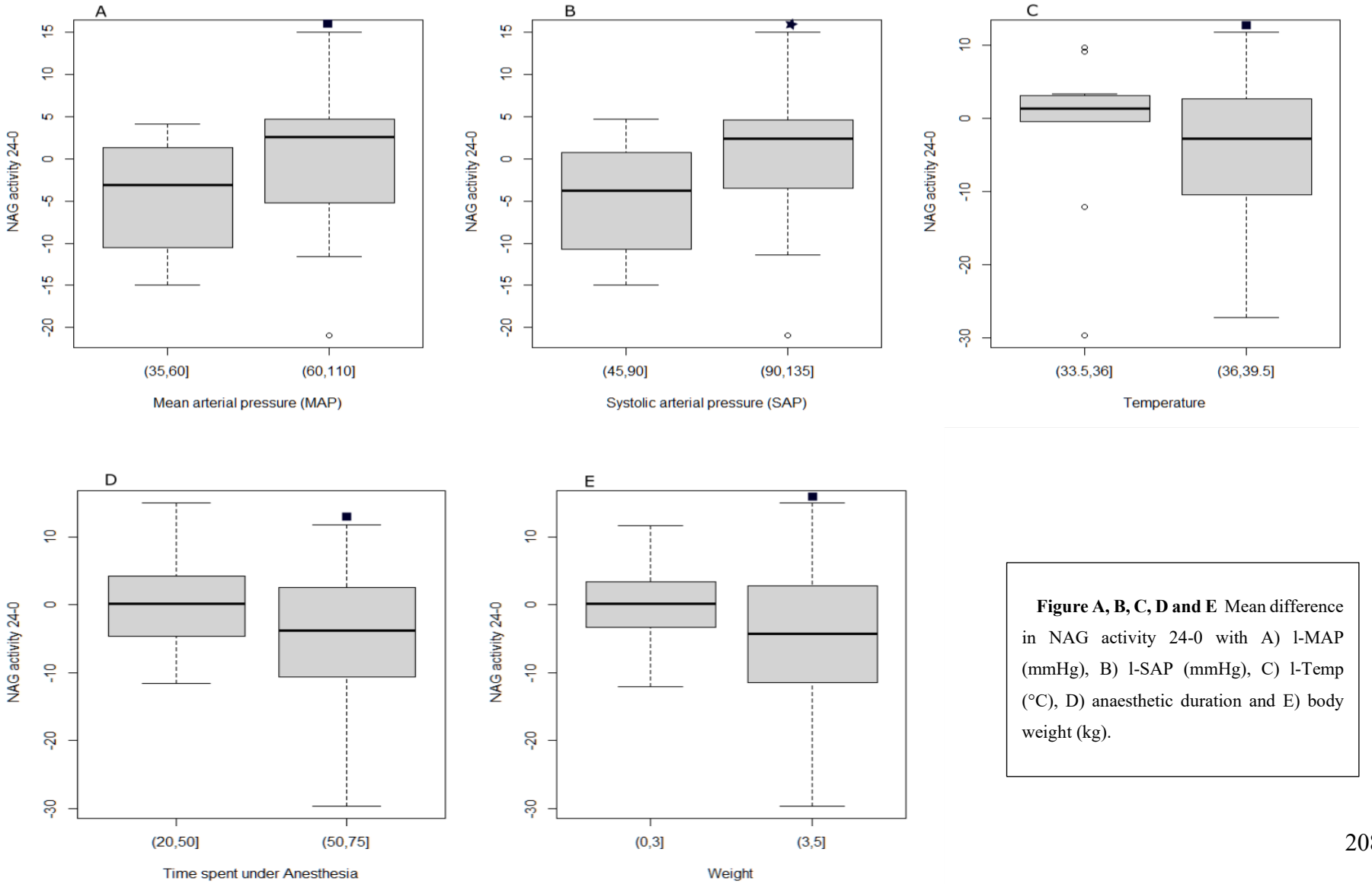


Figure A, B, C, D and E Mean difference in NAG activity 24-0 with A) l-MAP (mmHg), B) l-SAP (mmHg), C) l-Temp (°C), D) anaesthetic duration and E) body weight (kg).

NAG activity 48-0

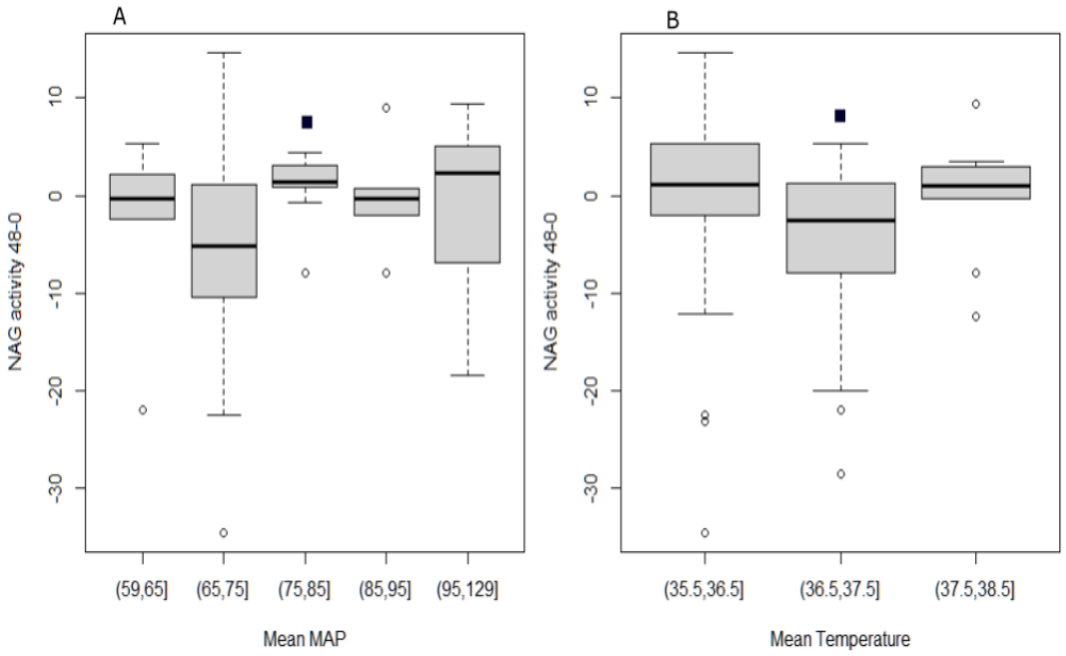


Figure A and B Mean difference in NAG activity 48-0 with m-MAP (mmHg) and B) m-Temp (°C).

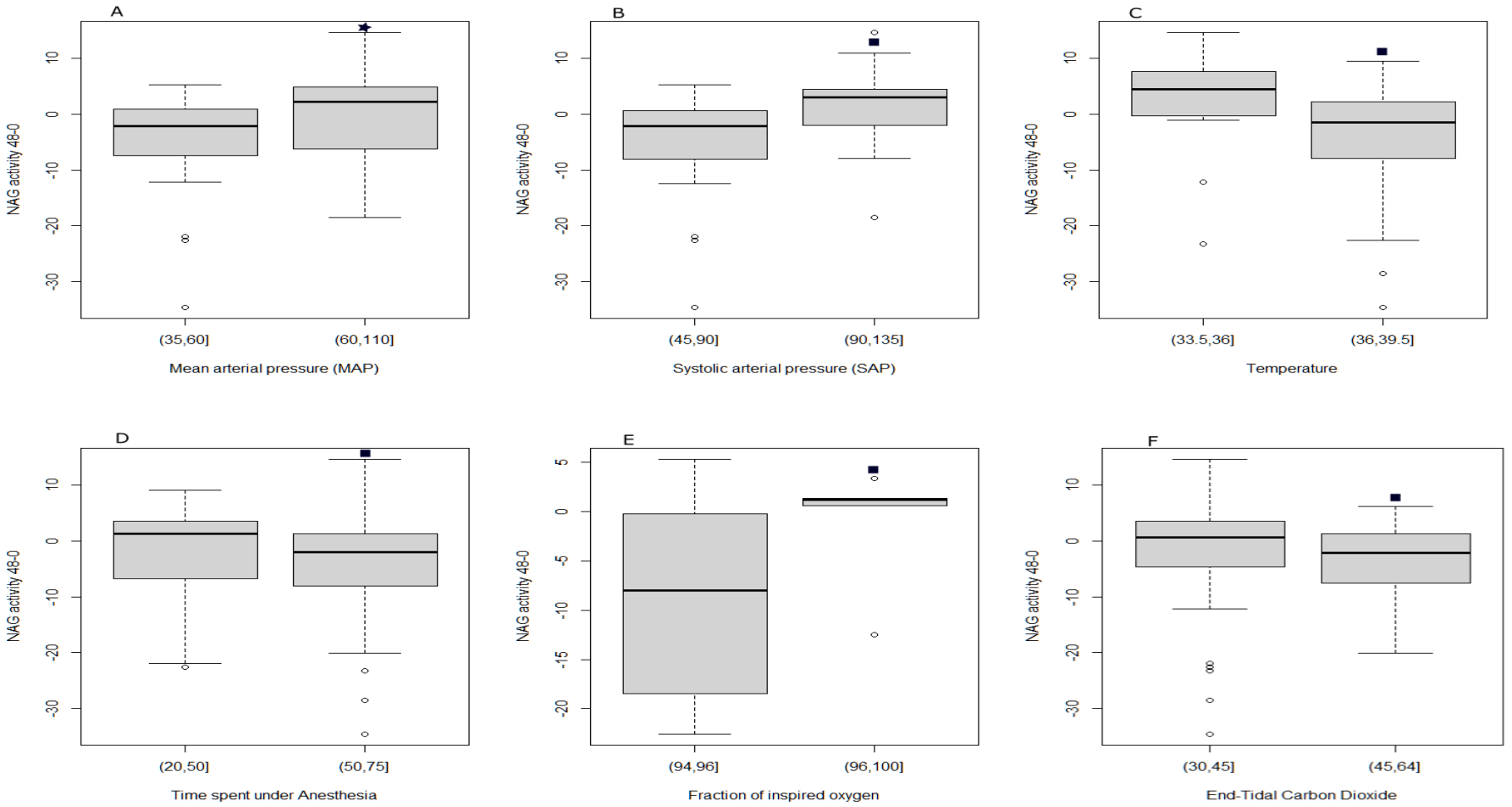


Figure A, B, C, D, E and F Mean difference in NAG activity 48-0 with A) l-MAP (mmHg), B) l-SAP (mmHg), C) l-Temp (°C), D) anaesthetic duration (mins), E) FiO_2 (%) and F) $ETCO_2$ (mmHg).

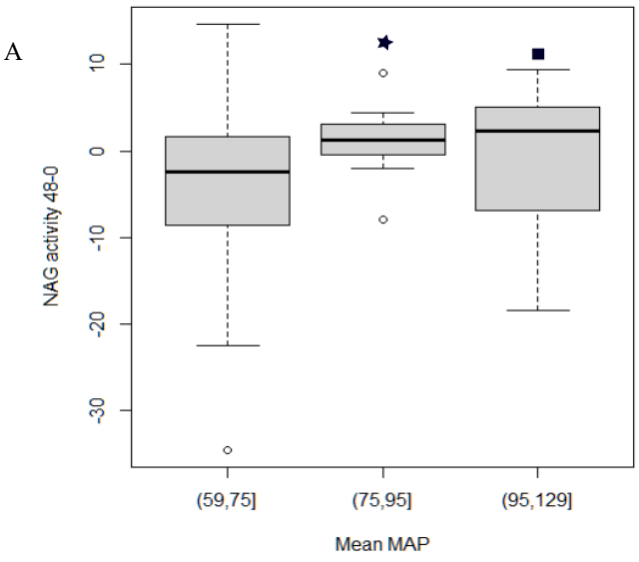


Figure A, B, C, D, E and F Mean difference in NAG activity 48-0 with m-MAP (mmHg).

NAG activity 48-24

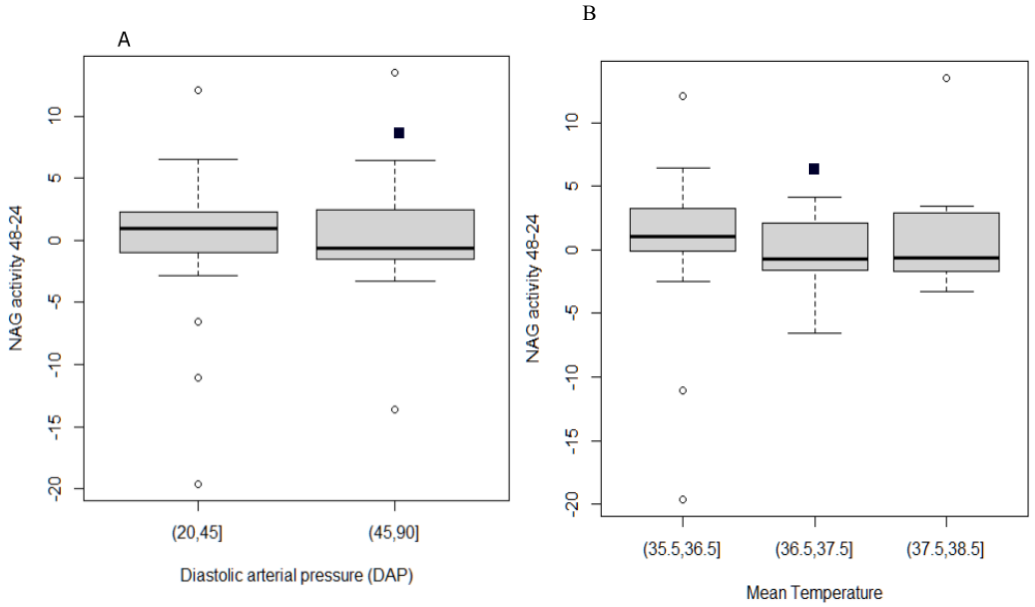


Figure A, and B Mean difference in NAG activity 48-24 with A) l-DAP (mmHg) and B) m-Temp (°C)

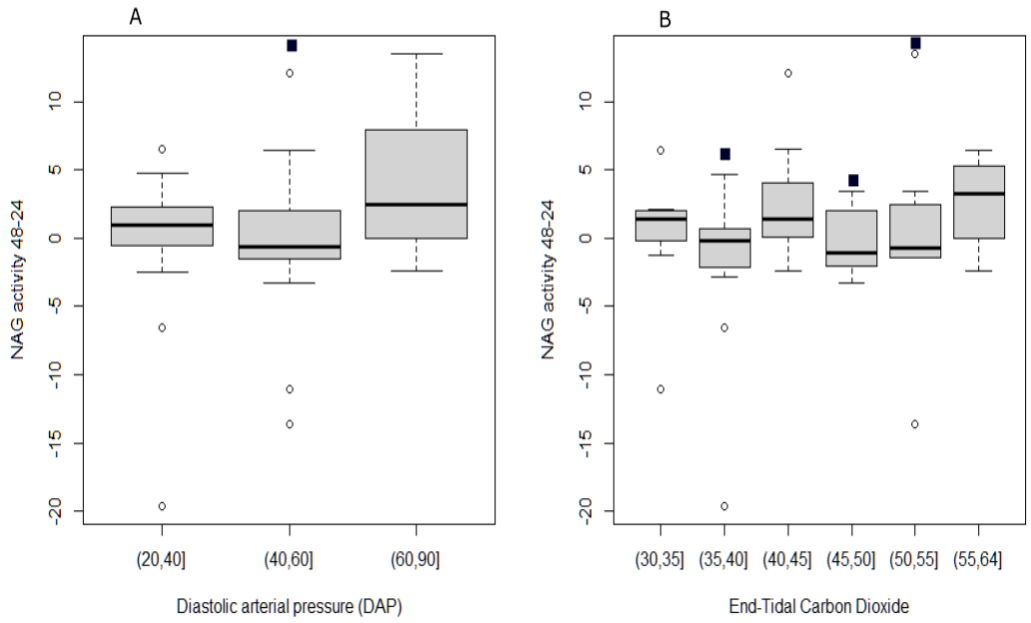


Figure A, and B Mean difference in NAG activity 48-24 with A) l-DAP (mmHg) and B) $ETCO_2$ (mmHg).

NAG index 24-0

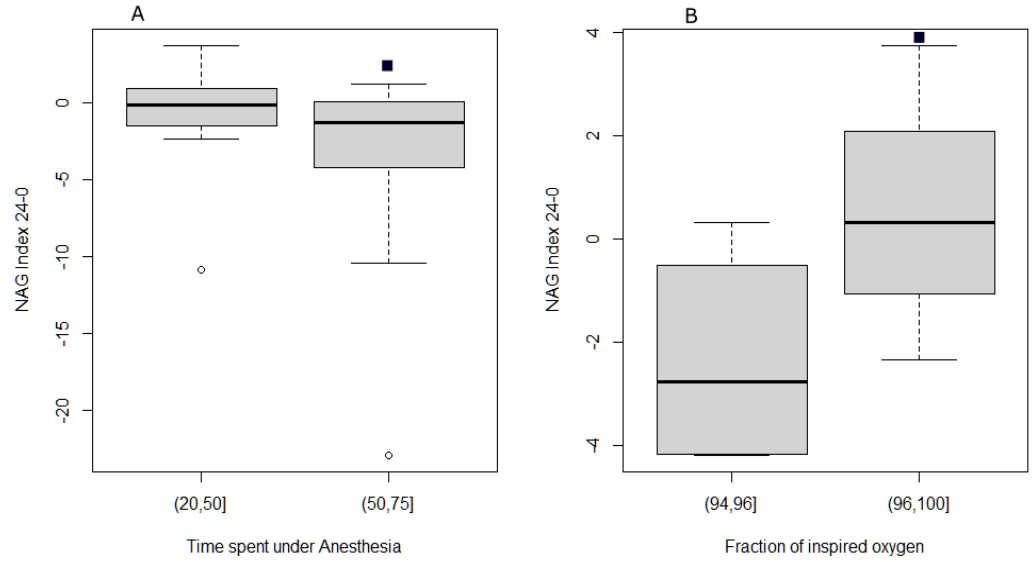


Figure A, and B Mean difference in NAG index 24-0 with A) anaesthetic duration (mins) and B) FiO_2 (%).

