Haematology and biochemistry effects of acepromazine or detomidine sedation in horses

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Abstract

Haematology and biochemistry effects of acepromazine or detomidine sedation in horses

Objective To determine the effects of acepromazine and detomidine standing sedation on haematological and biochemical variables in horses.

Study design A blinded randomised, comparative study.

Animal population Twelve Nooitgedachter horses and four Thoroughbred horses.

Methods Horses were enrolled into the study to receive one of two intravenous treatments (*n* = 8 per treatment group): acepromazine maleate (ACE; 0.05 mg kg^{-1}), or detomidine hydrochloride (DET; 0.01 mg kg^{-1}). One week later, eight of the horses were randomly assigned to undergo a control treatment (CON; received no drug, but underwent the same procedures). On the day of data collection, all horses underwent a routine physical examination and then placed in stocks or a stable to rest for 30 minutes. Baseline blood samples and clinical variables (sedation score, heart and respiratory rates, temperature) were obtained (time zero) and then the horse received the treatment, followed by blood sampling and clinical parameter measurements at 15-minute intervals until 60 minutes. Blood sampling at each time point involved the collection of jugular blood (single venous puncture, alternating between left and right jugular) into five vacuum storage tubes to measure: haematocrit, differentiated cell counts, total serum protein, albumin, globulins, cortisol, ACTH, insulin, glucose, AST, ALT, GGT, ALP, GLDH and CRP. Once the procedures were complete, the horses were observed until recovered from the effects of the sedative drugs (no signs of sedation or ataxia) before being allowed to continue with their daily routine. Quantitative data (clinical, hematological and biochemical values) were compared among treatments using a two-way analysis of variables. Significant findings were then compared using Dunnett's method of post-hoc

analysis where the baseline data within each treatment group was used as the control variable. Categorical data were compared among treatments using the Friedman Test (sedation scores).

Results The sedation scores were significantly higher, indicating sedation, in the horses that received DET or ACE when compared to CON, indicating that there was drug effect ($p < 0.01$ for both drugs). The haematocrit decreased in DET and ACE over time (both $p < 0.01$) but not in CON. The changes over time for the red blood cell count were similar to that of the haematocrit (both $p < 0.01$). The white cell count decreased significantly over time after DET and ACE compared to CON (both $p = 0.03$). The platelet counts were not different among groups and over time. Most of the biochemistry analytes remained unchanged over time, however, there were statistically and clinically relevant observations. The glucose concentration raised from 30 minutes onwards within DET compared to ACE and CON which remained constant over time ($p < 0.01$).

Conclusions and clinical relevance Acepromazine and detomidine induced standing sedation caused changes in haematological and some biochemical variables in the horses. These changes were detectable from 15 minutes of administration of the drugs and in some cases lasted the hour of observation. The present study highlights the clinical relevance of obtaining venous blood for analysis in equine patients prior to the administration of sedative drugs.

Keywords Horse, sedation, clinical pathology, haematology, serum biochemistry

List of abbreviations

%	percentage
°C	degree(s) Celsius
α-2	alpha-2
ACE	acepromazine
ACTH	adrenocorticotrophic hormone
ALB	albumin
ALP	alkaline phosphatase
ALT	alanine transaminase/aminotransferase
AST	aspartate transaminase/aminotransferase
BUN	blood urea nitrogen
CBC	complete blood count
CON	control
CRP	C-reactive protein
DET	detomidine
EDTA	ethylenediaminetetraacetic acid
g	gram(s)
GGT	gamma-glutamyl transferase
GLDH	glutamate dehydrogenase
GLOB	Globulin
HR	heart rate
hrs	hours
Ht	haematocrit
iv	intravenous
kg	kilogram(s)
L	litre(s)
LDH	lactate dehydrogenase
max	maximum
mg	milligram(s)
min	minimum
mins	minutes
mmol	millimole(s)
n	number
NEFA	non-esterified fatty acid (s)
ng	nanogram(s)
N:L	neutrophil: lymphocyte ratio
nU	nano unit(s)
OTAU	Onderstepoort Teaching Animal Unit
OVAH	Onderstepoort Veterinary Academic Hospital
OVAH-CPL	OVAH-Clinical Pathology Laboratory
p	probability value
PCV	packed cell volume
RR	respiratory rate
SDS	simple descriptive scale
T	time of data collection points in minutes (T0, T5, T10, T15, T20)
Temp	temperature in degrees Celsius
TSP	total serum protein
U	unit(s)
VUT-CPL	Vaal University of Technology Clinical Pathology Laboratory
WBC	white blood cell count

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Chapter 1: Literature review

1.1 Introduction

In clinical situations, horses are often sedated prior to complete clinical assessment. The sedative drugs may alter the variables being assessed, this could affect the clinical and diagnostic presentation and could result in an altered course of treatment. Some effects of the sedative drugs on haematology and serum biochemistry variables in horses have been described (Lumsden et al. 1975; Kullman et al. 2014). However, there remains debate as to what specific changes can be expected for some variables and when the onset of change occurs (Muir & Hubbell 2007a; Lemke 2007).

It has been noted that sedative and tranquilizer drugs do not have a sole effect of sedating a horse. It is established that these drugs could also cause physiological responses that include changes in heart rate and rhythm, respiratory depression, changes in blood pressure, increased urination and sweating, extrusion of the penis, ataxia, muscle tremors, antihistaminic effects, decrease in haematocrit and serum protein levels, to name but a few (Muir & Hubbell 2007b; Lemke 2007, Dugdale 2010). Different drugs and dosages used to sedate horses will have different degrees of clinical effects on the patient. While individual horse differences need to be taken into consideration, a predictive value on how the drugs will affect the haematological and serum biochemistry values will assist the equine practitioner in further assessment or evaluation and diagnosis of the patient.

The literature review for the present research project focuses on two drugs, detomidine hydrochloride and acepromazine maleate, and their known pharmacologic effects on horses. We focus on previous research investigating equine haematology and serum biochemistry and how these drugs affect these variables.

1.2 Literature review

Horses are often sedated prior to complete evaluation for a number of reasons, most often because the patient is excitable, too difficult or too painful to handle to be assess appropriately (Muir & Hubbell 2007b; Dugdale 2010). In South Africa, detomidine hydrochloride and acepromazine maleate are commonly used drugs to sedate horses.

Horse sedation

The commonly used groups of drugs horse sedation belong to the alpha-2-adrenoceptor agonists and the phenothiazine derivative tranquilizers (Clarke et al. 2014b). The alpha-2-adrenoceptor agonists are considered the most reliable sedative group of drugs currently licensed for use in the horse (Lemke 2007; Zeiler 2015). Of the alpha-2-adrenoceptor agonists, those which are licensed for use in horses in South Africa are detomidine, romifidine and xylazine. Detomidine is the more potent of these drugs due to a greater specificity for alpha-adrenoceptors and one of the most popular drugs used for equine sedation and premedication (Vainio 1985; Alitalo 1986; Clarke & Taylor 1986; Short et al. 1986). Furthermore, Wood et al. (1992) determined that detomidine has a dose-dependent effect comparable to phenothiazine derivative tranquilizers. The only licenced phenothiazine derivative tranquilizer in South Africa for horses is acepromazine maleate.

Detomidine hydrochloride

Detomidine (DET; 1H-imidazole,4-[2,3-dimethylphenyl]methyl-hydrochloride) is an alpha-2-adrenoceptor agonist (α-2 agonist). DET is highly protein bound and rapidly distributed, extensively metabolised in the liver and metabolites are eliminated in the urine (Muir & Hubbell 2007b; Grimsrud et al. 2009). The onset and degree of sedation, analgesia and the

other effects are rapid after the administration of DET. The duration of effect, time to peak effect and half-life are dose dependant (Reitemeyer et al. 1986; Wood et al. 1992).

The adrenoceptors are part of the sympathetic branch of the autonomic nervous system within the body and are made up of alpha- and beta-adrenoceptors. These receptors are found in most central (brain and spinal cord) and peripheral organ tissues within the body (Muir & Hubbell 2007b; Dugdale 2010; Clarke et al. 2014a). The nerve endings terminate in organ tissue and are globally named neuro-effect junctions. The synapsis of the neuro-effect junction comprises of three main structures: 1) a pre-synaptic sympathetic neuron, 2) a synaptic cleft and 3) a post-synaptic effect organ region. The endogenous agonist ligand for adrenoceptors is norepinephrine (noradrenaline) but, when necessary (stress response) epinephrine (adrenaline) can act as an agonist. Only the alpha-adrenoceptors will be discussed further because DET does not appear to interact with the beta-adrenoceptors.