NAG Index 48-0

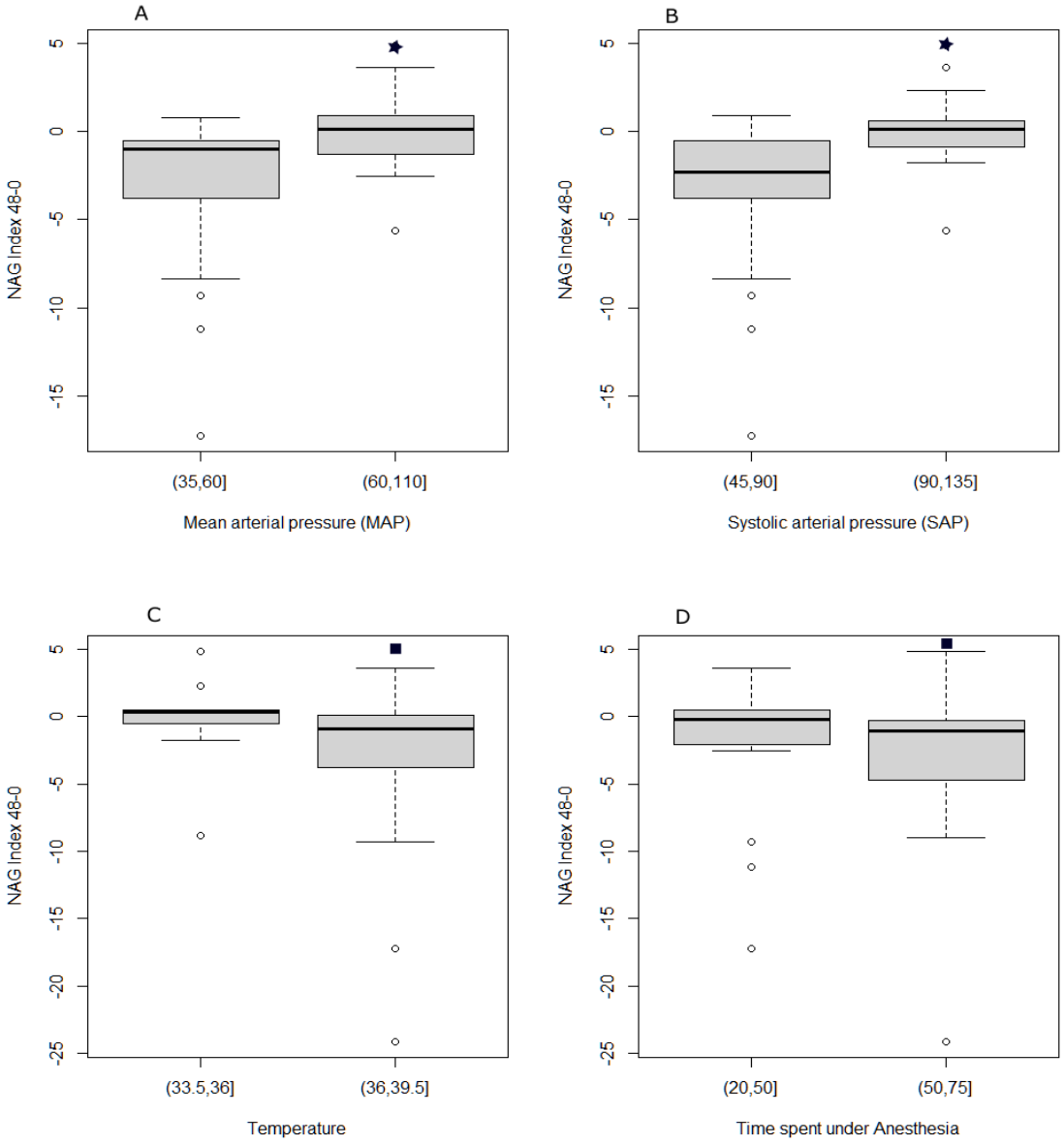


Figure A, and B Mean difference in NAG index 48-0 with A) l-MAP (mmHg), B) l-SAP (mmHg), C) l-temp (°C) and D) anaesthetic duration (mins).

NAG INDEX 48-24

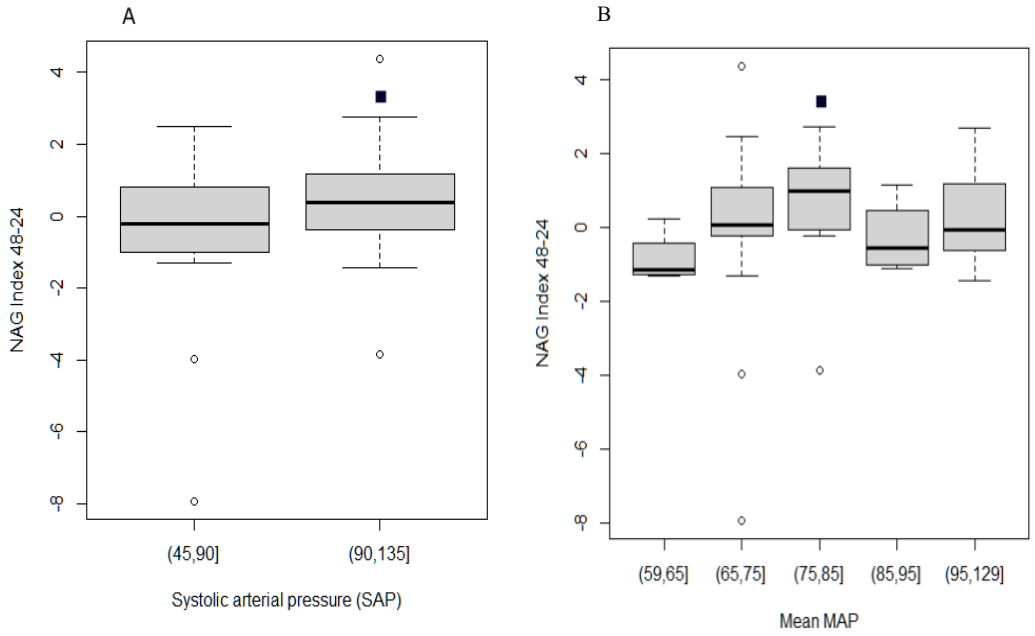


Figure A, and B Mean difference in NAG index 48-24 with A) l-SAP (mmHg), and B) m-MAP (mmHg).

NGAL 24-0

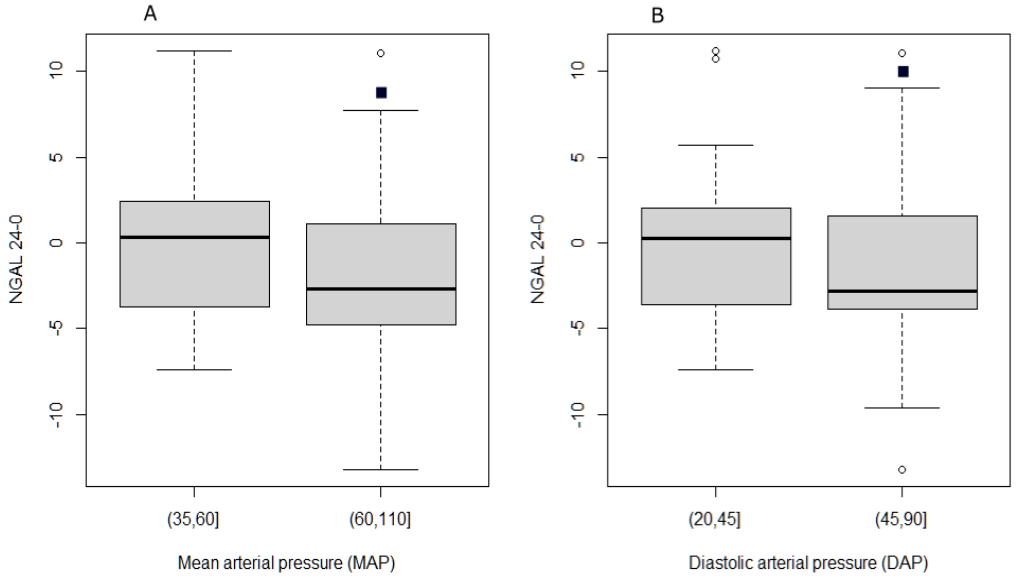


Figure A, and B Mean difference in NGAL 24-0 with A) l-MAP (mmHg), and B) l-DAP (mmHg).

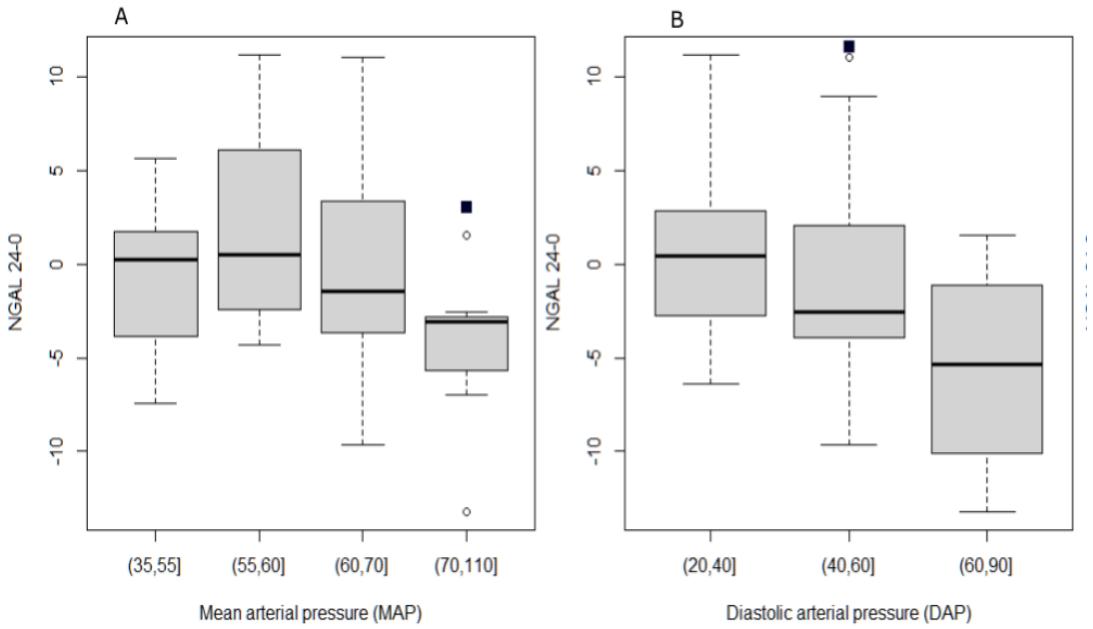


Figure A, and B Mean difference in NGAL 24-0 with A) l-MAP (mmHg), and B) l-DAP (mmHg).

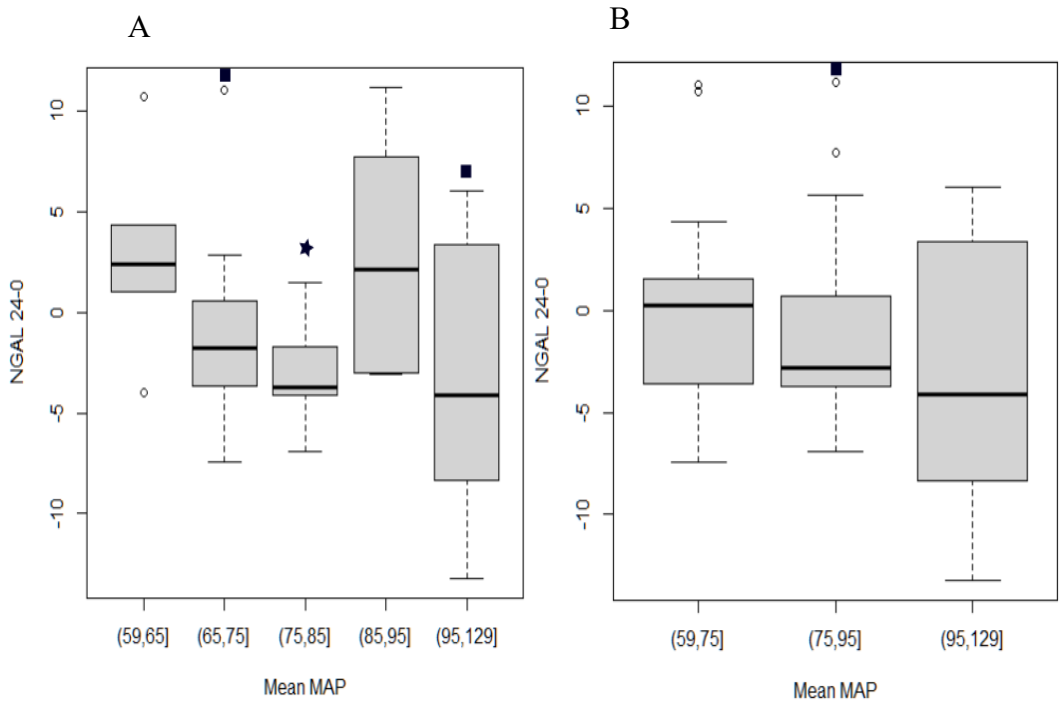


Figure A, and B Mean difference in NGAL 24-0 with A) l-MAP (mmHg), and B) l-DAP (mmHg).

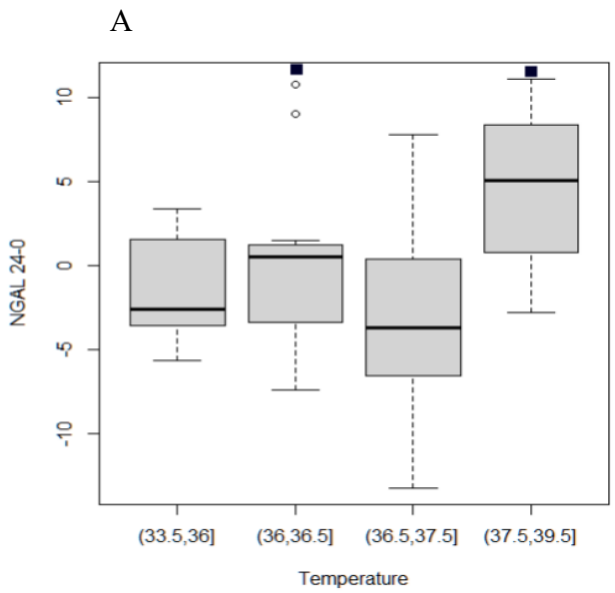


Figure A Mean difference in NGAL 24-0 with l-Temp (°C).

NGAL 48-0

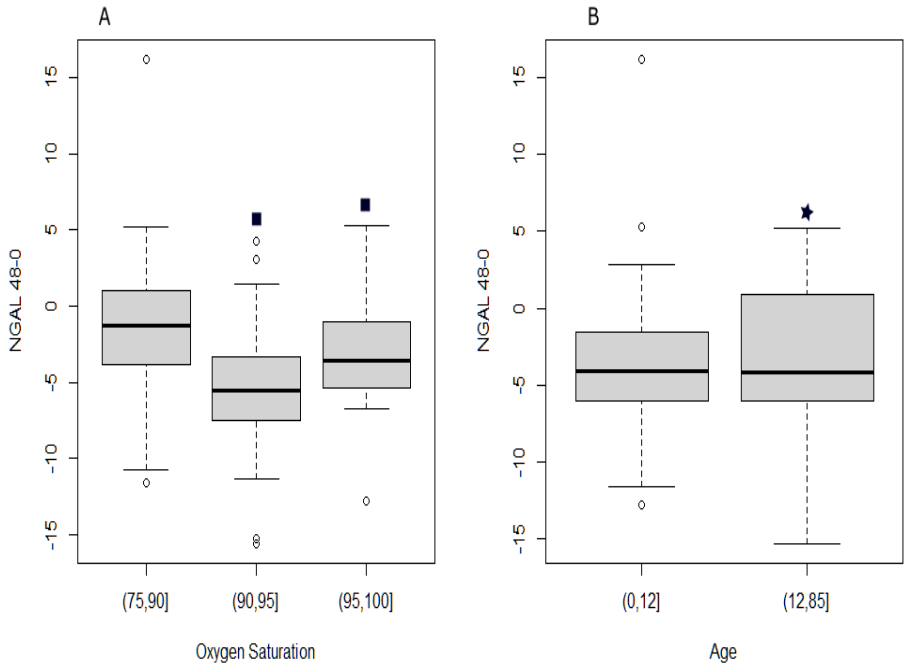


Figure A, and B Mean difference in NGAL 48-0 with A)SpO_2 (%), and B) age (m).

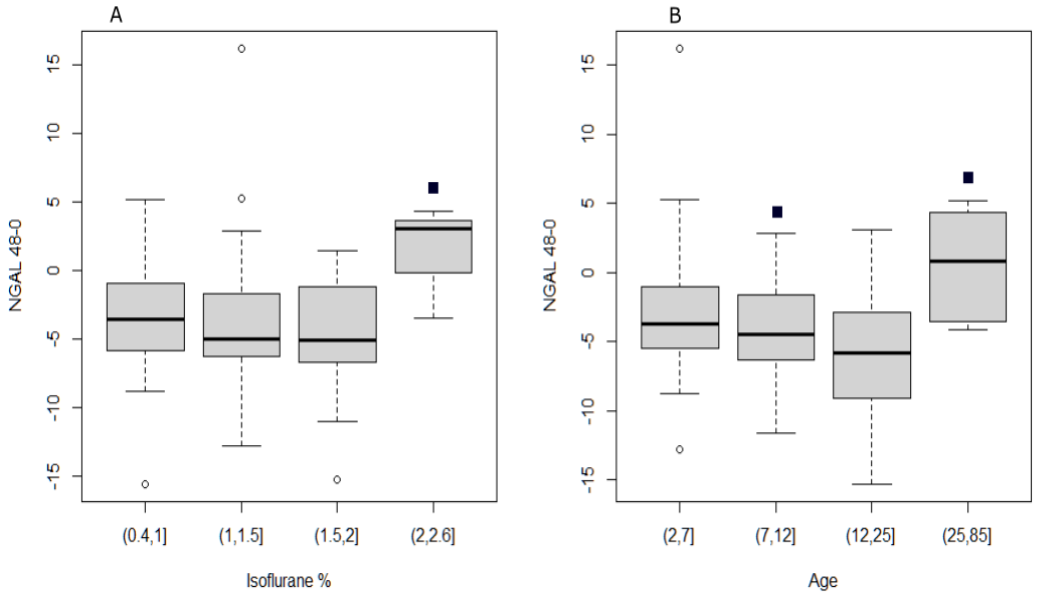


Figure A, and B Mean difference in NGAL 48-0 with A) isoflurane (%), and B) age (m).

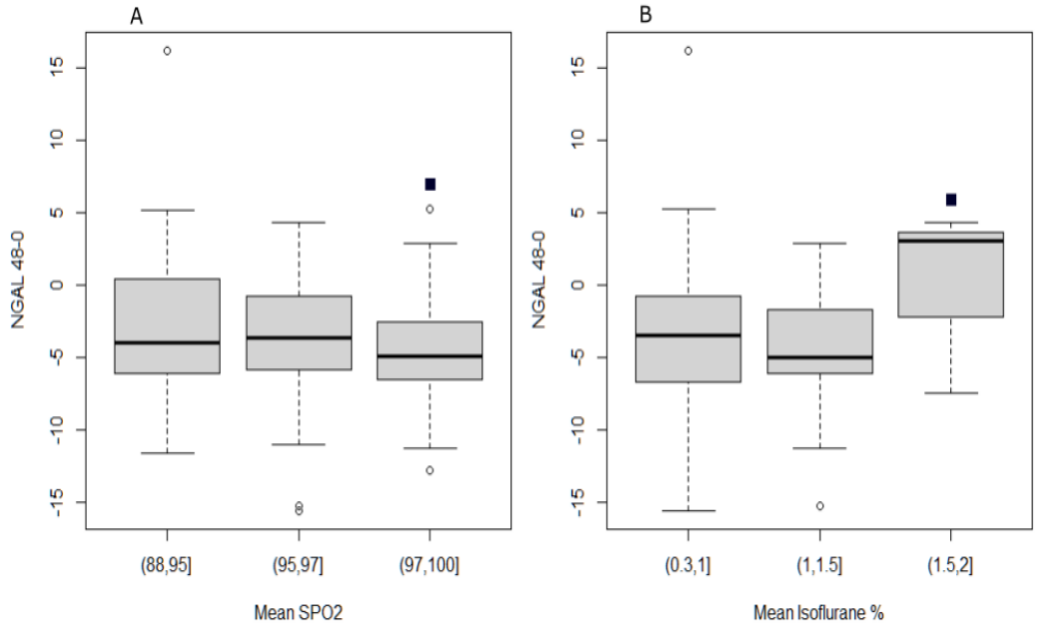


Figure A, and B Mean difference in NGAL 48-0 with A) mean SpO_2 (%), and B) m-isoflurane (%).

NGAL 48-24

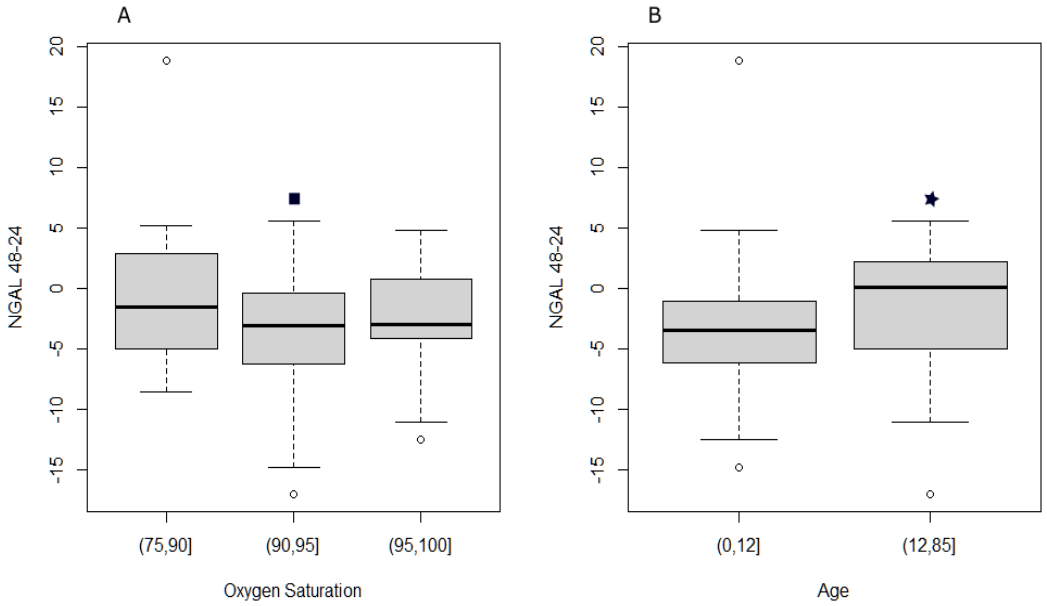


Figure A, and B Mean difference in NGAL 48-24 with A) SpO_2 (%), and B) age (m).

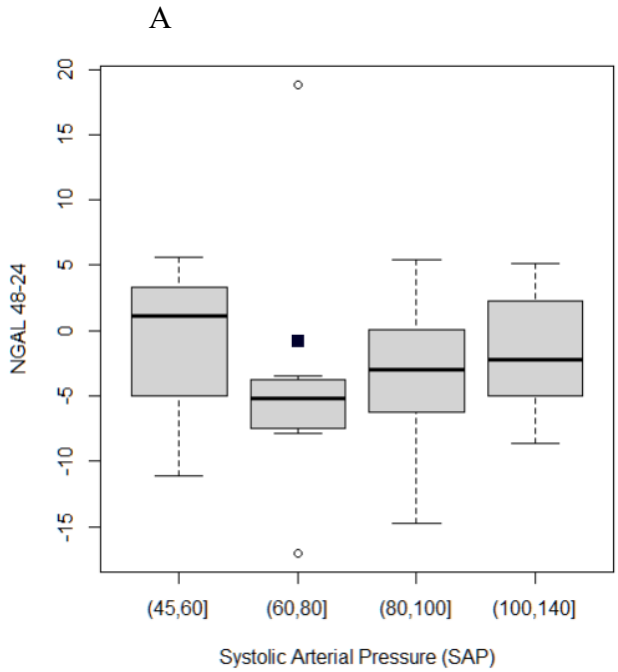


Figure A Mean difference in NGAL 48-24 with l-SAP (mmHg).

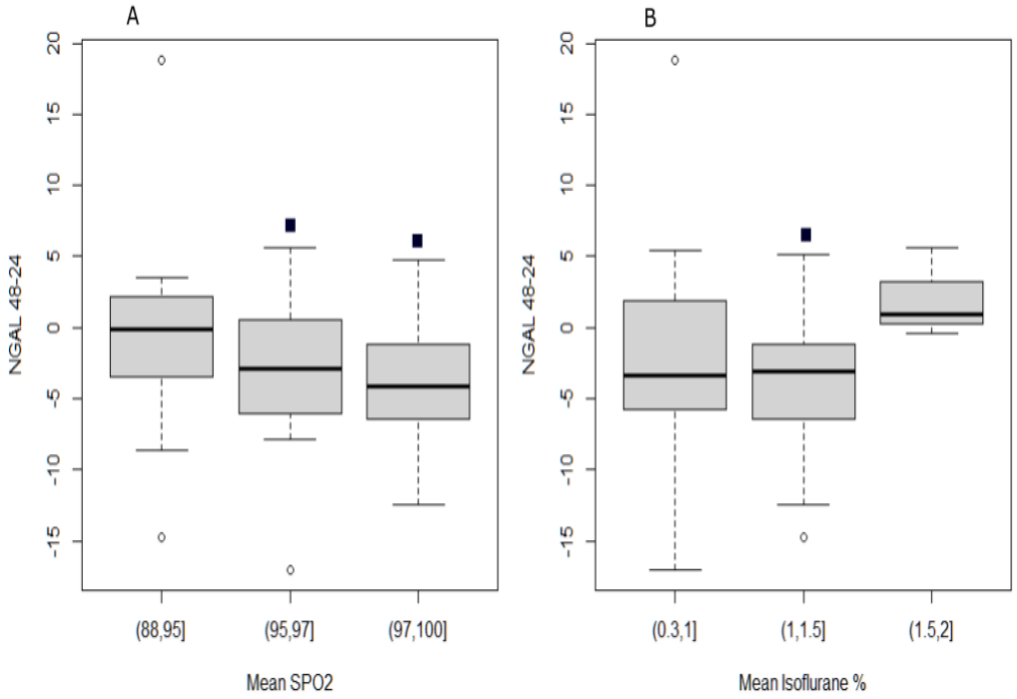


Figure A, and B Mean difference in NGAL 48-24 with A) m-SpO_2 (%), and B) m-isoflurane (%).

RBP 24-0

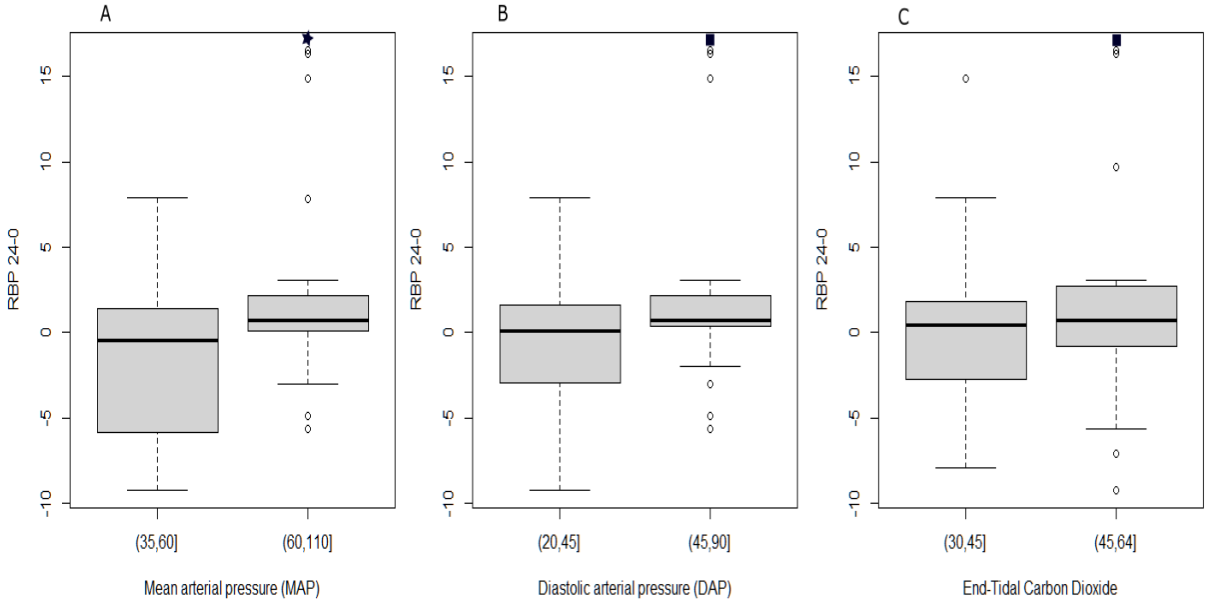
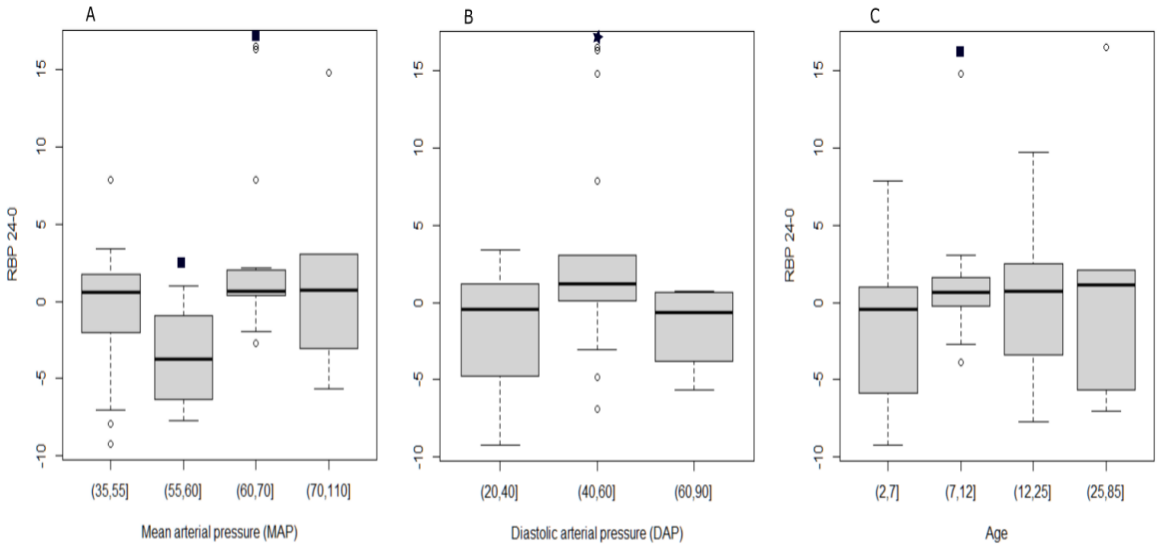


Figure A, B and C Mean difference in RBP 24-0 with A) l-MAP (mmHg), B) l-DAP (mmHg) and C) $ETCO_2$ (mmHg).



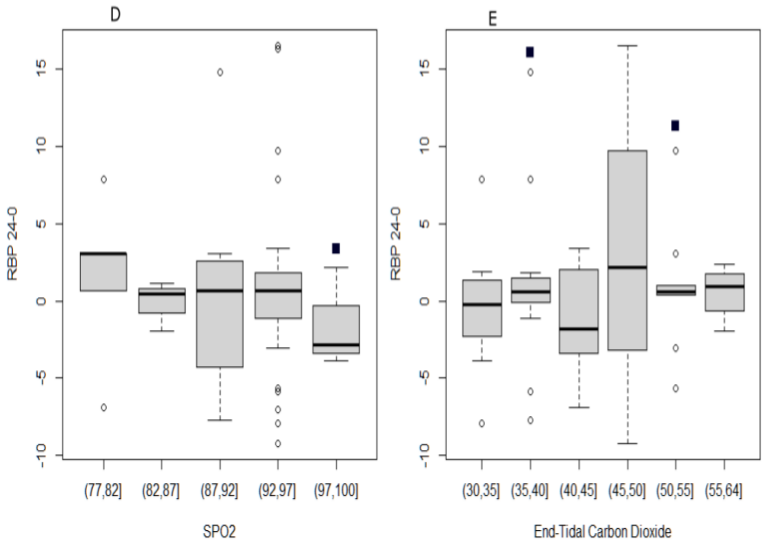


Figure A, B, C, D, and E Mean difference in RBP 24-0 with A) l-MAP (mmHg), B) l-DAP (mmHg), C) age (m), D) l-SpO_2 (%), and E) $ETCO_2$.

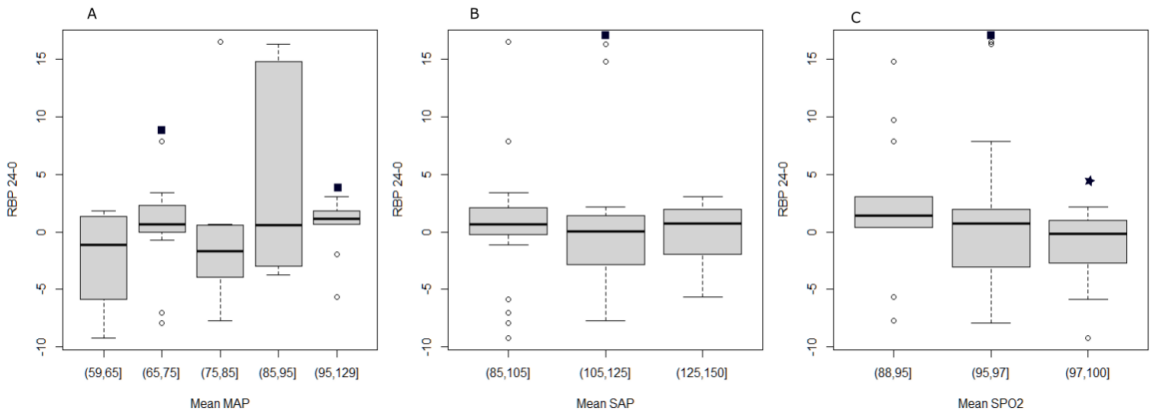


Figure A, B and C Mean difference in RBP 24-0 with A) m-MAP (mmHg), B) m-SAP (mmHg).

RBP 48-0

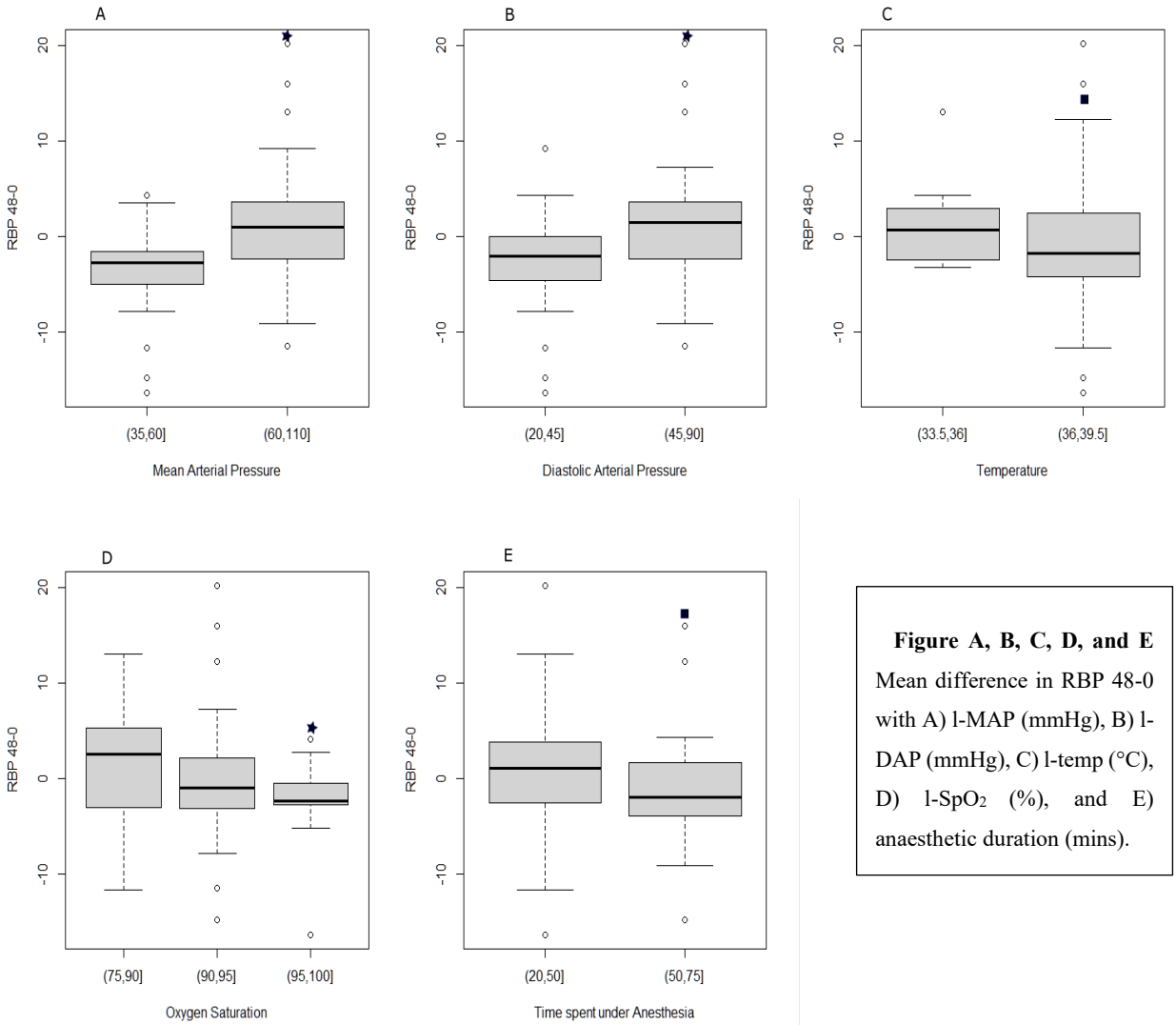


Figure A, B, C, D, and E Mean difference in RBP 48-0 with A) l-MAP (mmHg), B) l-DAP (mmHg), C) l-temp (°C), D) l-SpO_2 (%), and E) anaesthetic duration (mins).