Stimulation of the alpha-adrenoceptors, by the drugs or endogenous ligands, results in classic clinical signs that characterise the physiological response. As with other α-2 agonists, DET produces sedation and analgesia by its central effect on the alpha-2-adrenoceptors which causes a dose-dependent reduced central nervous system output and peripheral sympathetic tone (Lemke 2007; Muir & Hubbell 2007b). The reduction in sympathetic tone is caused by the α-2 agonist binding to pre-synaptic receptors which inhibit the release of catecholamines like norepinephrine, epinephrine and dopamine (Clarke & Taylor 1986; Wood et al. 1992). The activation of alpha-2-adrenoceptors cause inhibition of neurotransmission by activation of potassium channels at the postsynaptic level (Williams et al. 1985) and the inhibition of calcium channels at the presynaptic level; this process is mediated by protein G-coupled receptors which inhibit adenylate cyclase decreasing intracellular cyclic adenosine monophosphate (Lemke 2007). Peripheral dose-dependent effects that are well documented

with DET sedation include: bradycardia and decreased cardiac output; mild respiratory depression; increased intravascular fluid volume and frequency of urination due to inhibition of antidiuretic hormone and renin release, hyperhidrosis, increased salivation; hyperglycaemia and hypoinsulinaemia, reduced catecholamine and cortisol concentrations; muscle relaxation, muscle tremors, ataxia, piloerection, penile extrusion; occasional urticarial reactions and reduction in gastrointestinal activity (England et al. 1992; England & Clarke 1996; Lemke 2007; Muir & Hubbell 2007b; Serpa et al. 2015; Toribio 2010). When administered as a premedication some of these effects may be beneficial in the reduction of induction and maintenance of anaesthetic drug requirements and attenuation of the stress response to pain and surgery (England & Clarke 1996).

Of particular interest to our study are the documented changes in haemodynamics which are often observed with a systemic drug effect. Within the neuro-vascular endothelial junction, the pre-synaptic sympathetic neuron has alpha-2-adrenoceptors and the post-synaptic region has predominantly alpha-1-adrenoceptors and few alpha-2-adrenoceptors. The vascular endothelium has many extra-synaptic alpha-2-adrenoceptors that are found along the endothelium and are not associated with the synapsis (Ruffolo 1985). When DET is administered, there is an initial marked vasoconstriction due to the inactivation of the post- and extra-synaptic alpha-adrenoceptors (Knaus et al. 2007; Zeiler 2015). This vasoconstriction causes an increase in the arterial blood pressure which can last for a variable amount of time, depending on the individual and dose given (Clarke et al. 2014a). Carotid and aortic baroreceptors detect the increase in blood pressure and signal the cardiovascular centre within the brain to initiate mechanisms to return the blood pressure to a normal range. The initial response during this phase is by increasing vagal tone to decrease the heart rate which often results in a bradycardia (Daunt et al. 1993; Lemke 2007; Muir & Hubbell 2007a; Savola 1986). Horses may even develop atrioventricular blocks (usually 2nd degree atrioventricular blocks

Mobitz type I) within five minutes of administration (Lemke 2007) or occasionally even severe sinus bradycardia with ventricular escape beats (Muir & Hubbell 2007a). When the drug reaches the central nervous system, it will result in a decrease of norepinephrine release and cause suppression of the cardiovascular centre which could result in a prolonged bradycardia independent of the arterial blood pressure and baroreceptor response that is initially present (Lemke 2007).

Acepromazine maleate

Acepromazine maleate (ACE; 2-acetyl-10-[3-dimethylaminopropyl] phenothiazine) is a phenothiazine derivative tranquilizer, commonly used in horses as a pre-anaesthetic medication to cause anxiolysis or sedation, but a variety of behavioural, autonomic and endocrine effects have also been described (Gross & Booth 1995; Lemke 2007; Muir & Hubbell 2007b). ACE injected intravenously is widely distributed within the body in horses (Ballard et al. 1982; Marroum et al. 1994) and is highly bound to plasma proteins and results in a high tissue reservoir of ACE (Chou et al. 2002). The peak concentration of ACE has been reported within five to fifteen minutes of intravenous administration while the peak effects may only be evident after twenty minutes and sedation has been noted to last one to two hours (Ballard et al. 1982; Marroum et al. 1994; Muir & Hubbell 2007b; Clarke et al. 2014b). ACE is metabolised by the liver and both the conjugated and unconjugated metabolites are excreted in the urine (Lemke 2007). The duration of effect and elimination of the drug are dependent on the individual and the dose administered. The drug also exhibits a plateau effect whereby as the dose of ACE increases the effects are no longer increased but their duration is prolonged and the risks of potential side effects are increased (Clarke et al. 2014a).

Phenothiazine derivatives have a marked binding affinity for the adrenergic and muscarinic (autonomic) and other receptors. There is a higher binding affinity for the alpha-1-

adrenceptors, a blockade of which will result in a decrease in systemic vascular resistance (Lemke 2007). The tranquilizing effect of ACE occurs due to blocking of central and peripheral neurotransmitters (Lemke 2007) thereby blocking dopaminergic, cholinergic, histaminic, adrenergic and ganglionic activity (Muir & Hubbell 2007b). Dalton (1972) showed that ACE exerts an effect due to a depressant action on the central nervous system and an associated effect on the autonomic nervous system.

The effects of ACE on horses are not limited to calming or sedation. Reported effects of ACE include: reduced locomotor activity, limited muscle relaxation; mild respiratory system effects; vasodilation due to a direct effect on the blood vessels that may result in hypotension; increased sweating; delays in gastric emptying and reduced intestinal motility (Lemke 2007; Muir & Hubbell 2007b). Additionally, cardiovascular changes are reported to be variable and a lack of somatic analgesic effects has been noted. Visceral analgesia may occur because of the marked gastrointestinal spasmolytic effect on smooth muscle that is evident by the decreased tone, secretions, and peristalsis in the gastrointestinal tract (Muir & Hubbell 2007b). These effects are presumed to be due to the generalised suppression of the central nervous system and peripheral anticholinergic actions of phenothiazines (Muir & Hubbell 2007b). The cardiovascular effects are dose dependant anti-epinephrine and anti-norepinephrine effects whereby the direct competitors block alpha-1-adrenoceptors. Vasodilation and decrease in blood pressure (sometimes hypotension) result in the release of epinephrine from the adrenal medulla consequently causing a hyperglycaemia and hypothermia (Muir & Hubbell 2007a). The peripheral vasodilation and reduced arterial blood pressure are postulated to be related to a splenic dilation (Clarke et al. 2014b). Other properties of ACE include: anti-arrhythmic, antifibrillatory, anti-histamine, alpha-sympatholytic, and hypothermic effects (Wagner et al. 1991; Muir & Hubbell 2007a&b; Clarke et al. 2014a; Serpa et al. 2015).

Vasodilatory effects result in secondary reductions in hepatic and renal blood flow, prolonging the action, metabolism and elimination of ACE. These effects are more pronounced in geriatric, compromised or diseased horses, even those in which there is mild liver disease (Kerr et al. 1972; Clarke et al. 2014b). Prolonged courses of ACE are frequently used to manage difficult or stressed horses. The hepatic metabolism and reduced hepatic and renal blood flow secondary to hypotension (Muir & Hubbell 2007b) should be considered with long term administration and concurrent administration of other drugs to avoid inducing liver insult in horses. Penile extrusion is a well-known and documented dose-dependant side effect of phenothiazine derivatives (Ballard et al. 1982) and has been described with ACE. Prolonged prolapse or priaprism is an uncommon result of this effect, which is discussed in more detail by Perason & Weaver (1978), essentially this is why ACE is not recommended for use in breeding stallions (Lemke 2007). ACE is well-tolerated by normovolaemic horses. Rare negative effects such as excitement reactions or extrapyramidal effect have been reported to occur with large doses (Dugdale 2010, Muir & Hubbell 2007b).

Dosages of DET and ACE

The dosages used in this study needed to be those recommended to induce clinical sedation and be considered to produce an equivalent sedation. We considered that an equi-effective dose is one that produces a reliable state of clinical sedation. The intravenous dosage of DET administered in the present study was 0.01 mg kg^{-1}, the same used by Buhl et al. (2007) to investigate cardiovascular responses. This study reported that the horses were reliably sedated with this dosage.

Parry & Anderson (1983) administered ACE at dosages from 0.05 to 0.15 mg kg^{-1} to investigate the effects on haematocrit and total plasma protein concentration. Mostafa et al. (1995) performed a comparative study with different dosages of ACE in donkeys and found that 0.05

mg kg^{-1} resulted in a predictable sedative effect. ACE was administered intravenously at a dosage of 0.05 mg kg^{-1} in the present study because lower dosages (0.01 and 0.03 mg kg^{-1}) did not produce clinical sedation or had varying effects, particularly on the cardiovascular system (Chou et al. 2002).

Clinical variables

Assessment of the effects of these drugs on clinical variables such as heart rate and respiratory rate are well reviewed in the literature, most studies agreed that the rate of these clinical variables change with time following administration (Kerr et al. 1972; Muir et al. 1979; Ballard et al. 1982; Daunt et al. 1993; Short et al. 1986; Nilsfors et al. 1988; Wagner et al. 1991; England et al. 1992; Carroll et al. 1997; Buhl et al. 2007). These clinical effects can be used to indicate that there is a drug effect present and thus important to the present study. Sedation with DET has resulted in a bradycardia, within the first one to five minutes following administration (England et al. 1992; Mostafa et al. 1995; England & Clarke 1996). DET has an effect on the respiratory centre resulting in individual and dose-dependent inhibition of the respiratory drive. The inhibited respiratory drive often results in a decreased respiratory rate with an increased tidal volume resulting in a relatively stable minute volume (Muir & Hubbell 2007b). Sedation with ACE in horses is often accompanied by a mild elevation in heart rate, with no appreciable change in respiratory rate and an overall relaxation effect on the horse. (Muir & Hubbell 2007a; Clarke et al. 2014b).