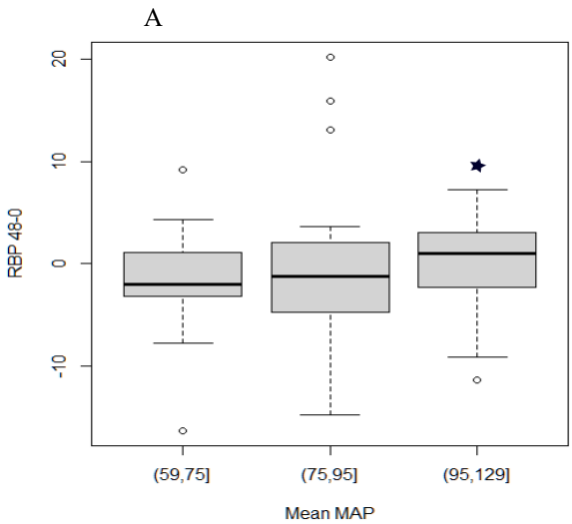
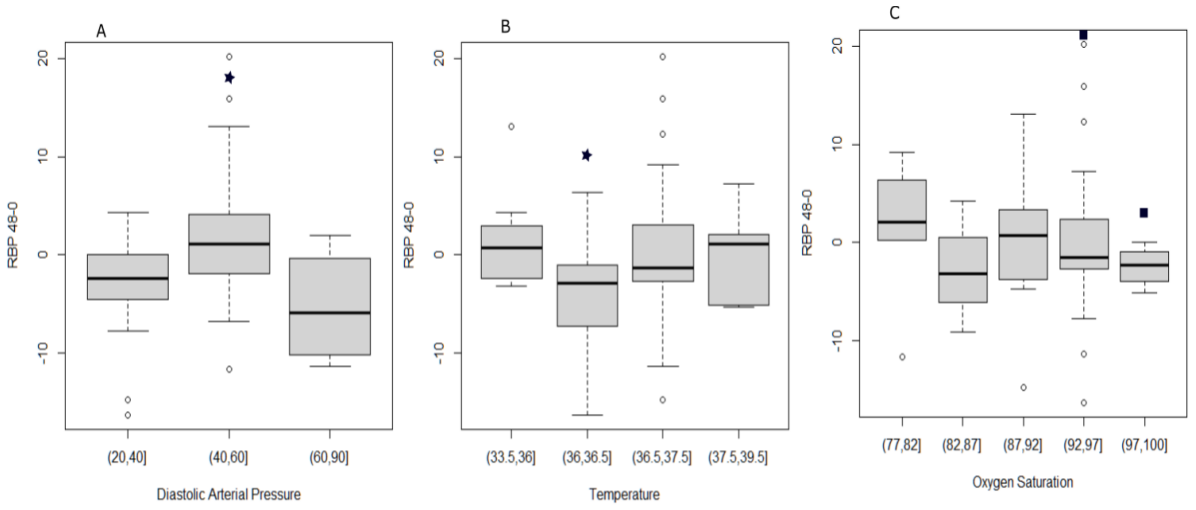


Figure A Mean difference in RBP 48-0 with m-MAP (mmHg).



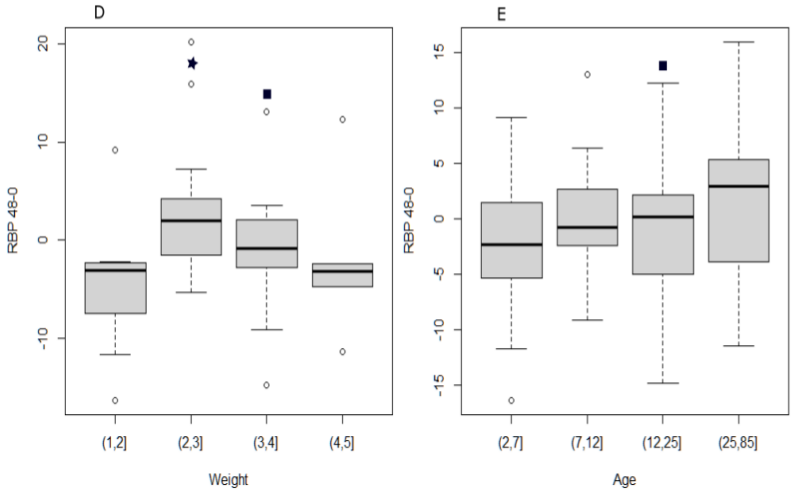


Figure A, B, C, D, and E Mean difference in RBP 48-0 with A) l-DAP (mmHg), B) l-temp (°C), C) l-SpO_2 (%), D) body weight (kg) and E) age (m).

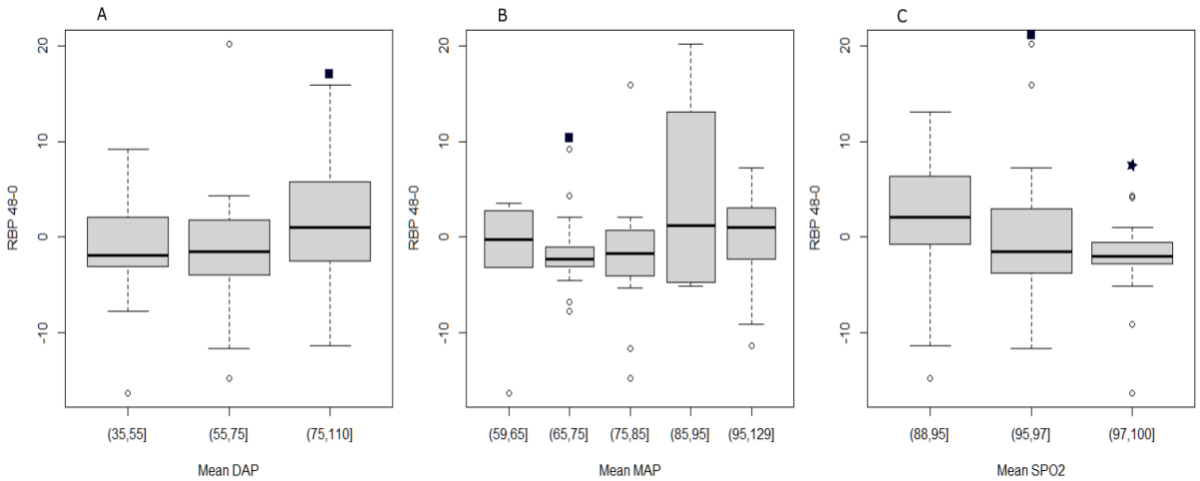


Figure A, B and C Mean difference in RBP 48-0 with A) m-DAP, B) m-MAP (mmHg) and C) m-SpO_2 (%).

RBP 48-24

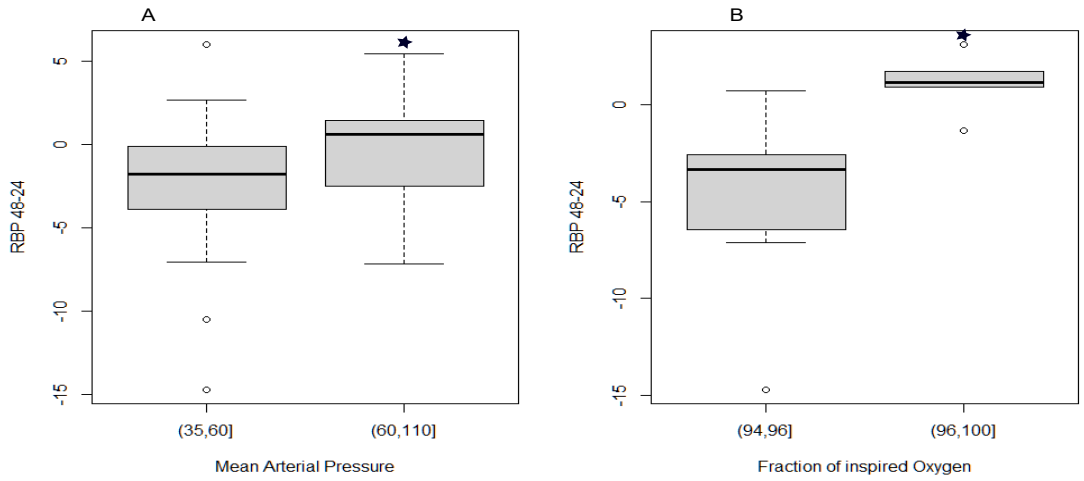


Figure A and B Mean difference in RBP 48-24 with A) l-MAP (mmHg) and B) FiO_2 (%).

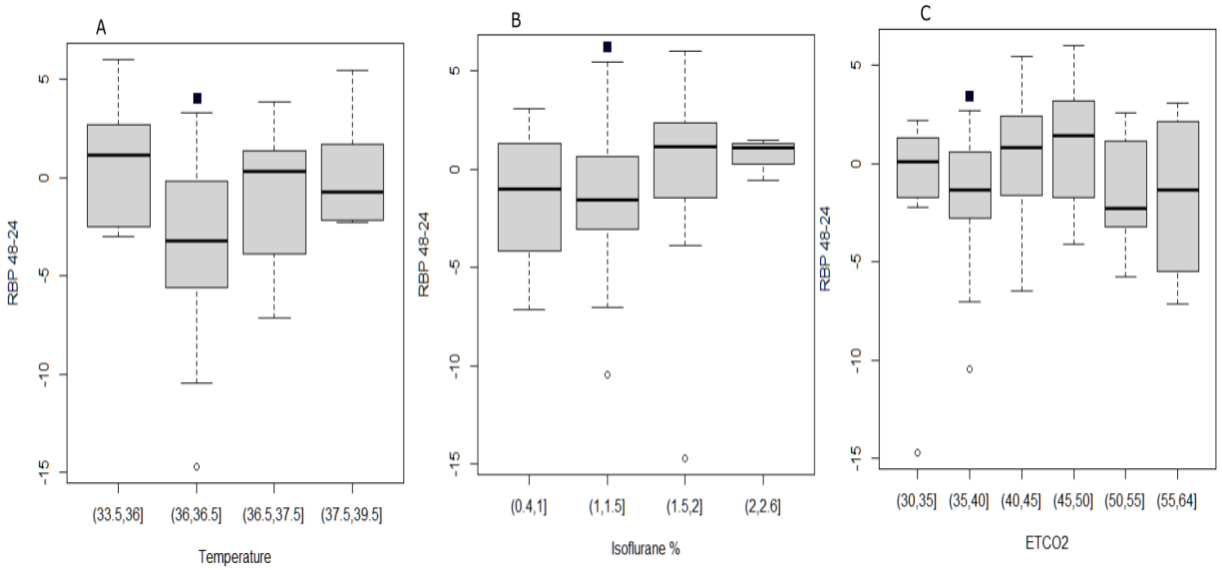


Figure A, B and C Mean difference in RBP 48-24 with A) l-Temp (°C), B) max isoflurane (%) and C) $ETCo_2$ (mmHg).

A

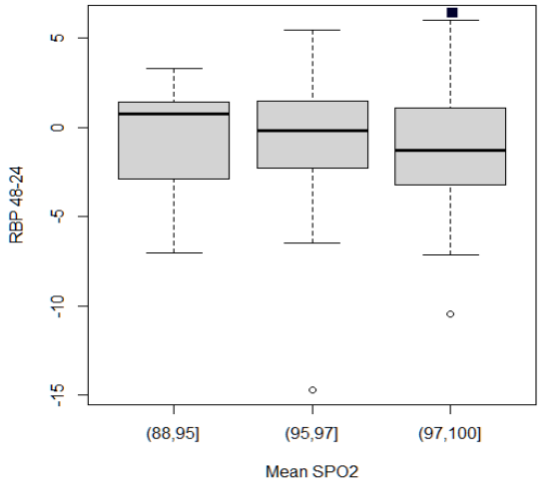


Figure A Mean difference in RBP 48-24 with m-SpO_2 (%).

SCr 24-0

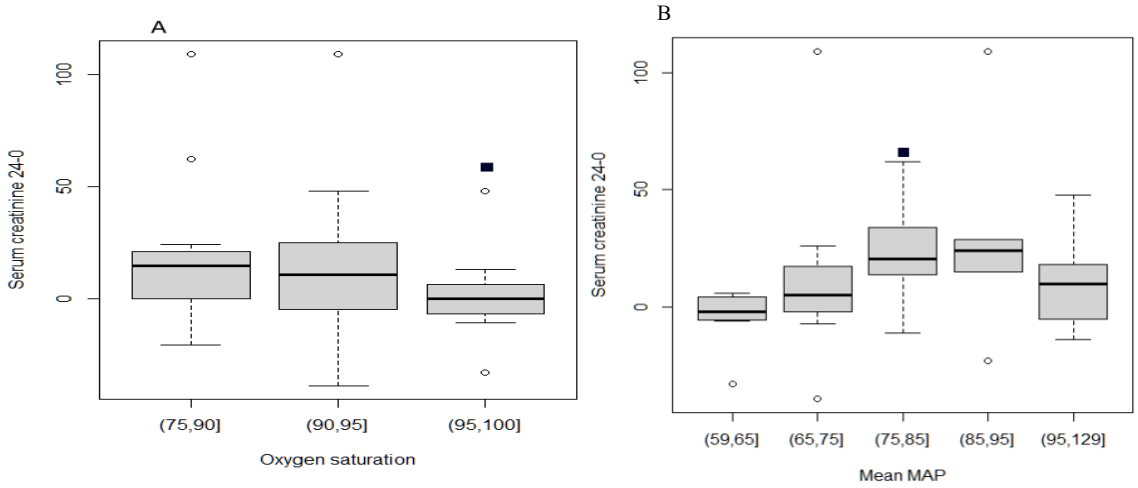


Figure A Mean difference in SCr 24-0 with A) l-SpO_2 (%) and B) m-MAP (mmHg).

A

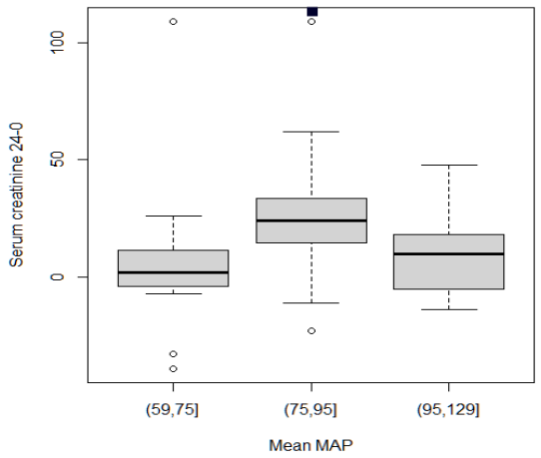


Figure A Mean difference in SCr 24-0 with m-MAP (mmHg).

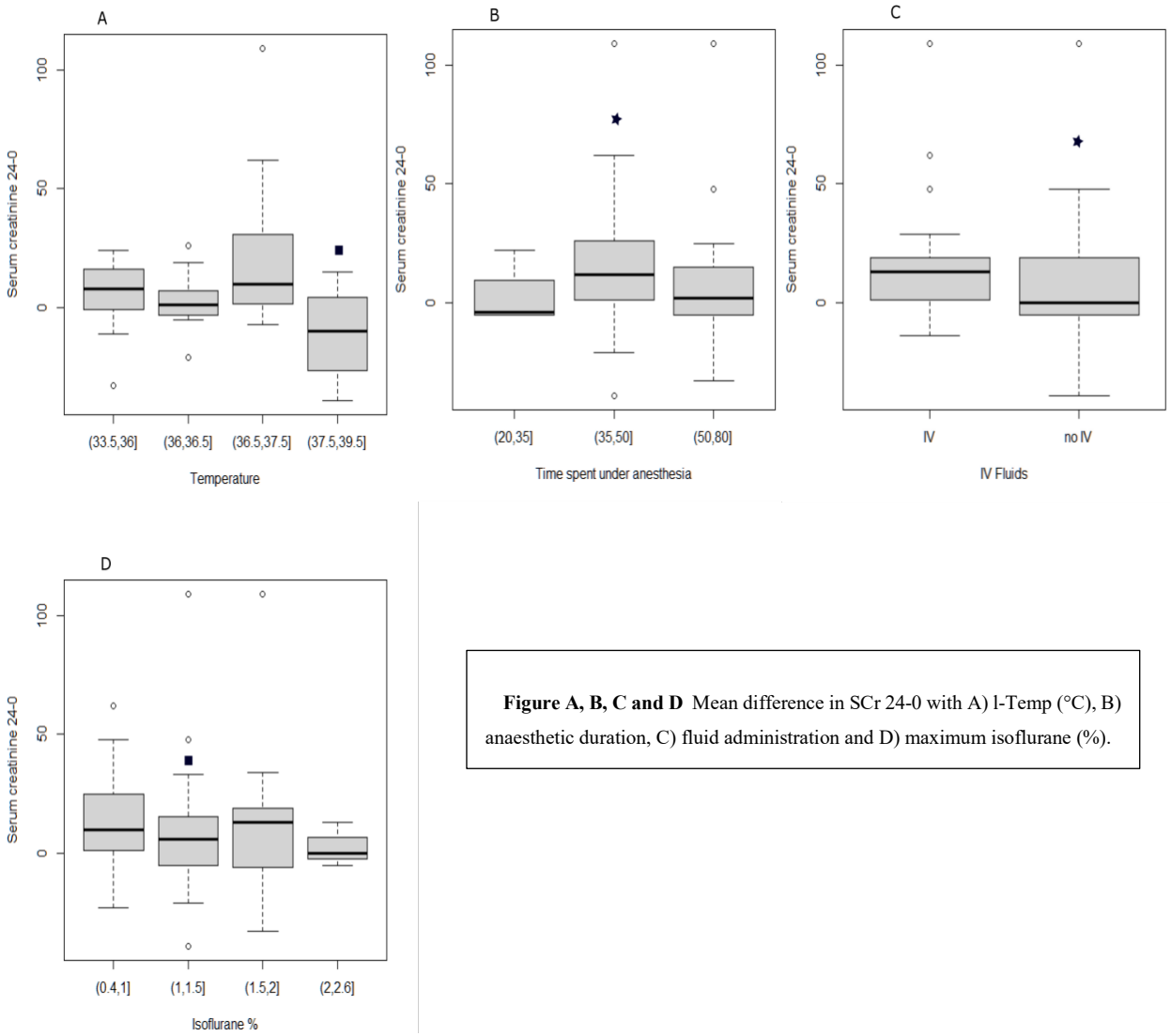


Figure A, B, C and D Mean difference in SCr 24-0 with A) l-Temp (°C), B) anaesthetic duration, C) fluid administration and D) maximum isoflurane (%).

SCr 48-0

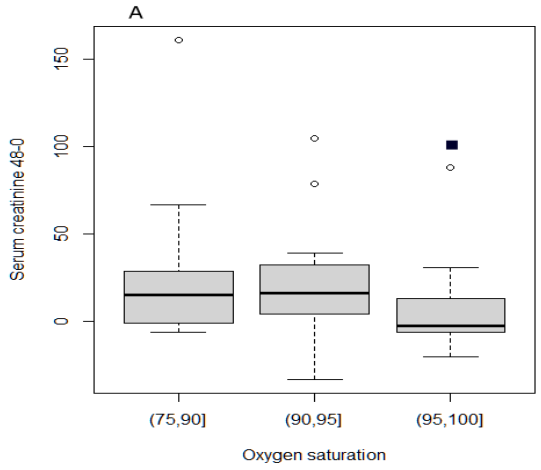


Figure A Mean difference in SCr 48-0 with l-SpO_2 (mmHg).

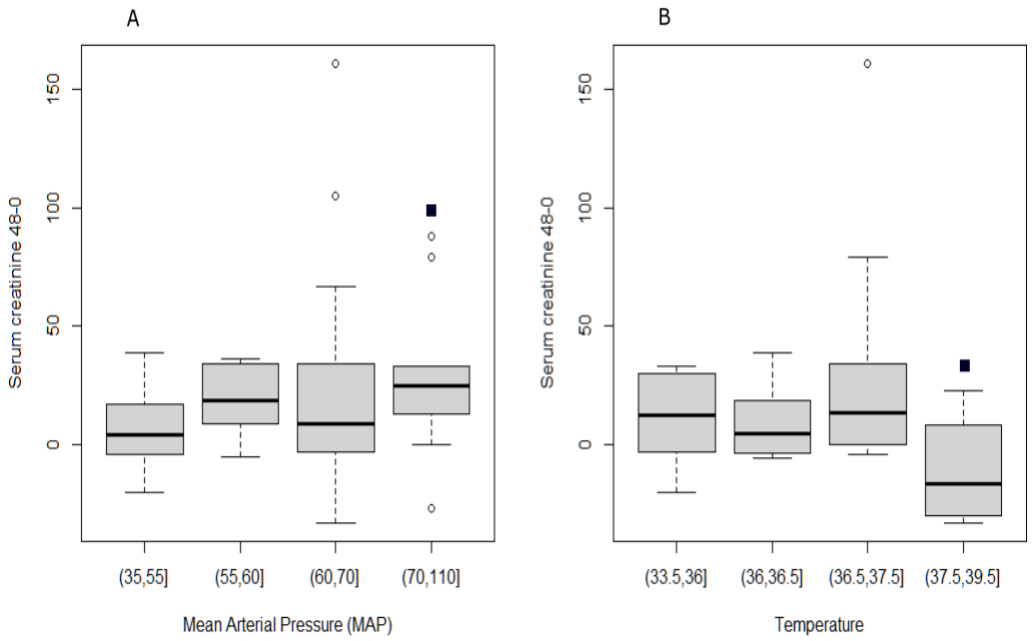


Figure A and B Mean difference in SCr 48-0 with A) l-MAP (mmHg) and B) l-Temp (°C).

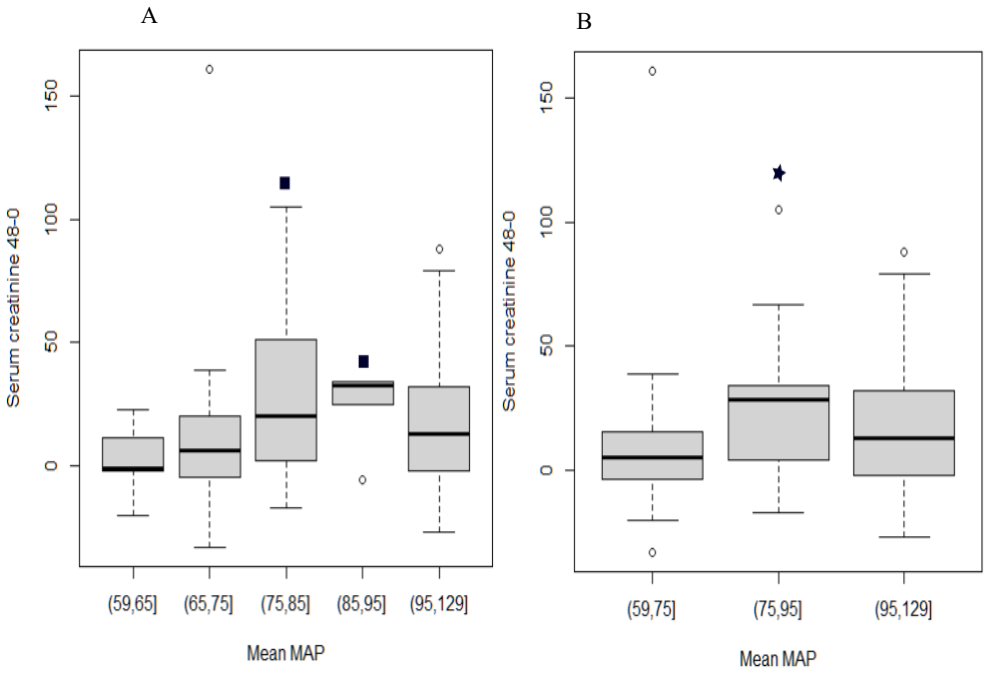


Figure A and B Mean difference in SCr 48-0 with A) l-MAP (mmHg) and B) l-Temp (°C).

SCr 48-24

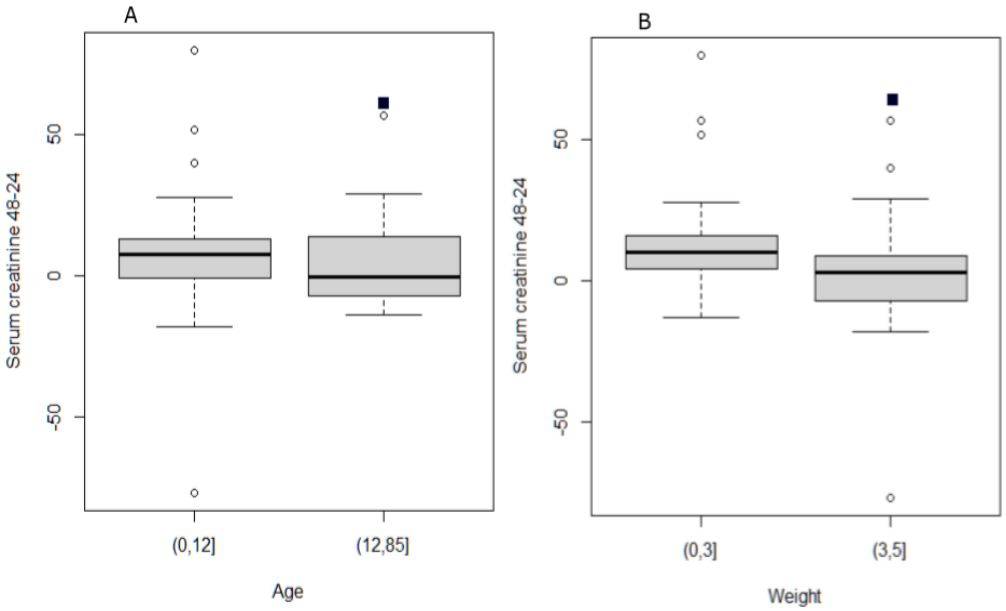


Figure A and B Mean difference in SCr 48-24 with A) age (m) and B) body weight (kg).

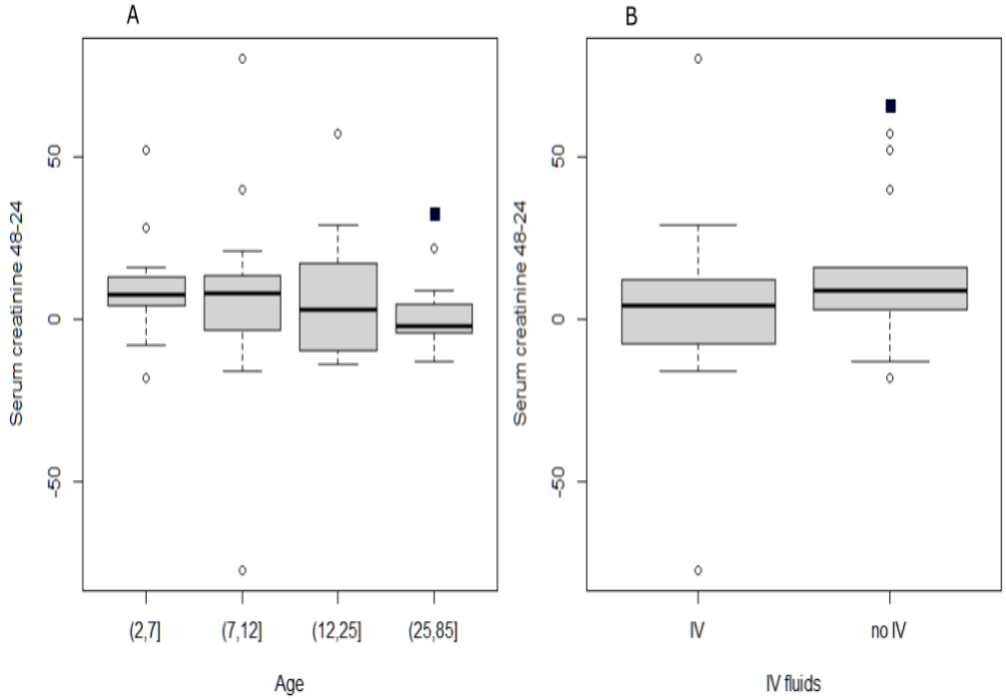


Figure A and B Mean difference in SCr 48-24 with A) age (m) and B) fluid administration.

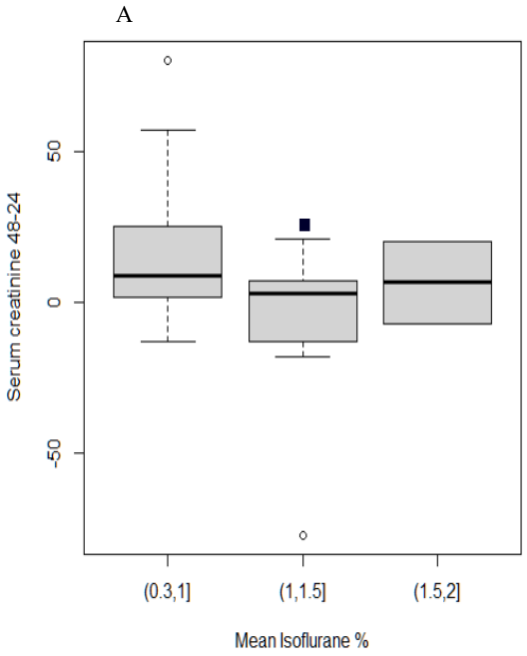


Figure A Mean difference in SCr 48-24 with m-isoflurane (%).

SDMA 24-0

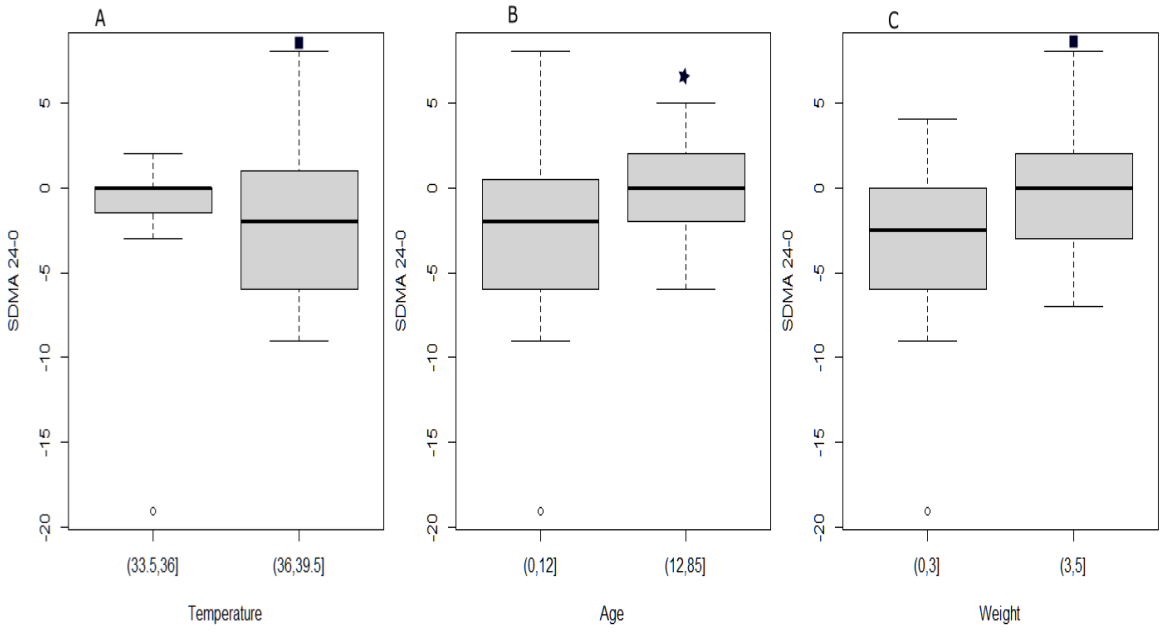


Figure A Mean difference in SDMA 24-0 with A) l-temp (°C), B) age (m) and C) body weight (kg).

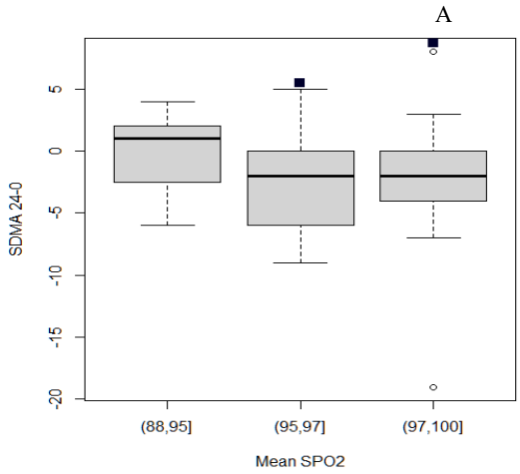
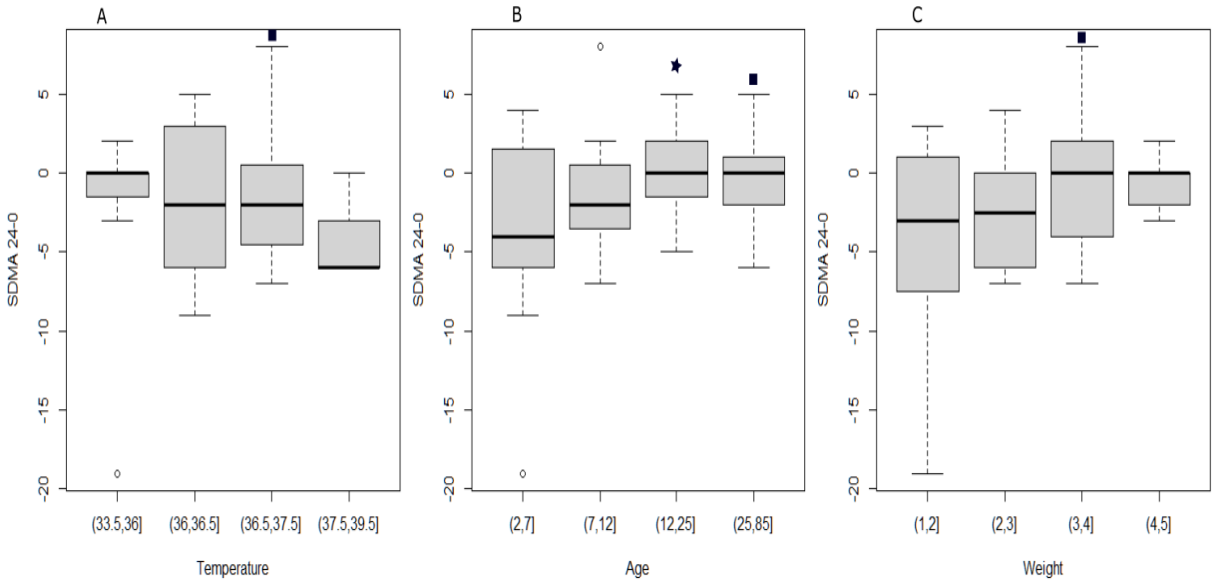


Figure A Mean difference in SDMA 24-0 with m-SpO_2 (%).