Haematology and biochemistry

Haematology

The haematological measurands from various studies that report the effect of sedation in horses is summarised in Table 1.2.1.

Table 1.2.1 Reported effects of sedation of horses on the haematological measurands.

Study/Article	Parameter	Change	Sedation to sampling (time)	Sedation (dose)
Gasthuys et al. 1987	PCV, RCC	Initial (non-significant) increase, followed by a decrease	30 mins, every 30 mins to 2.5 hrs, final collection 72 hrs	DET (0.04 mg/kg)
	Total WCC	No significant changes		
Mostafa et al. 1995	Ht, haemoglobin, RCC, total WCC, differential WCC	No significant changes	30, 60 & 90 mins	DET (0.05, 0.01, 0.02 & 0.04 mg/kg)
Serpa et al. 2015	PCV, haemoglobin	Significant decrease	60 mins	DET CRI 0.02 mg/kg/hr
Dalton 1972	Ht,	Decrease (16%),	[exercised, rested & sedated – no times given]	ACE (30 – 60 mg)
	plasma protein, erythrocyte sedimentation rate	decrease, Unchanged to increased (by up to 10mins)		
Lumsden et al. 1975	PCV,	Decreased (18%),	60 mins	ACE (0.006 mg/kg)
	Plasma protein	Unchanged		
Ballard et al. 1982	Ht	Significant decrease (20%)	[no times given for blood withdrawal]	ACE (0.02 mg/kg) (1 mg/horse)
Parry & Anderson 1983	Ht,	Decrease (14.5%),	60 mins	ACE (0.05 mg/kg)
	Total protein	Decrease (3.6%)		
Wood et al. 1992	Ht	Significant decrease	30 & 60 mins	ACE (0.01, 0.001 & 0.0001 mg/kg)
Chou et al. 2002	Htt, haemoglobin, RCC, total WCC, neutrophil & basophil counts	Significant decrease (Ht up to 30%)	1 to 8 hrs following sedation	ACE (25 mg)
Chou et al. 2002	MCV, RDW, MCH, MCHC, platelet concentration, Ht, basophil, eosinophil, monocyte, lymphocyte, CO_2, electrolytes, insulin and glucose	No significant changes	1 to 8 hrs following sedation	ACE (25 mg)
De Moor et al. 1978	Ht,	Decrease (16%),	15, 30 & 60 mins; and 1.5, 2, 3, 4, 5, 6, 8, 10, 12 & 24 hrs	Promazine-HCl (0.5 mg/kg)
	Plasma protein	Decrease (2%)		

DET: detomidine; ACE: acepromazine maleate; promazine-HCl: promazine-hydrochloride; min: minutes; hrs: hours; PCV: packed cell volume; Ht: haematocrit; WCC: white blood cell count; MCV: mean corpuscular volume; RDW: red cell distribution width; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; CO_2: carbon dioxide; mins: minutes; hrs: hours.
*PCV is a directly measured value after microhaematocrit centrifuge, while haematocrit is a calculated value carried out by haematology analysers [Ht = (MCV x RCC)/10] and is the value that we have investigated in the present study.

In contrast to most DET studies reporting a significant and marked decrease in haematocrit, Gasthuys et al. (1987) reported no significant change in the haematocrit. They reported an insignificant initial increase within thirty minutes, followed by a decline (Gasthuys et al. 1987). This effect on haematocrit was attributed to either an increase in urine production causing fluid loss from the body; or the activation of a "capillary fluid shift mechanism"; or the release of the red blood cells sequestered in the spleen. To date, the reason for haematocrit changes following sedation has not been fully elucidated, however, the fluid shift seemed to be more widely adopted and reasoned that increased blood pressure resulted in circulating intravascular fluids leaving the blood vessels in order to reduce the blood pressure. Other studies, such as Gasthuys et al. (1987), speculate the decline in haematocrit may be due to redistribution of interstitial and intracellular fluids to intravascular spaces or the sequestering of red blood cells to the spleen. Serpa et al. (2015) pointed to the decrease in haematocrit over time as corroboration of clinical evidence that alpha-2-adrenoceptor agonists cause relaxation of the splenic capsule, producing splenomegaly and red blood cell sequestration, in agreement with Daunt et al. (1993) and Wagner et al. (1991). Electron microscopic evidence of alpha-adrenoceptors has been identified within the pulp of the spleen of horses (Tablin & Weiss 1983), which may explain the drug related changes with respect to blood storage and expulsion from the spleen (Serpa et al. 2015). Wood et al. (1992) showed that ACE appears to have a greater effect on haematocrit compared to the current evidence on DET.

Dalton (1972) proposed that haematocrit and total protein changes after ACE administration were due to a plasma volume expansion or the haemodilution effect originally postulated by Hoe & Wilkinson (1957). However, Parry & Anderson (1983) showed that the mechanism was not as a result of haemodilution due to plasma volume expansion as originally proposed but rather caused by splenic sequestration of red blood cells. Parry & Anderson (1983) also determined that the magnitude of the haematocrit decrease was not necessarily as a result of

the dose or route of ACE administration. They further concluded that the duration of the response to ACE administration was dose, rather than route dependant. Parry & Anderson (1983) agreed with Lang et al. (1979) that the amount by which the haematocrit was decreased was likely dependant on the splenic storage capacity for red blood cells, therefore the haematocrit decrease would plateau, even with increasing ACE dosage.

Biochemistry

The effect of DET and ACE on the biochemical analytes in horses appear to be less well investigated compared to the haematological measurand effects. The effects of DET and ACE on liver-associated enzymes and insulin-glucose effects have been described (Gasthuys et al. 1987; Robertson 1987; Mostafa et al. 1995).

Liver-associated enzymes

Mostafa et al. (1995) determined that serum concentrations of aspartate amino transferase (AST) and alanine aminotransferase (ALT) fluctuated within the normal physiological ranges of the donkeys in the study, after DET administration. This outcome lead to the recommendation that DET can be safely administered to sedate horses suffering liver problems, which contradicted recommendations by Short (1992).

Studies determining the effect of ACE on equine liver enzymes are lacking. One study investigating premedication with ACE and changes in liver enzymes at induction, during and following anaesthesia Taylor (1989) reported "*considerable variation in enzyme activities and changes [*that*] were neither remarkable nor significant*".

Table 1.2.2 Studies reporting various serum biochemistry effects following sedation in horses.

Study	Parameter	Change	When sampled	Sedation (dose)
Gasthuys et al. 1987	Glucose	Significant increase (peak at 1.5 hrs, return to normal by 72 hrs)	30 mins post sedation & every 30 mins to 2.5 hrs, final collection 72 hrs	DET (0.04 mg/kg)
	Electrolytes (sodium, chloride, magnesium, potassium, calcium), metabolites (urea), enzyme concentrations	No significant changes		
Kullman 2011	Glucose, sodium	Significant increase over time	30, 45, 60, 75 & 90 mins	DET (0.01 & 0.02 mg/kg)
	BUN, calcium	Significant decrease over time		
	Potassium, chloride & lactate	No significant change over time		
Mostafa et al. 1995	AST, ALT, BUN, creatinine	No significant changes	30, 60 & 90 mins	DET (0.05, 0.01, 0.2 & 0.04 mg/kg)
Robertson 1987	Glucose	Fell significantly, but returned to normal by 20 mins	Between 15 and 20 mins post sedation	ACE (0.1 mg/kg)
	Lactate	Significant increase		
	NEFA	Significant increase		
	Cortisol	Large individual variations, no significant changes		
	Insulin	Significant depression		
Thurmon et al. 1982	Glucose	Increased to peak by 45 min, still increased at 180 min	15, 30, 45, 60, 90, 120, 150 & 180 mins	Xylazine (1.1 mg/kg)
	Insulin	Decreased at 15 min, thereafter increase to peak at 150 min		

DET: detomidine; ACE: acepromazine maleate; mins: minutes; hrs: hours; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; NEFA: non-esterified fatty acid

Insulin, glucose and cortisol

Robertson (1987) reported hypoglycaemia following ACE administration, this significant decrease was, however, corrected within 20 minutes of the sedation. The mechanism for this drop was postulated to be as a result of either splanchnic vasoconstriction which the author then postulated would alter glycogenolysis and glucose release, or due to anaerobic glycolysis as lactate concentrations were likewise increased.

More often, however, hyperglycaemia following sedation with α-2 agonists is described and has been suggested to be dose-dependent (Gasthuys et al. 1987, Clarke 1988). The mechanism for the hyperglycaemia is suspected to be caused by either a direct action on the *beta* cells of the islets of Langerhans of the pancreas which inhibits insulin release; or by an inhibition of the oxidation of glucose at the cellular level (Thurmon et al. 1982; Gasthuys et al. 1987). Regardless of the cause, both can result in a decreased energy supply to the muscles. According to Serpa et al. (2015) the *"impact and extension of these changes has still not been fully elucidated"*, even though changes can be mild, they could be a problem in animals with muscular or other metabolic dysfunctions. Furthermore, contrary to earlier evidence, Serpa et al. (2015) concluded that DET induced hyperglycaemia regardless of the dose administered. The proposed inhibition of insulin release could also produce an impairment of blood glucose uptake in insulin-dependent tissues, such as muscle and adipose tissue.

Exton (1982) found that alpha-2-adrenoceptors are present in the membrane of *beta* cells of the islets of Langerhans of the pancreas, and if stimulated, could inhibit the release of insulin. Glucose concentrations increased in response to xylazine, another type of α-2 agonist drug, and took 210 minutes from sedation to return to control concentrations. ACE appears to have a minimal effect on glucose metabolism and concentration (Dugdale 2010).

Cortisol levels have been shown to either decrease (Carroll et al. 1997, Raekallio et al. 1992) or not change (Carroll et al. 1997) in the horse following DET administration. The results of the decreased cortisol level study, however, could also be attributed to resting cortisol levels that were too low to note significant changes and/or the small samples size (Raekallio et al. 1992).

Storage of blood samples prior to analysis

Biochemistry results are affected by pre-analytical variables, such as collection methods, sample handling, and sample storage (details in Materials and Methods section); as well as analytical methodology. In this study these variables were all standardised from collection to processing of samples. Biochemical analyte analyses were conducted in batches on stored samples. The haematology measurands were analysed on non-stored samples, and as such storage of the samples is not relevant. Here, we consider previously determined effects of storage on results of analyte measurements, summarised in Table 1.2.3.

Table 1.2.3 Studies reporting effect of storage on equine serum biochemical analyte measurements.

Study	Parameter	Stability and storage
Grenn et al. 1976	PCV and plasma protein	Significant increase in couriered samples (unreported delay from sample collection to processing and analysis for all species tested).
	Haemoglobin	No statistical significance in couriered samples (for any species tested).
Rico et al. 1977	GGT	Not affected by moderate dilution or slight haemolysis. Activity only slightly decreased by storage at -30°C. Duration of storage was not reported.
Lohni 1977	LDH	For storage periods of up to 3 weeks, freezing at -20°C was most satisfactory; for 24-48 hrs storage at room temperature is optimal for repeatability. Most unsuitable temperature for lengthy storage was 4°C. Storage of clotted blood for up to 48 hrs may result in haemolysis and unexpected rises in activity.
Dorner 1981	ALT	Significant changes in serum ALT after addition of haemoglobin. Duration and temperature of storage was not reported.
West 1989	GGT	Stable in heparinized plasma for 1 month at -20°C.
Reimers 1991	Cortisol Insulin	Concentrations in equine plasma not affected by haemolysis. Concentration decreased at 2-4°C and 20-22°C, effect is exacerbated by haemolysis. Duration of storage was not reported.
Lindner 1992	AST, LDH, ALP, TP and ALB	No differences were found between serum samples and sodium-heparinised samples. Duration and temperature of storage was not reported.

Ferrante & Kronfeld 1994	Glucose	Concentration lower when blood was preserved with sodium fluoride/potassium oxalate compared with lithium heparin. Duration and temperature of storage was not reported.
Rendle et al. 2009		Separation of serum prior to storage is protective against effects of time delays before processing.
	AST, LDH	Delays in processing (72 hrs) of whole blood resulted in significant increases in activities.
	Glucose	Intense decrease from the first day when stored at 20-25°C.
Oberg 2012	Insulin	No significant decrease in insulin concentration for non-haemolysed samples when stored at 6-8°C for 30 days or at -20°C for 1 year.
Rendle et al. 2015	ACTH	Significant decreases in ACTH concentration in PPID horses from 4 to 8 hrs. ACTH should be measured within 48 hrs of sample collection if not frozen.
Prutton 2015	ACTH	Concentration significantly reduced at 24 hrs but remained stable when plasma was frozen at -20°C and -80°C for 30 days. Beyond 24 hrs samples stored at 21°C showed greater reduction in ACTH concentration than those stored at 4°C. Can be stored for 8 hrs without centrifugation, or frozen for 30 days without appreciable reductions in ACTH concentrations.
Collicutt 2015	Glucose	Storage at 4°C limits serum glucose declined for at least 4 hrs (all species tested) and up to 8 hrs in the horse (and alpaca). At 25°C serum-clot contact time should not exceed 1 hr in equine samples.
Carslake 2016	Insulin	Storage at room temperature for 72 hrs (serum or whole blood) has minimal effect on measured serum insulin concentration in horses.

PCV: packed cell volume; PP: plasma protein; TP: total protein; hrs: hours; °C: degrees Celsius; RBC: red blood cell; WBC: white blood cell; ACTH: adrenocorticotropic hormone; PPID: pituitary pars intermedia dysfunction; GGT: gamma-glutamyl transferase; LDH: lactate dehydrogenase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; ALT: alanine aminotransferase; hrs: hours.

Cuhadar et al. (2013) determined that human AST, ALT, creatinine kinase and glucose remained stable for up to 90 days when stored at -20°C. However, significant changes in total protein and albumin were reported and the changes were attributed to problems related to the stability of the proteins following freezing and thawing (Cuhadar et al. 2013).

Storage of serum samples for glucose and insulin testing has proved problematic in some instances. Clark et al. (1990) showed that human plasma glucose concentrations were found to decrease during storage by up to 11% if samples were frozen for longer than 24 hours (and up to fourteen months). The losses reported and the variability of losses in this study endorsed that samples frozen and then analysed would not give accurate results, and could not even be accurately calculated "backwards" with any confidence due to the high variability (Clark et al. 1990). Rosato et al. (2009) found that in cooled conditions canine proteins and enzymes (total

proteins, ALB, AST, ALT, ALP) remained stable, with the exception of GGT, for up to 30 days at -20°C. Equine CRP stability studies are lacking but samples frozen at -20°C and -80°C have been used in studies after being stored for an unreported length of time (Takiguchi et al. 1990; Leclere et al. 2015), 3 months (Hillström et al. 2015), and even frozen and thawed for four cycles (Hillström et al. 2014) and the values were deemed to provide clinically significant results. Other human studies have stored samples for CRP testing at -70°C for undetermined length of time until analysis (Roberts et al. 2001).

The effects of storage on equine biochemical analyte analyses is lacking or has not been recently reported on using modern techniques and analysers. To mitigate the unknown effects of storage at -20°C, general recommendations are to 1) reduce pre-analytical variability (same sampling technique and handling), and 2) conduct the analyses of the analytes as a batch (at the same time, using the same machine, technique and clinical pathologist or technician), should be done to provide clinically meaningful results (Rendle et al. 2009). In veterinary practice, the average time taken from collection to analysis has a tendency to be greater in horses and production animals when compared with people and small companion animals (Grenn et al. 1976). We concluded therefore that in order to obtain the most accurate results, standardised sample collection, handling, methods and storage were essential and as such adopted for the course of the study by the primary investigator.

The equine spleen

The spleen of the horse is highly innervated by the adrenergic system (Ballard et al. 1982) and its capsule is controlled by smooth muscular tissue. ACE is an antagonist of the adrenergic receptors and DET causes a central sympatholytic effect and therefore both may contribute to relaxation of the splenic capsule and result in the sequestration of circulating blood cells (Lang et al. 1979; Kullman et al. 2014). Parry & Anderson (1983) discussed the “dynamic storage

capacity of the spleen" which permits systemic mobilisation of red blood cells, during excitement and physical stress, by splenic contraction (Torten & Schalm 1964). "The alpha-adrenolytic activity" of phenothiazine tranquilizers, with vasomotor centre depression causes hypotension (Byck 1975) – probably causes splenic relaxation with consequent red blood cell storage and Parry & Anderson (1983) therefore surmised that the decrease in haematocrit was principally caused by splenic sequestration of red blood cells. Studies in which splenectomies were performed suggested that red blood cell mobilisation was prevented and eliminated the decrease in haematocrit following intravenous administration of promazine (De Moor et al. 1978).

Shortfall in the literature and problem statement

Previous studies have highlighted that administering a sedative drug to a horse results in haematological and biochemical changes. However, what is not clear is how soon these effects happen. Furthermore, there are some rarely reported haematological measurands (total WBC, platelet concentrations) and biochemical analytes (ACTH, LDH, Cortisol) that warrant investigation. To address the shortfall in the literature, we have therefore proposed to investigate the scantly described haematological and biochemical variables in horses that are administered DET and ACE. We also proposed to take serial blood samples soon after administering the drugs to determine whether the anticipated haematological and biochemical shifts take place earlier than commonly reported and as a form of simulating normal field conditions of sampling.

1.3 Aim and objective

The present study aimed at determining what the effects of DET and ACE administration would be on haematological and biochemical variables in horses within one hour of undergoing standing sedation.