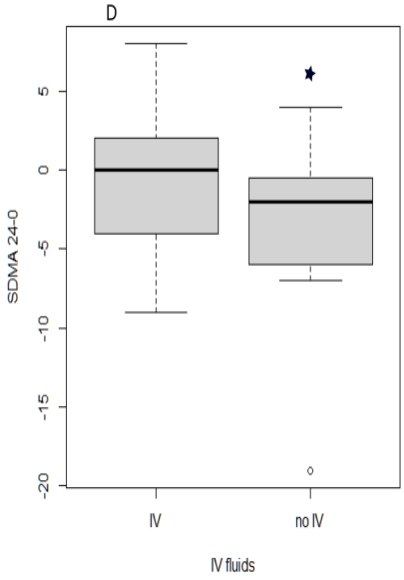


Figure A, B, C and D Mean difference in SDMA 24-0 with A) l-temp (°C), B) age (m), C) body weight (kg) and D) fluid administration.

SDMA 48-0

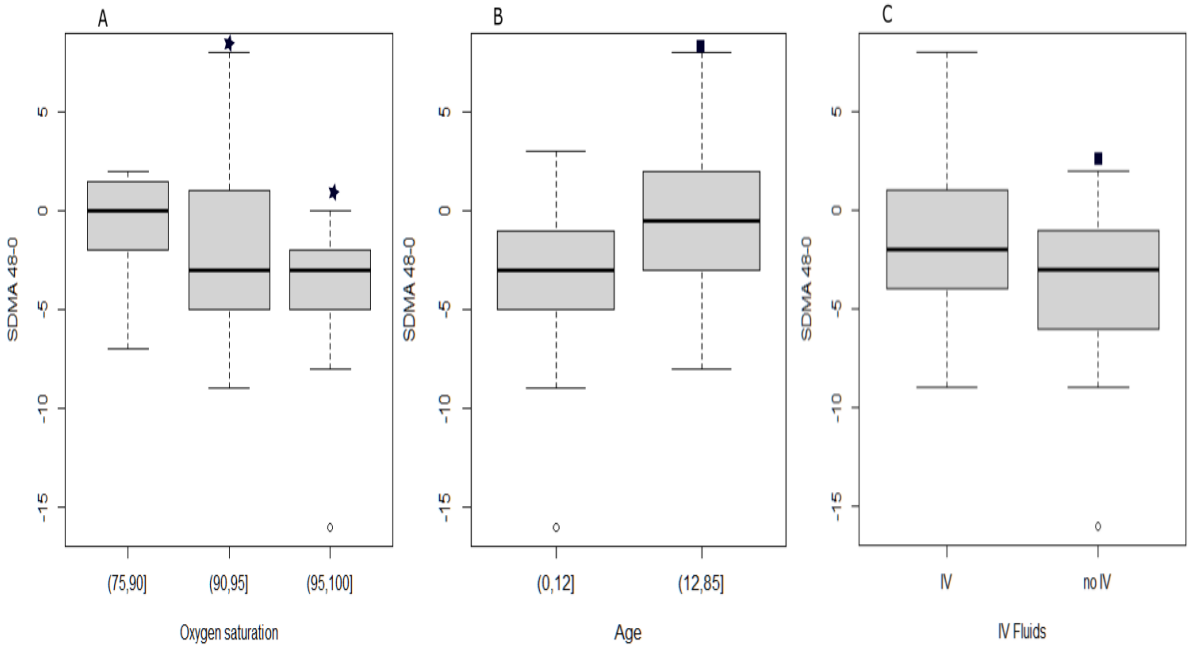


Figure A, B, and C Mean difference in SDMA 48-0 with A) l-SpO_2 (%), B) age (m), and C) fluid administration.

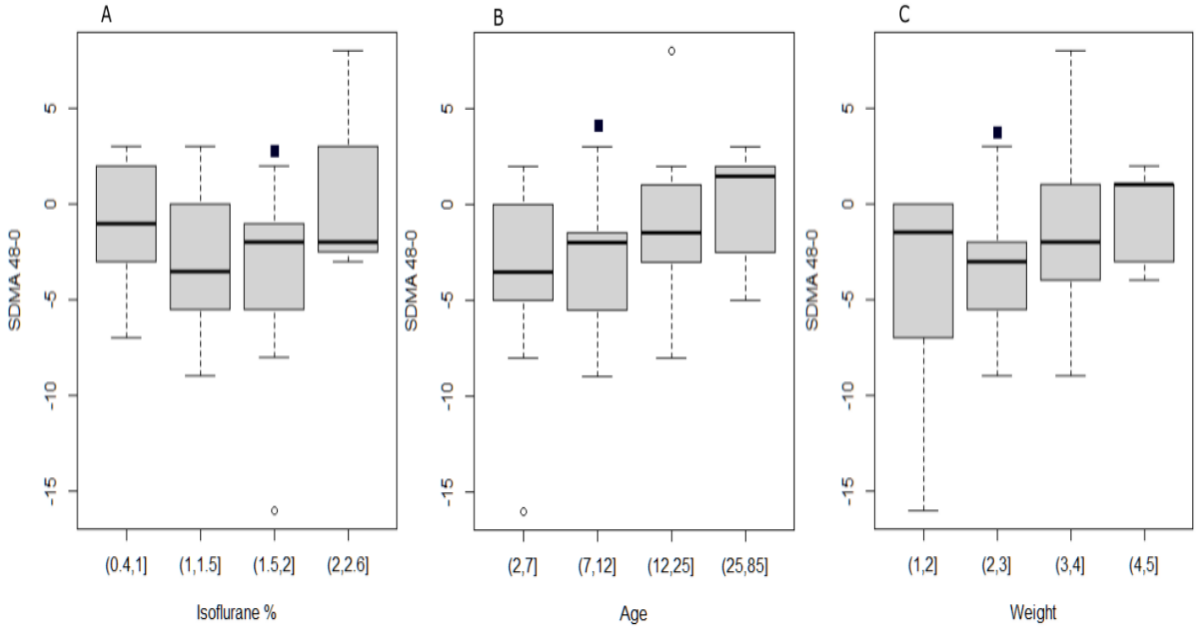


Figure A, B, and C Mean difference in SDMA 48-0 with A) max isoflurane (%), B) age (m), and C) body weight (kg).

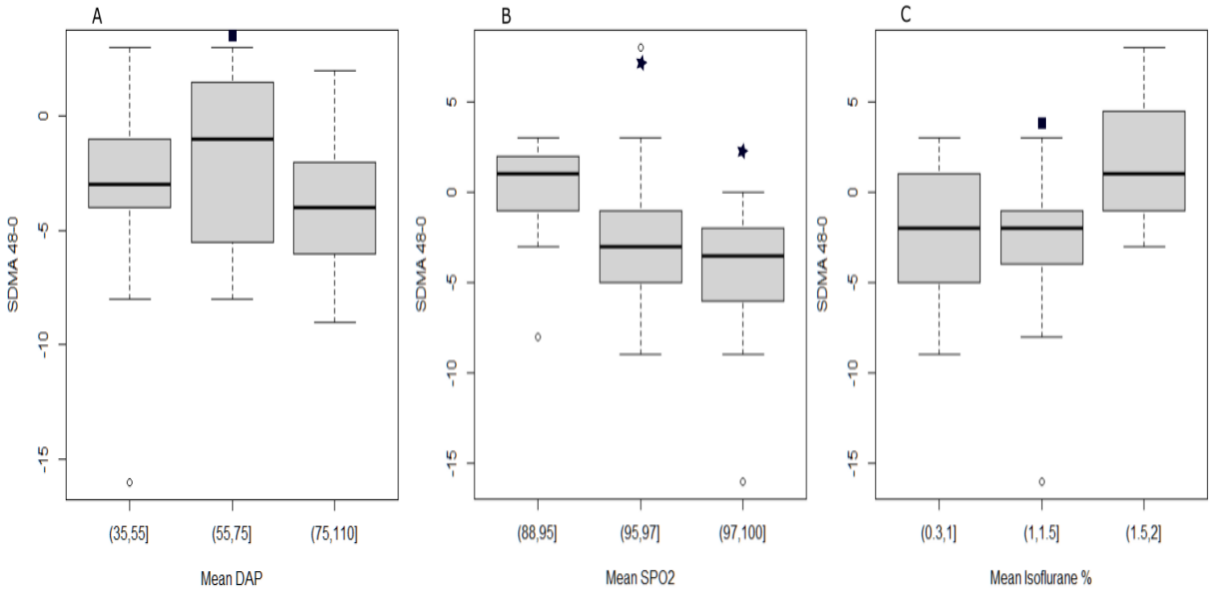


Figure A, B, and C Mean difference in SDMA 48-0 with A) m-DAP, B) m-SpO_2, and C) m-isoflurane (%).

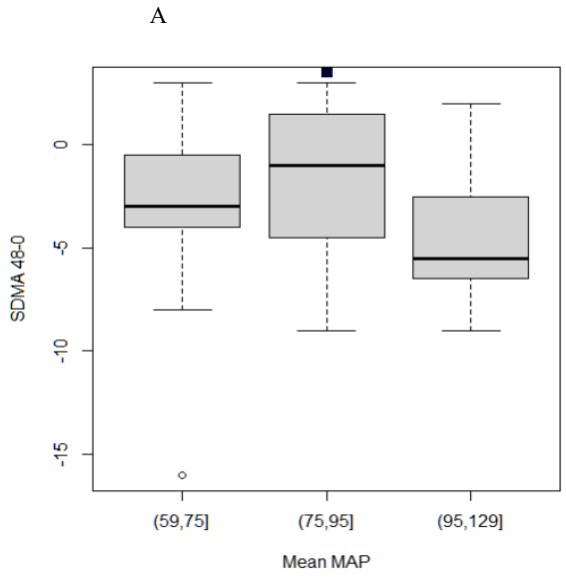


Figure A Mean difference in SDMA 48-0 with m-MAP (mmHg).

UCr 24-0

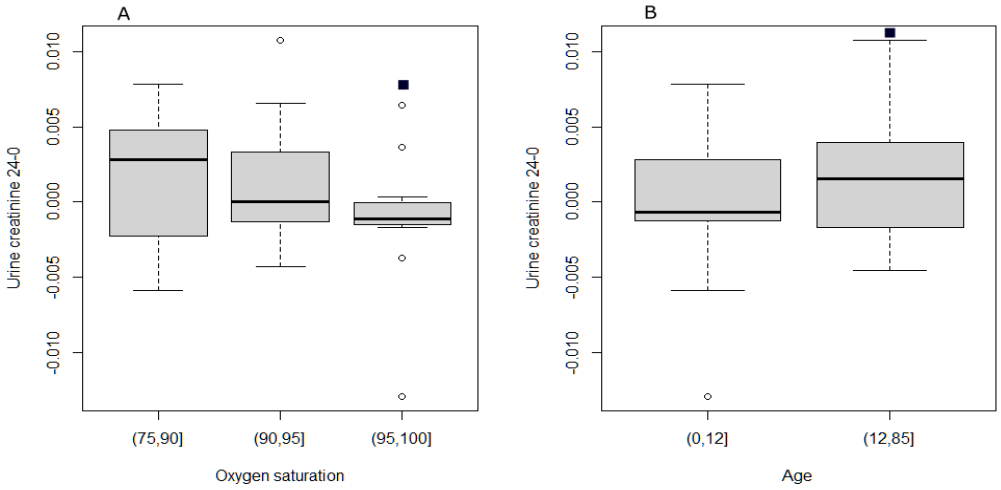


Figure A and B Mean difference in UCr 24-0 with A) l-SpO_2 (%) and B) age (m).

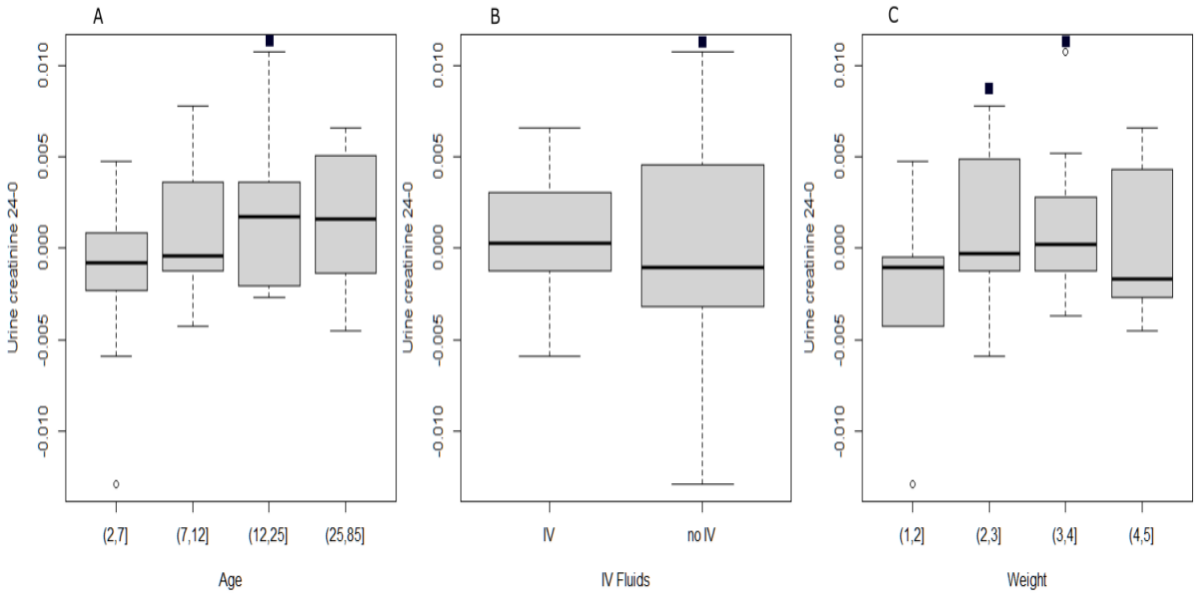


Figure A, B and C Mean difference in UCr 24-0 with A) age (m), B) fluid administration and C) body weight (kg).

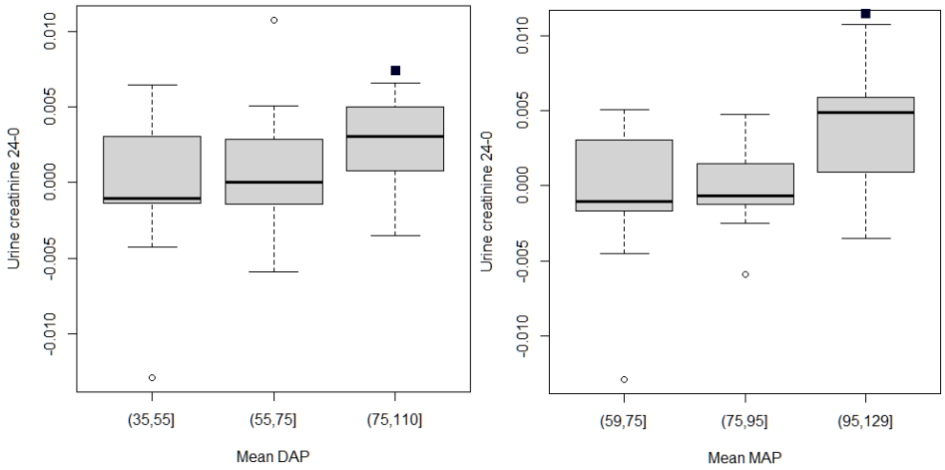


Figure A, and B Mean difference in UCr 24-0 with A) m-DAP (mmHg) and B) m-MAP (mmHg).

UNCR 24-0

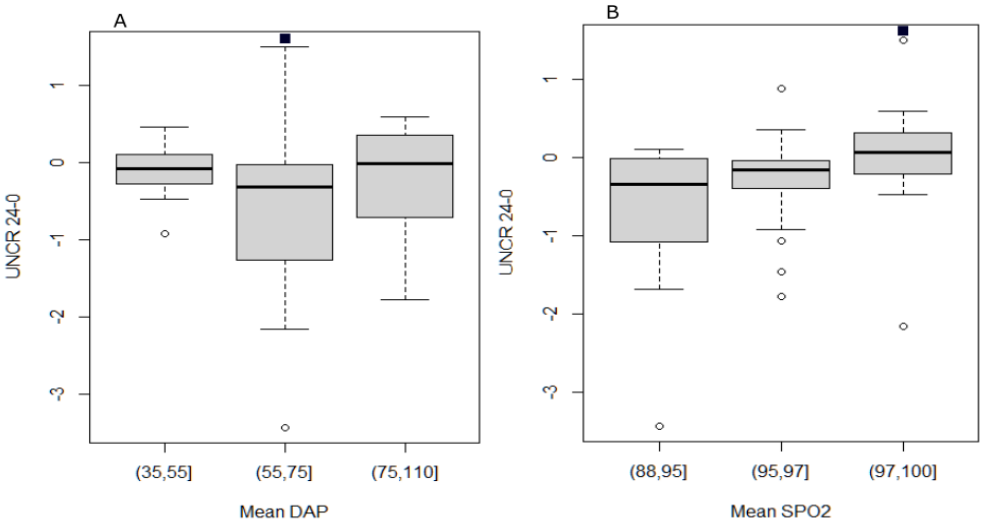


Figure A, and B Mean difference in UNCR 24-0 with A) m-DAP (mmHg) and B) m-SpO_2 (%).

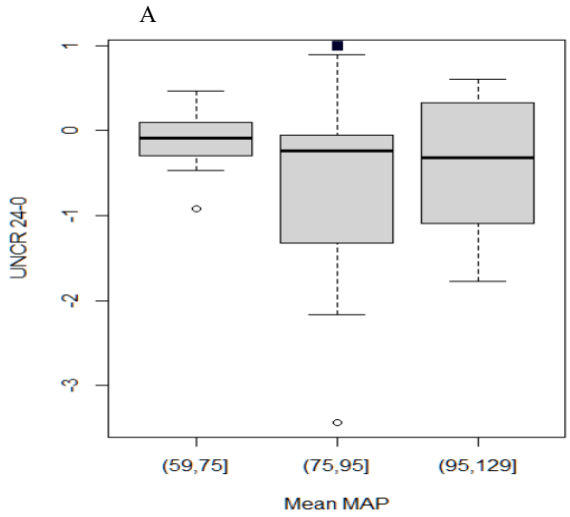


Figure A Mean difference in UNCR 24-0 with m-MAP (mmHg).