The aim was accomplished by obtaining control blood samples before administering DET or ACE and then obtaining repeated blood samples at standardised time intervals until 60 minutes. These blood samples were then analysed in order to determine what the drug effects on haematological and biochemical variables were in horses.

1.4 Hypotheses

The primary hypothesis was:

> H0: There will be no difference in haematological and biochemical variable values over time in horses administered DET or ACE.
>
> H1: There will be a difference in haematological and biochemical variable values over time in horses administered DET or ACE.

The secondary hypothesis was:

> H0: There will be no difference in haematological and biochemical variable values after DET compared to ACE administration in horses.
>
> H1: There will be a difference in haematological and biochemical variable values after DET compared to ACE administration in horses.

Chapter 2: Materials and methods

2.1 Study design and experimental procedures

Study design

A collaborative blinded randomised, comparative study was conducted between the University of Pretoria and the Vaal University of Technology (Animal Ethics Committee approval certificate number V099-16).

Animals

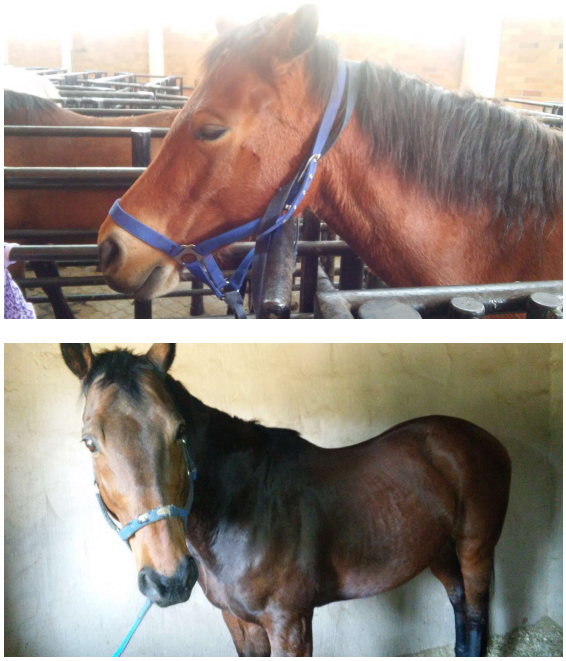

Image 1 An OTAU (above) and privately owned (below) horse prior to blood collection

Twelve healthy Nooitgedachter horses (mean ± standard deviation age: 8 ± 1 years; weight 413 ± 77 kg) from the Onderstepoort Teaching Animal Unit (OTAU) and four healthy privately-owned Thoroughbred horses (age: 18 ± 5 years, estimated weight: 450 ± 30 kg) were enrolled (Image 1). Owner consent was obtained prior to enrolment. All horses were managed according to their daily routine for the duration of the study. We calculated a minimum of 8 horses per treatment group (standard deviation: 2; margin of error: 2 units; alpha: 0.05; minimum power: 0.8).

Experimental procedures

Prior to experimental procedures, the horses were randomly assigned (online randomisation website http://www.randomization.com) to receive a single intravenous treatment bolus of either:

- detomidine hydrochloride (DET; 0.01 mg kg^{-1}; *n* = 6 OTAU horses and *n* = 2 private horses; Domosedan, Zoetis)
- acepromazine maleate (ACE; 0.05 mg kg^{-1}; *n* = 6 OTAU horses and *n* = 2 private horses; Neutrotranq PRO INJ, Alfasan)

The treatment drugs were drawn up into a 2 mL syringe and wrapped with opaque 25 mm adhesive tape to conceal the contents of the syringe in an effort to blind the primary investigator of the treatment.

One week later, a randomly selected control group was formed from within the horses enrolled in the study (CON; n = 6 OTAU and n = 2 private horses). This control group received no treatment drug at this assessment point but underwent the same experimental procedures. The experimental procedures and data collection were done at the same time of day on each of the data collection days for all horses.

OTAU horses:

On day one, six of the 12 OTAU horses were fetched from their paddocks and lead to the Onderstepoort Veterinary Academic Hospital (OVAH) where they were weighed (equine electronic floor scale) and then lead to a herringbone crush facility where they were allowed to rest for 30 minutes.

After the rest period, the study procedures commenced in the following steps:

1) collection of baseline data,

2) intravenous administration of the randomly assigned treatment (Time zero: T0),

3) data collected at 15-minute intervals until 60 minutes (T15, T30, T45 and T60),

4) recovery from treatment before being returned to their respective paddock and allowed to continue their daily routine (Image 2).

On day two, the remaining six of the 12 OTAU horses were enrolled into the study and completed the procedures steps 1 to 4 as already described. One week later, six of the 12 OTAU horses were randomly selected as a control group. The procedures were repeated as described but with the exception of step 2 where no treatment was administered.

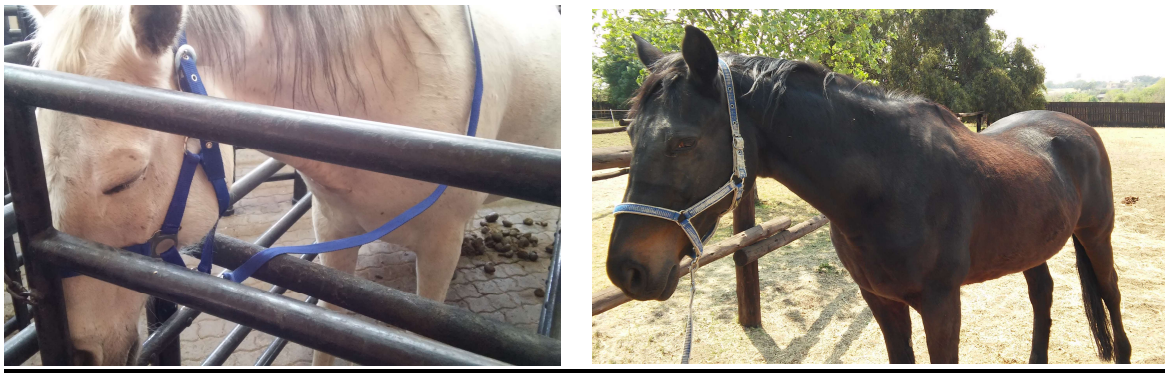

Image 2 An OTAU (left) and privately owned (right) horse recovering after the data collection phase.

Privately-owned horses:

Four horses from Randjesfontein were enrolled into the study. The yard was 65 kilometres from Onderstepoort and required a 60 to 80-minute travel time by car, depending on traffic. On the day of data collection, the horses were brought from grass paddocks into their stables and then allowed to rest for 30 minutes. During the rest period the body weight was estimated by an experienced equine clinician taking girth and shoulder height measurements into consideration. After the rest period they underwent the same procedure steps 1 to 4 as already described. One week later, two of the four privately owned horses were randomly selected as a control group. The procedures were repeated as described but with the exception of step 2 where no treatment was administered. Both data collection sessions were completed within a single day.

2.2 Data collection and analysis

Image 3 Preparation for blood collection.

On the mornings of the data collection days, prior to blood sampling a self-sealing waterproof bag (Ziploc; Glad; South Africa) with blood collection vacuum tubes (BD Vacutainer tubes; BD-Plymouth) was prepared for each time point per horse (Image 3). A cooler box was prepared by placing ice and water inside an insulated polystyrene cooler box (Image 4) to achieve an environmental temperature below 10°C (monitoring using an analogue thermometer every 15 minutes). Furthermore, ethylenediaminetetraacetic acid (EDTA) tubes (BD vacutainer K2E [EDTA],

Image 4 Cooler box with blood samples in self-sealing waterproof bags, ice and thermometer.

BD-Plymouth), were chilled overnight in a standard freezer and then placed in the cooler box to keep them chilled prior to sampling.

At each data collection time point, first the level of sedation was evaluated using the subjective simple descriptive scale (Table 2.1.1, Taylor et al. 2014).

Table 2.1.1 Simple Descriptive Scale (SDS) monitoring system used in the study. Adapted from Taylor et al. (2014).

SDS scoring system for sedation and ataxia

SEDATION	**0**	No sedation. Animal is alert, with normal posture and response to contact with assessor. Normal objection to intervention
	1	Mild sedation. Low head carriage, relaxed facial muscles and pendulous lower lip. Some response to intervention
	2	Moderate sedation. Head lowered towards ground and swaying of hind legs. Slight response to intervention
	3	Marked sedation. Attempts to or becomes recumbent. No response to intervention
ATAXIA	**0**	No ataxia. Animal stands and walks normally; is able to turn lightly
	1	Mild ataxia. Animal able to walk, but some lack of limb control
	2	Moderate ataxia. Animal can walk only with support, staggers but saves itself from falling
	3	Marked ataxia. Animal is unable to walk without danger of falling, staggers, falls if turned

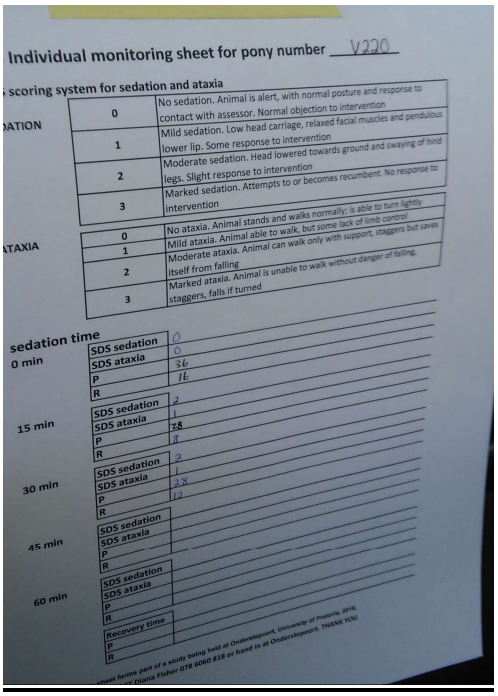

Image 5 Data collection form used in the present study.