UNCR 48-0

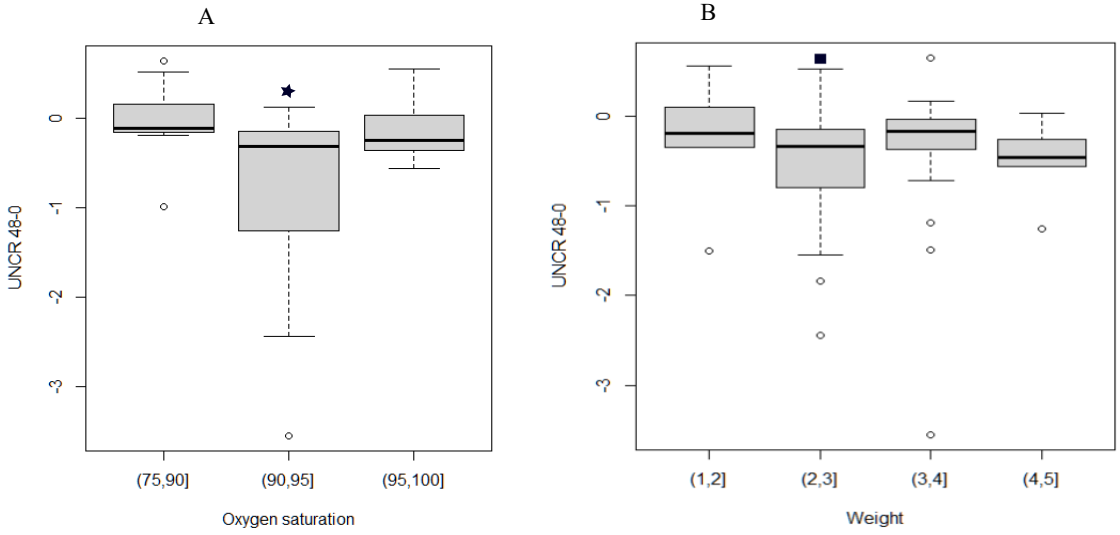


Figure A and B Mean difference in UNCR 48-0 with A) l-SpO_2 (%) and B) body weight (kg).

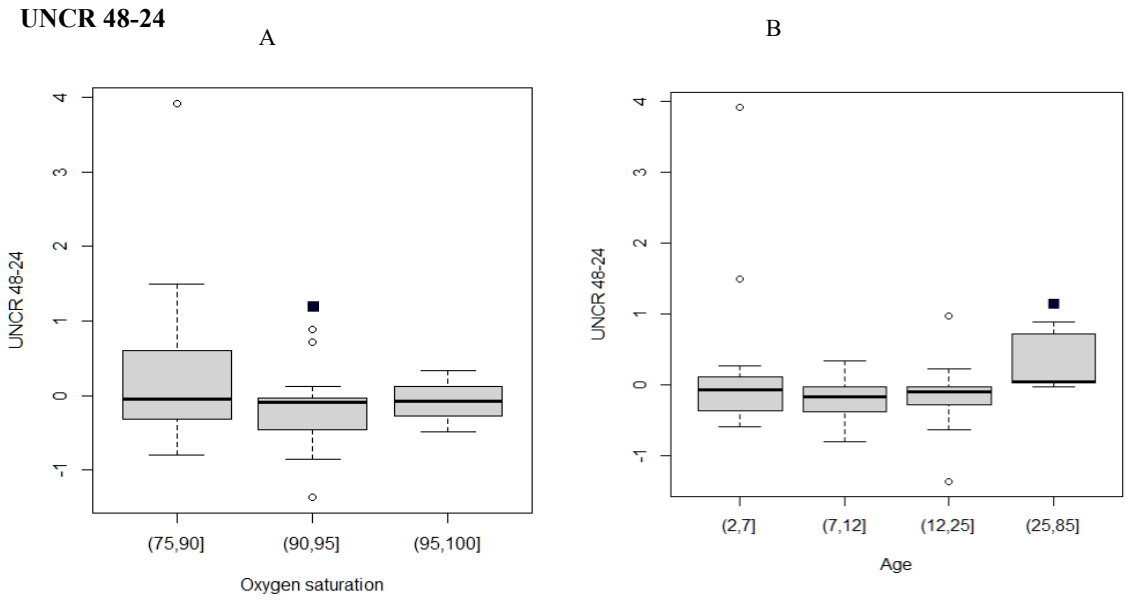


Figure A and B Mean difference in UNCR 48-24 with A) l-SpO_2 (%) and B) age (m).

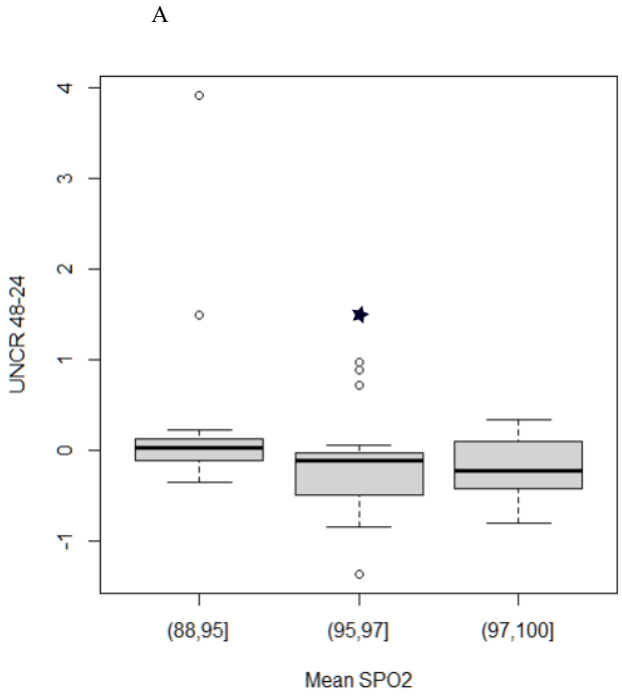
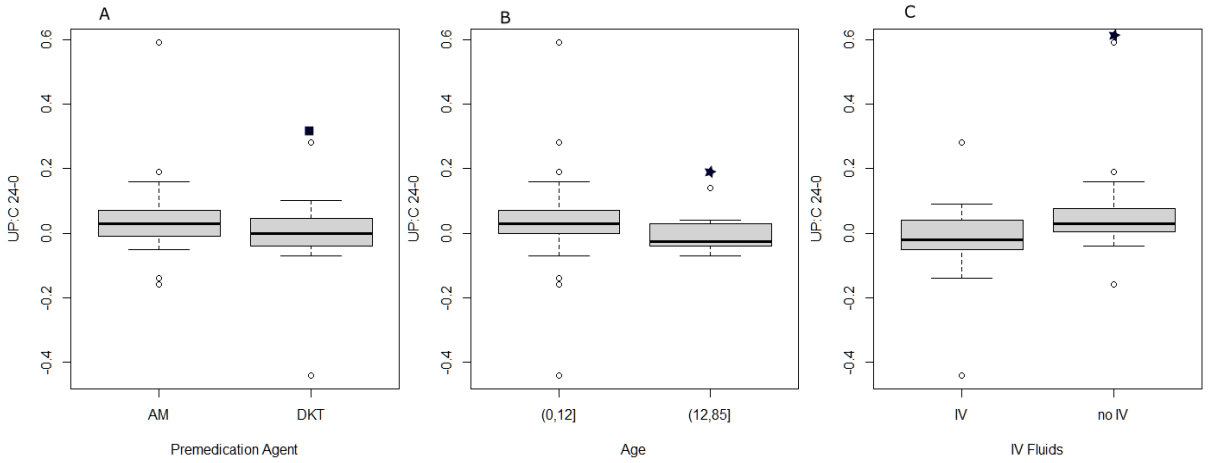


Figure A Mean difference in UNCR 48-24 with m-SpO_2 (%).

UP:C 24-0



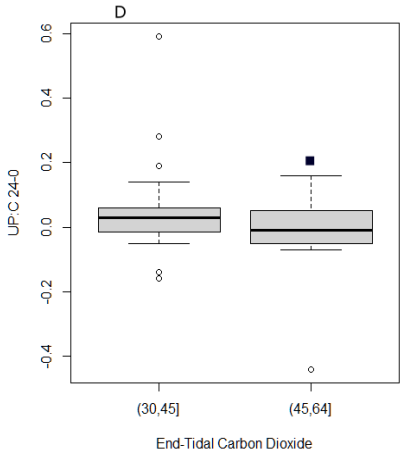


Figure A, B, C and D Mean difference in UNCR 48-24 with A) premedication agent, B) age (m), C) fluid administration and D) $ETCO_2$ (mmHg).

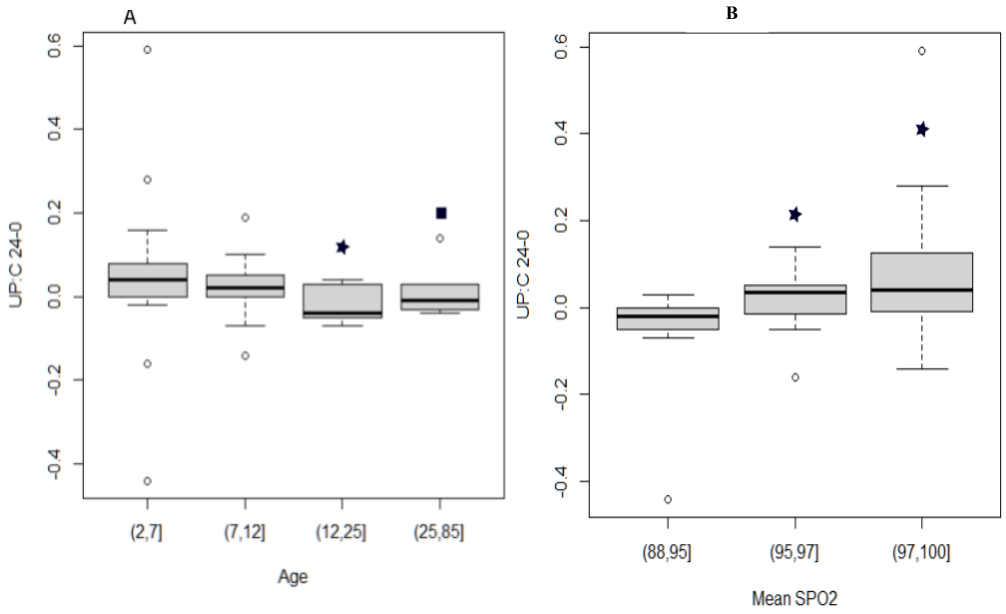


Figure A, and B Mean difference in UP:C 24-0 with A) age (m), B) m-SpO_2 (%)

UP:C 48-0

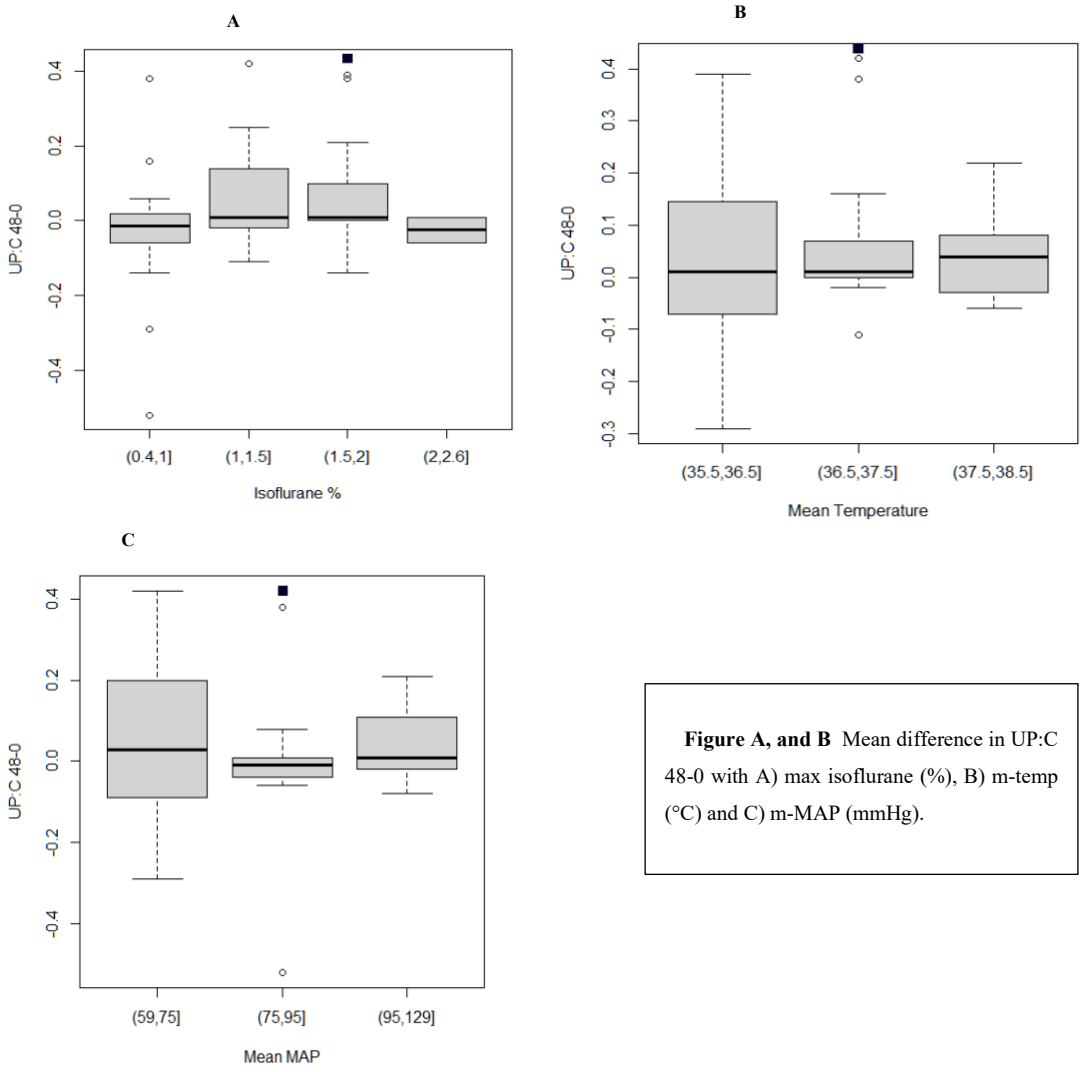


Figure A, and B Mean difference in UP:C 48-0 with A) max isoflurane (%), B) m-temp (°C) and C) m-MAP (mmHg).

UP:C 48-24

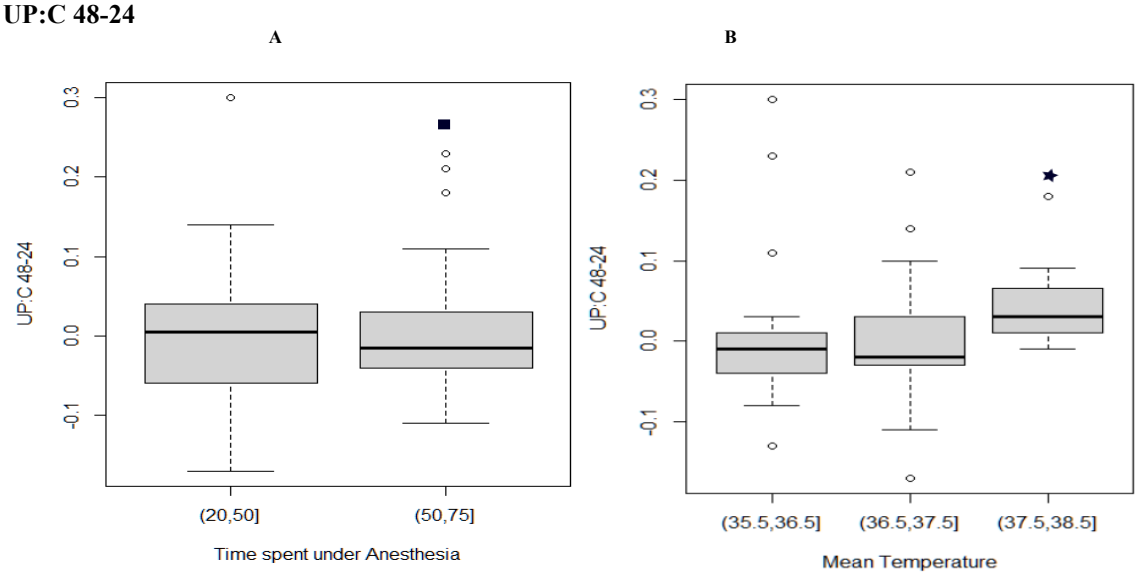
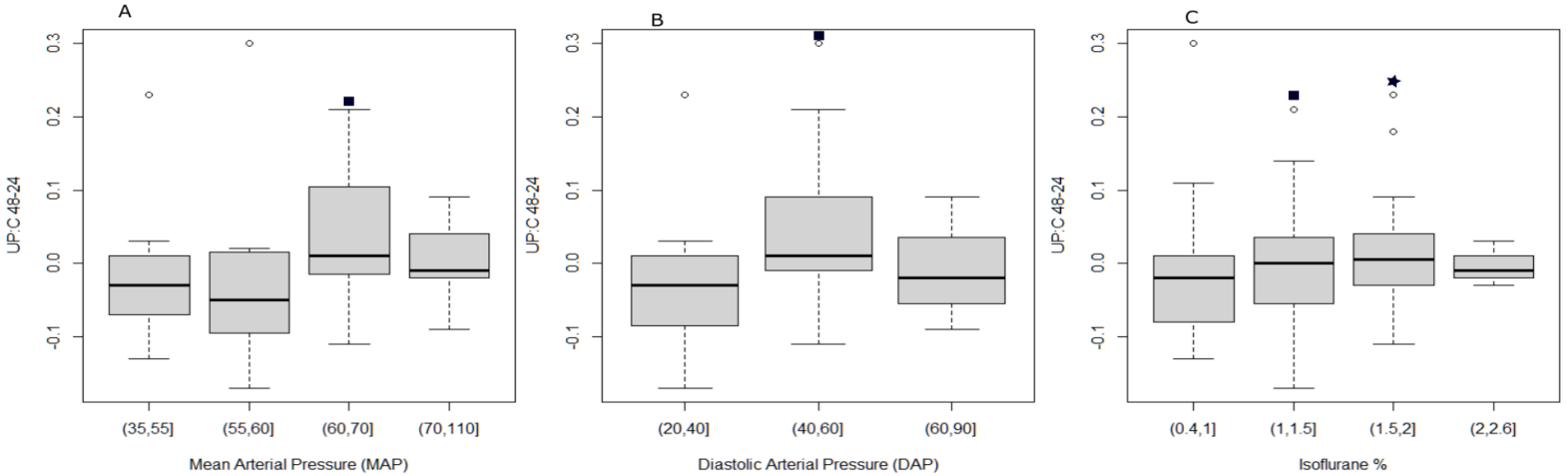


Figure A, and B Mean difference in UP:C 48-24 with A) anaesthetic duration (mins)and B) m-temp (°C).