The SDS score was recorded (Image 5) along with the clinical variables from a basic clinical examination to determine the heart and respiratory rate by thoracic auscultation. The form in Image 5, used to record these findings, is Data Collection Form 3.4 and can be found in the Appendix.

Then, jugular venous blood was sampled using a proprietary blood collection system (BD vacutainer precision glide multiple sample needle and holder/shoulder). A single venepuncture into the jugular (alternating left and right for each collection) using a 20 gauge needle attached to a shoulder and then blood was collected into the following 5ml vacuum tubes, as follows in order (Clinical and Laboratory Standards Institute Recommendations H21):

- Serum x 2 (BD vacutainer CAT [clot activator tube], BD-Plymouth)
- Citrate x 1 (SG vacutainer Sodium Citrate, BD-Plymouth)
- Chilled EDTA x 1 (BD vacutainer K2E [EDTA], BD-Plymouth)
- EDTA x 1 (BD vacutainer K2E [EDTA], BD-Plymouth)

A total of 25 ml of blood was then sampled per horse per collection time point. Blood samples collected from the OTAU horses were sealed in individually marked (horse and time) waterproof bags and placed into the cooler box (Images 3 & 4). Once all six horses were sampled, for each collection time point (T0, T15, T30, T45 and T60), then the cooler box was taken to the onsite OVAH Clinical Pathology Laboratory (OVAH-CPL) for processing. One

serum tube, chilled EDTA tube and citrate tube were immediately placed into a chilled centrifuge and spun, at 5000 revolutions per minute for five minutes, to separate the serum or plasma from the cellular content. The serum or plasma was pipetted into Eppendorf cryovials and stored in a -20°C freezer until being transported to the Vaal University of Technology Clinical Pathology Laboratory (VUT-CPL) for measurand analysis. The remaining EDTA and serum tubes were processed and analysed by the OVAH-CPL on every collection date.

Blood samples collected from the privately-owned horses were handled differently. Once all blood samples had been collected at each collection time point, the chilled EDTA and citrate tubes were centrifuged immediately at 5000 revolutions per minute for five minutes. Once centrifuged the plasma was pipetted into Eppendorf cryovials. The serum tubes, remaining EDTA tube and the Eppendorf cryovials were sealed in individually marked (horse and time) waterproof bags and placed into the cooler box before the next data collection time point. Once complete, the cooler box was transported to the OVAH-CPL for processing. The OVAH-CPL processed and analysed the remaining EDTA and serum tube within four hours of collection. The remaining serum tube was centrifuged and the serum pipetted into Eppendorf cryovials. All Eppendorf cryovials were stored in a -20°C freezer until being transported to the VUT-CPL for measurand analysis.

Prior to analysis samples were visually assessed for haemolysis, lipaemia, and icterus by the primary investigator and the quality control manager of the OVAH-CPL. Quality control and quality assurance protocols were followed as per OVAH-CPL routine and these were found to be satisfactory.

The -20°C samples were transferred to the VUT-CPL by the technicians working in the laboratory and who were experienced with handling and transport of frozen samples. They used a purpose-built cooler box containing dry ice to keep the temperature below 0°C and a

thermometer to maintain a constant temperature and ensure no sample thawing occurred during transport.

Haematology and biochemistry

Haematological measurands and biochemical analytes of interest were analysed by experienced lab technologist and specialist veterinary and human clinical pathologists at the two laboratories as follows:

OVAH-CPL

- Haematology using differential cell counts (ADVIA 2120 Hematology System; Cobas, Integra 400 Plus; Roche Products (Pty) Ltd, South Africa)
    - Blood smears were Diff-Quik-stained and checked by OVAH-CPL lab technologists; results were confirmed by clinical pathologists in the OVAH-CPL, as per standard OVAH-CPL protocol.
- Serum cortisol using automated solid-phase, competitive chemiluminescent enzyme immunoassays (IMMULITE/IMMULITE 1000 Cortisol analyser; Siemens, Isando, South Africa)

VUT-CPL

- ACTH using ELISA (DEMEDITEC ACTH Immunoassay, Demeditec Diagnostics GmbH, Kriel, Germany) and immunoturbidity assay (Tosoh AIA-600II Automated Enzyme Immunoassay Analyzer, Tosoh Corporation, Tokyo, Japan)
- ALP, ALT, AST, GGT, LDH using kinetic colorimetric enzymatic assays (Indiko Clinical and Speciality Chemistry System, Thermo Fisher Scientific Massachusetts, USA)

- Glucose using Glucose Hexokinase reagent assays (Indiko Clinical and Speciality Chemistry System)
- ALB using Bromocresol Green and TSP using biuret reaction assays (Indiko Clinical and Speciality Chemistry System)
- Serum insulin using one-step and two-step sandwich and competitive fluorescence enzyme immunosorbent assays (Tosoh AIA-600II Automated Enzyme Immunoassay Analyzer)
- CRP using microparticle enhanced immunoturbidimetry assays (Indiko Clinical and Speciality Chemistry System).

- Immunochemical cross-reactivity of human CRP and equine CRP has recently been substantiated in a review of CRP (Pathak & Agrawal, under peer-review).
- ACTH was determined from the plasma from the chilled EDTA sample tube, glucose from the serum from the citrate sample tube, and other biochemistry analytes from the serum from a serum tube.
- Methods of analysis for the analytes analysed at the VUT-CPL have not all been validated for use in equines as yet. These samples were analysed as part of a validation study at the VUT-CPL.

The reference intervals used for each analyte in this study were then not specific to the assays for each analyte; but rather taken from well-documented reference intervals, as indicated in the Results Table (Table 3.3.1). However, the results provide a comparative framework for the overall interpretation of the short-term effects of sedation on the various serum biochemistry analytes and whether their respective concentrations are increased, decreased, or not significantly altered.

Statistical analysis

Data were assessed for normality by evaluating descriptive statistics, plotting of histograms, and performing the Anderson-Darling test for normality. Quantitative data (clinical, hematological and biochemical variables) were compared among treatments using a two-way analysis of variables. Significant findings were then compared using Dunnett's method of post-hoc analysis where the baseline data (T0) within each treatment group was used as the control variable. Categorical data were compared among treatments using the Friedman Test (sedation scores). Data were reported as mean (minimum, maximum), to appreciate the entire range of values obtained, unless otherwise stated. Data of variables demonstrating a statistical or clinically relevant change over time were plotted on 95% confidence interval plots for visual evaluation. Data were analysed using commercially available software (MiniTab 17.1.0; MiniTab Incorporated) and results interpreted at the 5% level of significance ($p < 0.05$).

2.3 Limitations

The OTAU horse unit could only allow the enrolment of 12 horses on a set amount of days during the study data collection period. Therefore, we had to enrol an additional four privately owned horses into the study to meet the minimum sample size calculation. The sixteen horses provided an adequate sample size when compared with similar studies investigating drug effects and determining measurands and analytes in equines (Buhl et al. 2007, Carroll et al. 1997, Freeman & England 2000, Haritou et al. 2008, Hemmann et al. 2011, Thurman et al. 1982). The number of horses enrolled in these studies varied from four (Gasthuys et al. 1987 and Kullman et al. 2014 measuring haematological, biochemical, and other variables) to 30 (Freeman & England 2000 studying the effects of romifidine and detomidine) in number but most report the enrolment of eight horses. Due to OTAU horse unit time constraints, we were unable to conduct a crossover study. We also acknowledge that the sample of horses were predominantly Nooitgedachter horses. According to historical haematological and biochemical records of OTAU horses obtained from the OVAH-CPL, we did not anticipate any breed-specific peculiarities in the various haematological measurands and biochemical analytes. While horses from the OTAU unit were weighed prior to sedation, no scale was available at the privately owned horses' yard and as such the weights of these four animals were estimated in order to calculate the dose of sedation required.

Samples had to be frozen at -20°C and were then stored for up to 90 days prior to being transferred to the VUT-CPL. Upon arrival at the VUT-CPL samples were thawed and analysed as a batch. Although the prolonged storage time is not ideal, as discussed in the literature review, all samples were treated uniformly, and as such measurements were surmised to be acceptable and accurate enough for comparative clinical interpretation of the sedative drug effects. As previously mentioned, not all of the biochemistry analysis methods used are

currently validated in equines, while validated for other species these samples were run as part of a validation study that was being conducted by the VUT-CPL.