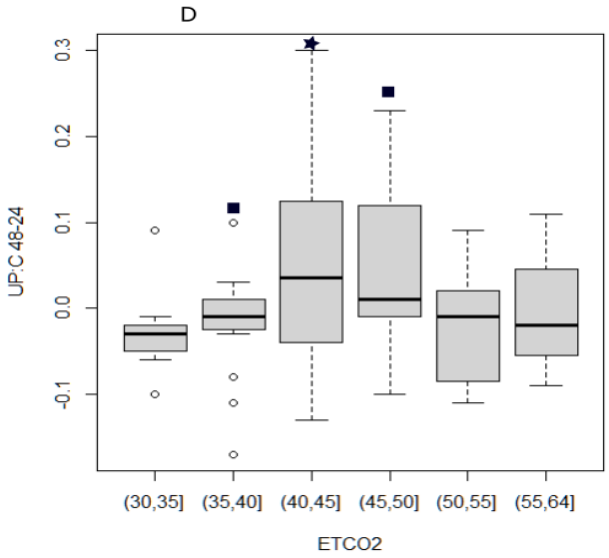


Figure A, B, C and D Mean difference in UP:C 48-24 with A) l-MAP (mmHg), B) l-DAP (mmHg), C) max isoflurane (%) and D) $ETCO_2$ (mmHg).

uRBP:Cr 48-24

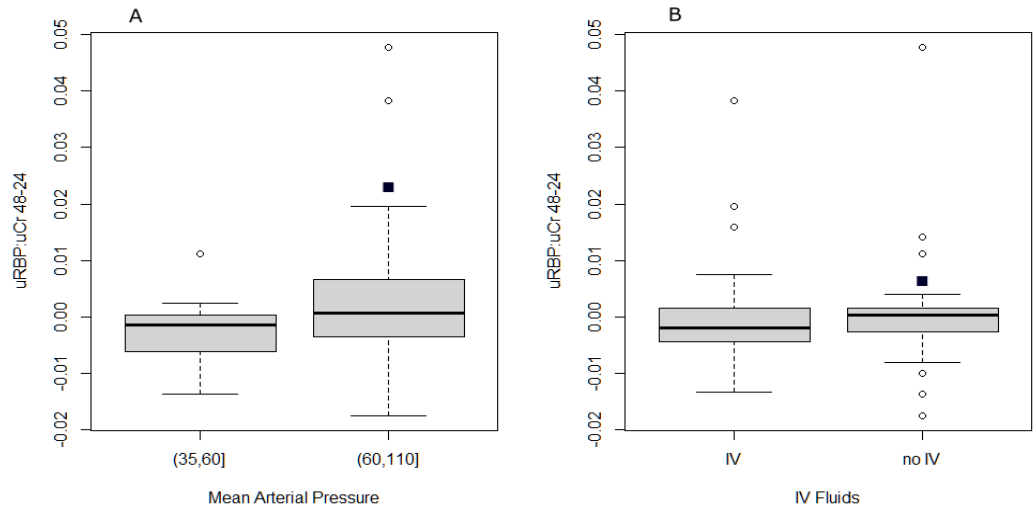


Figure A, and B Mean difference in uRBP:Cr 48-24 with A) l-MAP (mmHg), B) fluid administration.

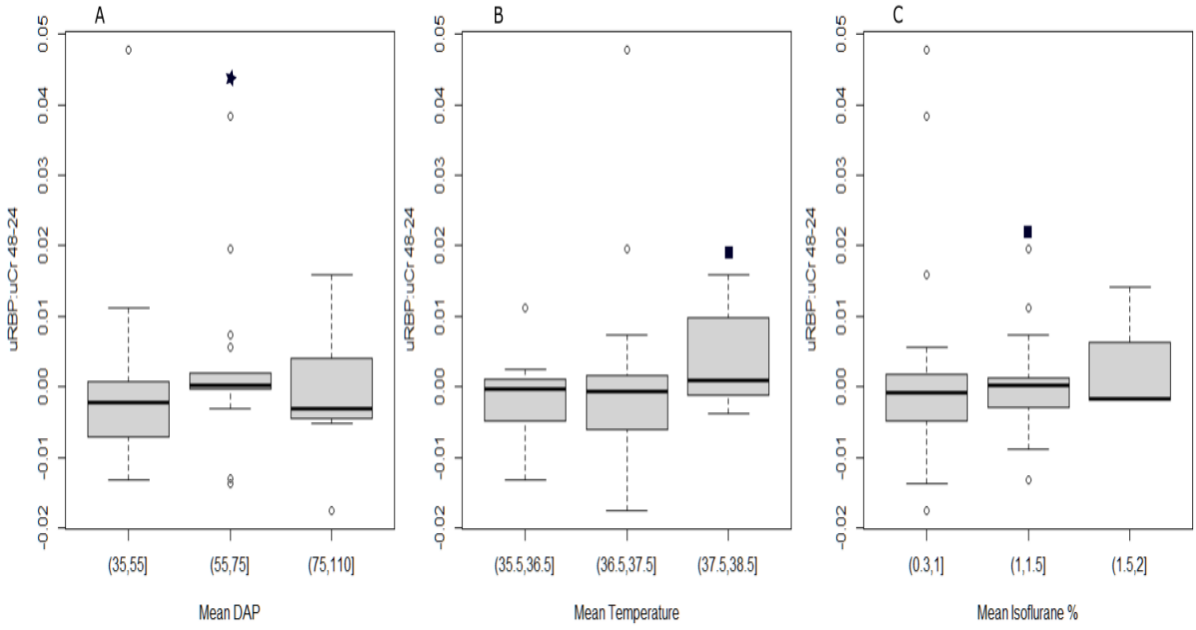


Figure A, B and C Mean difference in uRBP:Cr 48-24 with A) m-DAP (mmHg), B) m-temp (°C) and C) m-isoflurane (%).

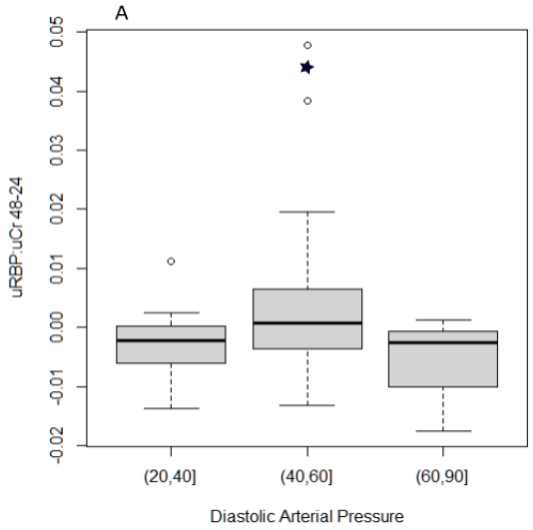


Figure A Mean difference in uRBP:Cr 48-24 with m-DAP (mmHg).

UTP 24-0

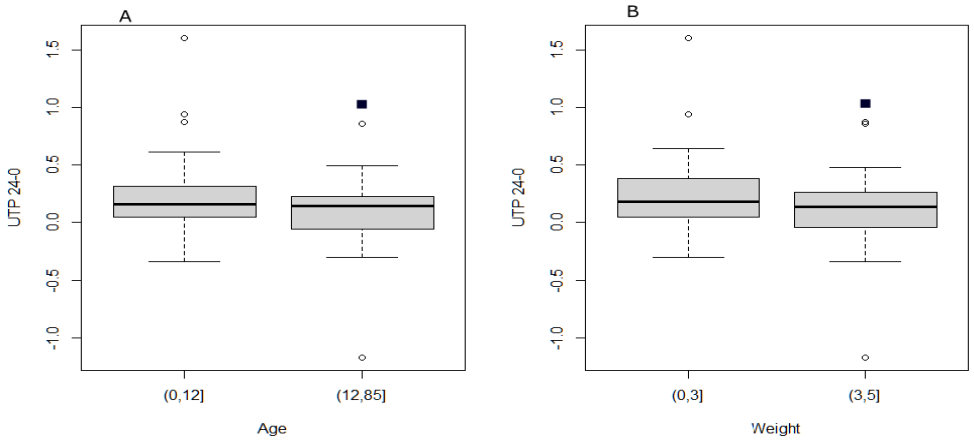


Figure A, B and C Mean difference in UTP 24-0 with A) age (m) and body weight (kg).

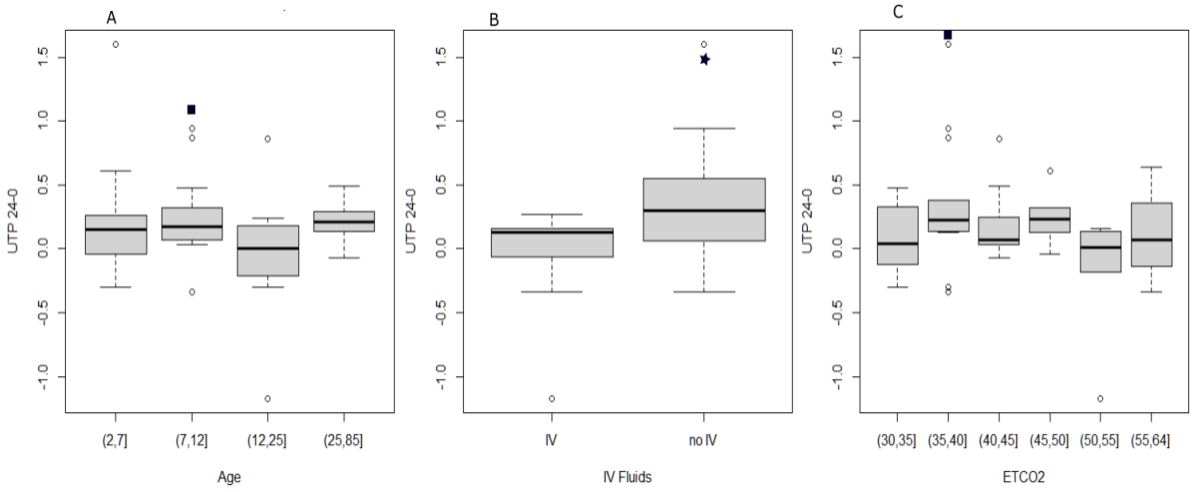


Figure A, B and C Mean difference in UTP 24-0 with A) age (m), B) fluid administration and C) $ETCO_2$ (mmHg).

UTP 48-0

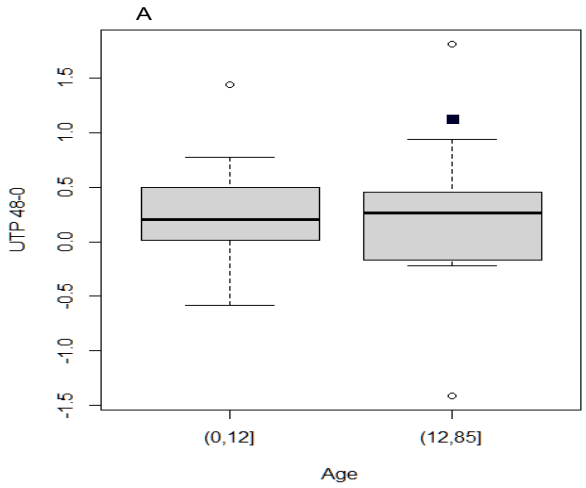


Figure A Mean difference in UTP 48-0 with age (m).

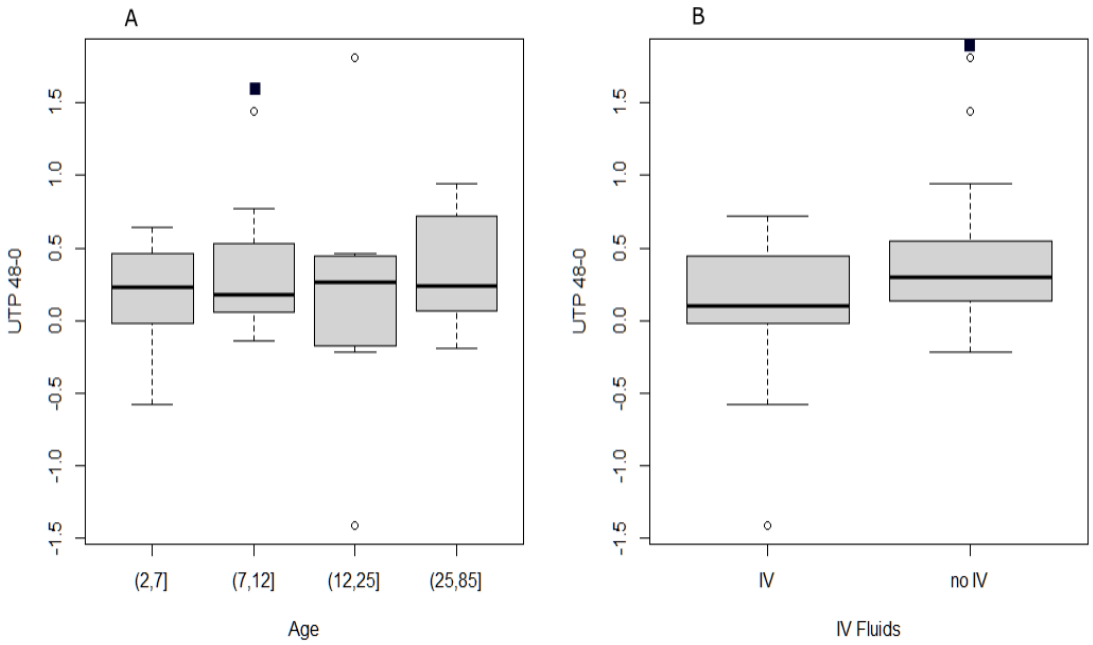


Figure A and B Mean difference in UTP 48-0 with A) age (m) and B) fluid administration.

UTP 48-24

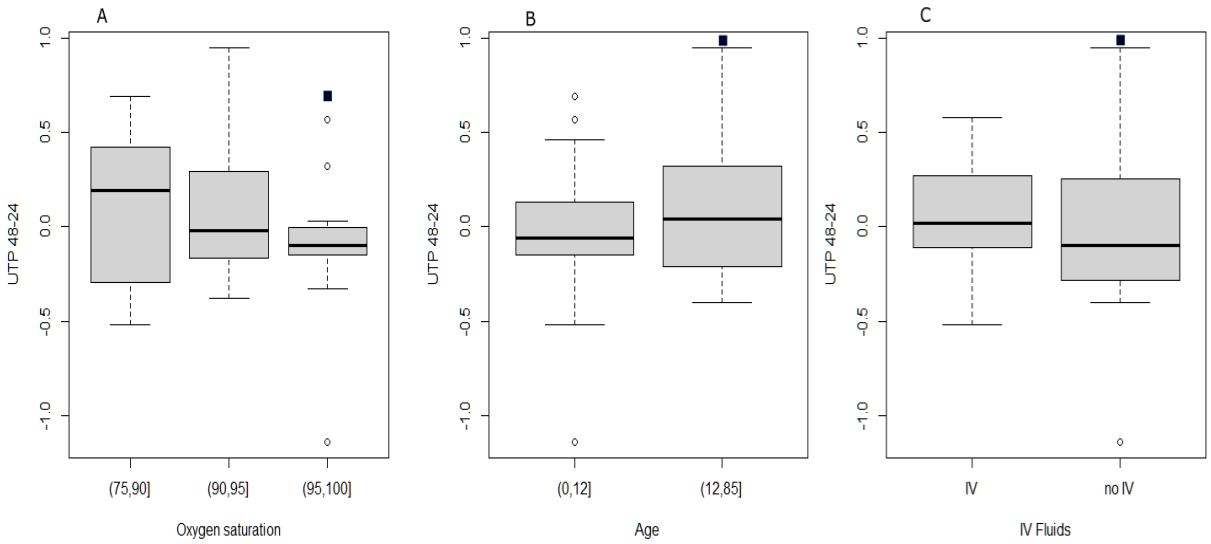


Figure A, B and C Mean difference in UTP 48-24 with A) l-SpO_2 (%), B) age (m) and C) fluid administration.

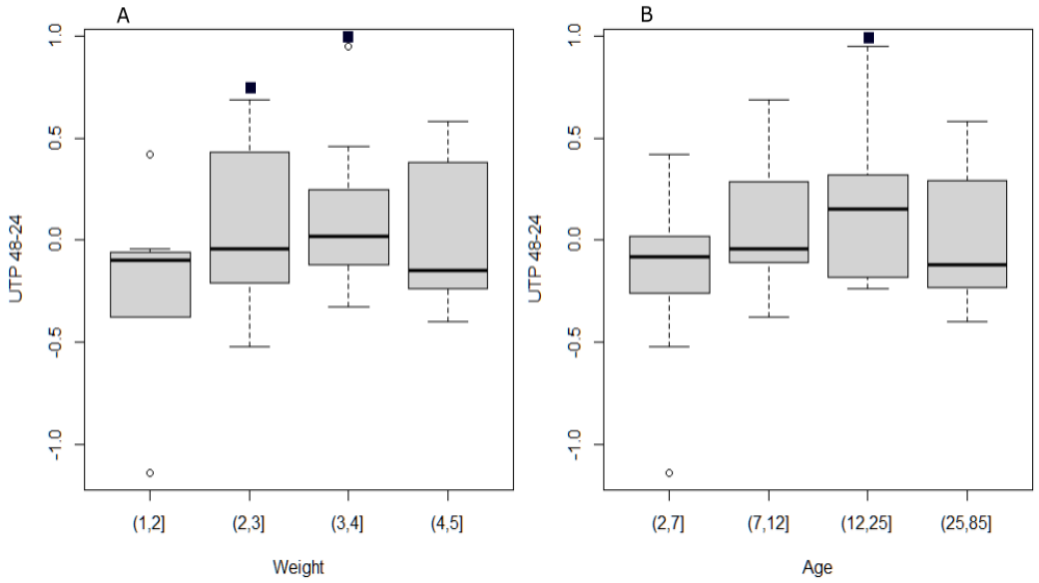


Figure A, and B Mean difference in UTP 48-24 with A) weight (kg) B) age (m).