2.4 Ethics

The study was approved by the Animal Ethics Committee of the University of Pretoria. The welfare and safety of the horses was always a priority. Horses that were not regarded as clinically healthy, based on a basic clinical evaluation (rectal temperature, pulse rate and respiration rate) and visual inspection, were excluded from the study. Potential major complications that could arise during the study procedures included:

1) Excitement or anxiety associated with being confined in the herringbone crush or stall;
2) Excessive sedation where the horse becomes recumbent;
3) Haematoma formation during venepuncture;
4) Repeated (or inappropriate) venepuncture may also result in thrombosis or jugular vein thrombophlebitis which may result in loss of normal blood flow in a jugular vein.

If any of these major complications occurred, then the following rescue interventions were provided:

1) Horses were excluded from the study if they could not adapt to the temporary confinement. The excluded horse could be replaced with another horse;
2) If due to DET then a single bolus of atipamezole (5 mg mg^{-1} detomidine dose) was administered intramuscularly. If due to ACE then an intravenous catheter was placed and a bolus of isotonic crystalloid fluid was administered. In all cases, the positioning of the horse was rapidly determined to identify trapped limbs, or body positions that compromised breathing efforts. If these complications were noted, then the horse was

manually moved outside of the crush before administering other appropriate treatments. Then the horse was excluded from the study;

3) Manual pressure was immediately applied to the venepuncture site of the vein for 5 minutes.

If any of these major complications occurred, then the event was reported to the Animal Ethics Committee.

Chapter 3: Results

All of the horses completed the study procedures. However, four horses sustained a jugular haematoma that resolved with rescue intervention and without further complication.

The measured variables changed over time but mostly within laboratory reference intervals, or where these were not available, published reference intervals for the variable. However, there were variables, even in the control group, where the values were either less or greater than the reference interval. Values outside the reference interval are indicated in bold in Tables 3.2.1 and 3.3.1 for easy reference.

3.1 Clinical variables

The sedation scores, heart and respiratory rates and temperature changed in predictable patterns over time (Table 3.1.1). The sedation scores were significantly higher, indicating sedation, in the horses that received DET or ACE when compared to CON, indicating the drugs exerted a sedative effect ($p < 0.01$ for both drugs).

Table 3.1.1 Clinical variables measured over time of horses receiving acepromazine maleate (ACE) and detomidine hydrochloride (DET) or no treatment (CON).

Variable	Reference interval		Treatment	T0		T15		T30		T45		T60	
	min	max		mean	min max	mean	min max	mean	min max	mean	min max	mean	min max
Sedation score	0	6	CON	0	0 0	0‡	0 0	0‡	0 0	0‡	0 0	0‡	0 0
			ACE	0†	0 0	2†	1 2	2†	2 2	2†	1 2	1†	1 2
			DET	0†	0 0	2†	2 3	3†	2 3	2†	2 3	2†	0 3
Heart rate (Beats minute^{-1})	30	40	CON	43	36 48	35	32 36	37	28 44	37	32 44	35	28 40
			ACE	32	28 40	36	30 44	35	32 38	33	28 42	34	28 38
			DET	32	24 44	28‡	20 34	28‡	20 36	28‡	24 32	31	24 36
Respiratory rate (Breaths minute^{-1})	8	16	CON	15	12 16	13	12 16	13	12 16	11	8 12	13	12 20
			ACE	13†	10 16	14	8 20	11†	8 18	13	8 28	13	8 20
			DET	18†	12 25	13†	8 20	11†	8 12	10	8 16	10	8 12

CON: control; ACE: acepromazine maleate; DET: detomidine hydrochloride; †: $p < 0.05$ time effect; ‡: $p < 0.05$ time x treatment effect
Sedation score reference: Taylor et al. (2014), see Table 2.1.1 (*supra*)
Reference for heart and respiratory rate: Corley & Stephen (2008)
Bold values are outside the reference intervals.

3.2 Haematology

As indicated in Table 3.2.1, the haematocrit decreased following DET and ACE over time (both $p < 0.01$) but not in CON ($p = 0.09$; Figure 3.2.1a). The changes over time for the red blood cell count (RCC) were similar to that of the haematocrit (Ht, both $p < 0.01$; Figure 3.2.1b). The total white cell count (WCC) decreased significantly over time after DET and ACE compared to CON (both $p = 0.03$; Figure 3.2.1c). The platelet concentrations were not different among groups and over time.

Table 3.2.1 Haematological measurands over time of horses receiving acepromazine maleate (ACE) and detomidine hydrochloride (DET) or no treatment (CON).

Variable (unit)	Reference interval		Group	T0		T15		T30		T45		T60	
	min	max		mean	min max	mean	min max	mean	min max	mean	min max	mean	min max
Ht ($L\ L^{-1}$)	0.30	0.45	CON	0.38	0.33 0.40	0.36	0.33 0.39	0.38	0.33 0.42	0.36	0.32 0.41	0.36	0.31 0.40
			ACE	0.39†	0.34 0.46	0.34†	0.31 0.42	0.32†	**0.29** 0.39	0.32†	**0.28** 0.39	0.32†	**0.29** 0.39
			DET	0.40†	0.34 0.44	0.35†	0.32 0.37	0.33†	0.30 0.37	0.33†	0.30 0.35	0.33†	0.31 0.36
RCC ($x10^{12}\ L^{-1}$)	6.0	10.4	CON	7.5	6.7 7.9	7.3	6.4 7.9	7.7	6.1 8.5	7.3	**5.9** 8.2	7.8	**5.8** 8.0
			ACE	8.0†	6.6 9.2	6.8†	**5.8** 8.3	6.5†	**5.6** 7.7	6.5†	**5.7** 7.7	6.5†	**5.5** 7.6
			DET	7.9†	7.1 8.7	7.0†	6.5 7.4	6.5†	**5.9** 7.5	6.5†	**5.6** 7.1	6.6†	**5.5** 7.3
Total WCC ($x10^{9}\ L^{-1}$)	5.5	12.1	CON	10.9	10.1 **13.0**	10.8	9.5 **12.2**	11.3	9.8 **12.3**	10.9	9.2 12.1	10.6	9.9 11.7
			ACE	9.2†	**4.6** **12.4**	7.6†	**3.9** 9.8	7.3†	**3.9** 8.9	7.1†	**3.8** 8.8	7.2†	**3.7** 9.2
			DET	8.6†	6.8 10.6	7.1†	5.6 8.9	6.5†	**5.0** 8.2	6.7†	**4.8** 8.4	7.0†	**4.8** 9.5
N:L	1.1	1.5	CON	0.9	0.7 1.3	0.8	0.5 1.1	0.9	0.6 1.2	0.9	0.5 1.4	0.8	0.6 1.1
			ACE	0.9	0.6 1.4	0.9	0.6 1.4	1.0	0.8 1.4	0.9	0.6 1.4	1.0	0.8 1.5
			DET	1.2	0.370 2.000	1.3	0.6 2.5	1.2	0.6 2.3	1.2	0.700 2.220	1.2	0.7 2.1
Platelet concentration ($x10^{9}\ L^{-1}$)	100	350	CON	**96**	**26** 177	**99**	**28** 176	**104**	**30** 175	**102**	**25** 174	**89**	**25** 166
			ACE	126	**79** 194	121	**76** 161	131	**75** 171	123	**75** 171	124	**71** 179
			DET	129	**38** 239	128	**36** 220	121	**35** 212	120	**40** 197	130	**39** 216

CON: control; ACE: Acepromazine maleate; DET: detomidine hydrochloride; Ht: haematocrit; RCC: red cell count; WCC: white blood cell count; N:L: Neutrophil to Lymphocyte ratio; †: $p < 0.05$ time effect. Bold values are outside the reference intervals.
Reference interval for haematocrit, red and total white cell count: OVAH Clinical Pathology reference interval established from a local population of horses
Reference interval for N:L and platelet concentration: Corley & Stephen (2008)

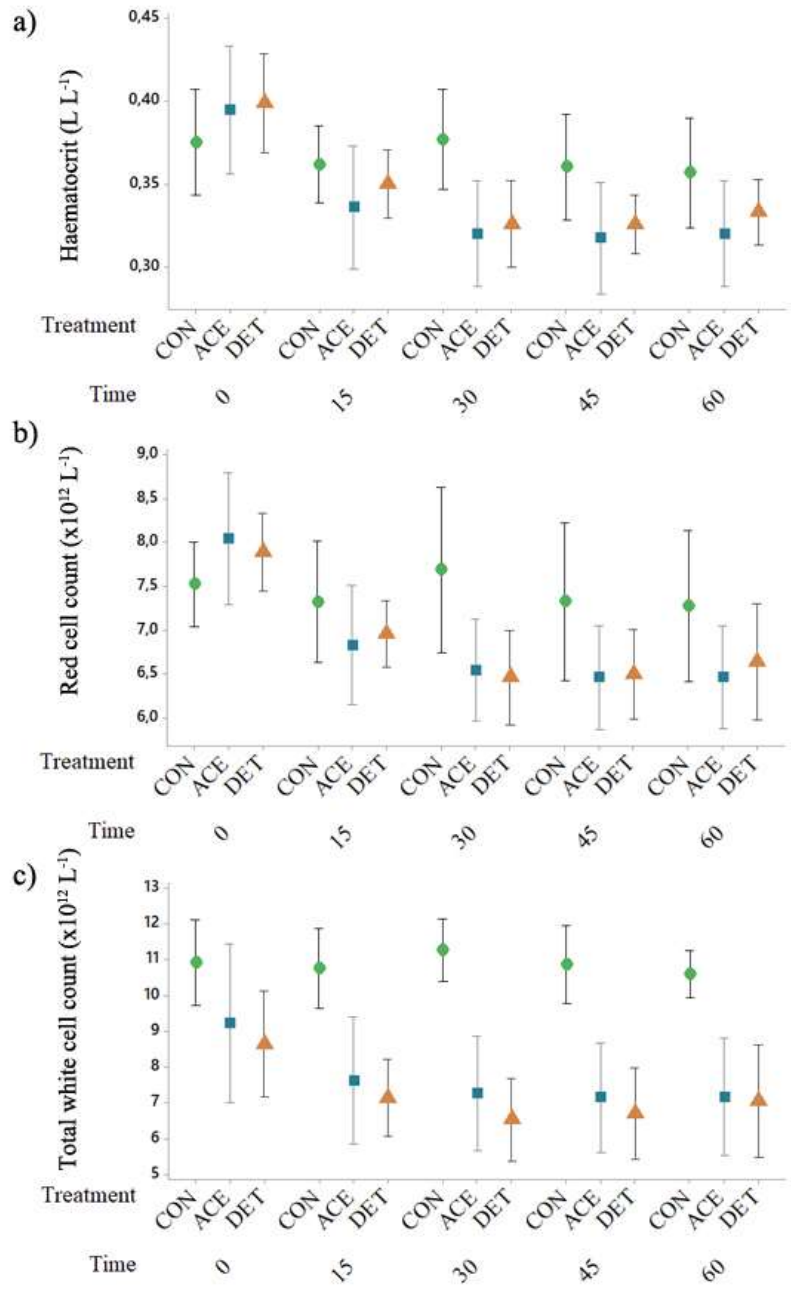

Figure 3.2.1 Confidence interval (95%) plots of mean (a) haematocrit, (b) red cell count and (c) total white cell count over time of horses receiving acepromazine maleate (ACE) and detomidine hydrochloride (DET) or no treatment (CON).

3.3 Biochemistry

Most of the biochemical analytes remained unchanged over time, however, there were statistically and clinically relevant observations. The total serum protein concentration (TSP, Figure 3.3.1a) and albumin concentration (ALB, Figure 3.3.1b) were similar between groups and remained consistent over time. The C-reactive protein (CRP) concentration decreased over time in all groups (p = 0.02; interaction: time). Serum enzyme activity of gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), AST and ALT did not show a difference among the groups and over time (Figure 3.3.2a, b & c). The endocrine (cortisol and ACTH) and metabolic analyte insulin did not demonstrate a change in concentration among groups and over time. The glucose metabolic analyte concentration, however , was raised from 30 minutes onwards within DET compared to ACE and CON which remained constant over time ($p < 0.01$; interaction: treatment x time; Figure 3.3.3a).

Table 3.3.1 Serum biochemistry results of over time of horses receiving acepromazine maleate (ACE) and detomidine hydrochloride (DET) or no treatment (CON).

Variable (unit)	Reference interval		Group	T0		T15		T30		T45		T60	
	min	max		mean	min max	mean	min max	mean	min max	mean	min max	mean	min max
Proteins													
TSP (g L^{-1})	58	77*	CON	68	**51** 73	64	**57** 72	59	**32** 72	66	60 73	66	60 72
			ACE	57	**52** 63	59	**49** 64	58	**49** 63	57	**48** 62	57	**48** 64
			DET	62	60 63	60	58 63	59	**54** 62	59	**54** 62	58	**52** 60
ALB (g L^{-1})	23	36*	CON	27	**18** 32	29	26 31	31	28 34	30	29 32	30	29 31
			ACE	29	26 31	28	27 41	28	26 30	28	26 29	28	27 29
			DET	30	28 31	31	29 39	30	28 33	29	28 32	29	28 32
GLOB (g L^{-1})	17	47*	CON	41	33 41	35	31 41	28	**4** 39	36	31 41	36	31 41
			ACE	28	26 32	31	22 23	30	23 33	29	22 33	29	21 35
			DET	32	32 32	29	24 29	29	26 29	30	26 30	29	24 28
CRP (mg L^{-1})	-	-	CON	79†	41 148	38†	11 78	65†	24 112	102†	8 207	72†	8 136

			ACE	32†	2 98	24†	0.1 77	33†	7 65	39†	21 78	18†	4 55
			DET	38†	10 67	26†	5 71	49†	34 89	60†	20 83	43†	12 83
Liver-associated enzymes													
ALP (IU L^{-1})	86	285*	CON	182	128 214	188	153 239	192	150 235	189	151 231	192	155 237
			ACE	127	89 170	124	80 164	123	88 148	130	84 163	123	83 163
			DET	167	92 252	156	89 238	148	84 228	142	82 222	151	83 231
ALT (IU L^{-1})	0.0	6.0#	CON	**8**	**8** **10**	**8**	6 **10**	**7**	6 **9**	**7**	6 **9**	**8**	**7** **11**
			ACE	5	4 6	5	4 6	5	4 **7**	5	3 6	5	3 **7**
			DET	6	5 **9**	5	4 6	5	4 6	5	4 6	5	4 6
AST (IU L^{-1})	138	409*	CON	318	271 406	303	249 **411**	308	254 **409**	305	251 377	303	250 378
			ACE	254	201 338	252	200 319	251	197 327	251	195 315	250	196 316
			DET	267	232 322	254	221 304	249	210 307	246	213 287	249	213 293
GGT (IU L^{-1})	8	22*	CON	15	**6** 19	12	**5** 17	13	**7** 19	13	**7** 18	12	**7** 17
			ACE	12	**6** **25**	12	**6** **25**	12	**6** **24**	12	**7** 22	12	**5** 22
			DET	10	**7** 14	10	**7** 15	9	**5** 16	9	**7** 14	10	8 14
LDH (U L^{-1})	172	403#	CON	**446**	400 **526**	**473**	401 **567**	**432**	323 **512**	**463**	389 **547**	**434**	260 **622**
			ACE	390	203 **522**	370	204 **489**	372	247 **507**	395	235 **518**	386	228 **496**
			DET	**446**	347 **520**	379	251 **501**	389	257 **500**	368	249 **471**	391	241 **474**
Endocrine and metabolic													
ACTH (pg mL^{-1})	9	41#	CON	16	11 19	13	10 17	12	**4** 20	14	11 22	13	**8** 19
			ACE	10	**4** 22	11	**5** 30	10	**4** 19	11	**5** 22	9	**4** 16
			DET	12	**5** 15	16	**7** 19	21	9 **61**	18	**8** 26	13	**6** 20
Cortisol (nmol L^{-1})	50	640$	CON	89	56 106	98	69 122	105	73 130	105	56 155	98	64 120
			ACE	80	50 135	95	53 159	100	52 172	112	63 173	123	90 184
			DET	94	65 162	99	63 144	111	69 160	103	70 159	101	70 165
Glucose (mmol L^{-1})	4.9	6.2*	CON	4.9	**4.6** 5.1	**4.5**	**4.2** **4.8**	**4.5**	**4.3** **4.8**	**4.6**	**4.3** **4.8**	**4.6**	**4.3** 4.9
			ACE	**4.5**	**4.0** 5.2	**4.6**	**4.1** 5.4	**4.5**	**4.1** 5.0	**4.4**	**4.2** 4.9	**4.5**	**4.1** 5.1
			DET	**4.4**	**4.1** 5.1	**4.8**‡	**4.0** 5.3	5.6‡	**4.6** **6.5**	5.5‡	**3.9** **6.5**	5.2‡	**4.2** **6.0**
Insulin (µU ml^{-1})	2.0	32.5#	CON	6.4	2.7 8.9	5.2	2.2 9.0	5.1	2.7 6.9	5.6	2.0 9.4	4.7	2.6 7.2
			ACE	6.4	**1.9** 22.9	6.5	**1.5** 26.3	4.6	**1.3** 15.1	4.4	**1.9** 10.4	4.8	2.0 13.1
			DET	6.3	**1.0** 15.5	**1.7**	**1.0** 3.1	2.2	**1.2** 3.7	3.1	2.1 4.9	5.6	3.1 12.1

CON: control; ACE: Acepromazine maleate; DET: detomidine hydrochloride; †: $p < 0.05$ over time; ‡: $p < 0.05$ time x treatment interaction. Bold values are outside the reference intervals.
Reference intervals:
*Corley & Stephen (2008).
#VUT Clinical Pathology reference interval established according to manufacturer's recommendations to obtain minimum and maximum ranges.
$OVAH Clinical Pathology reference intervals established from a local population of horses.

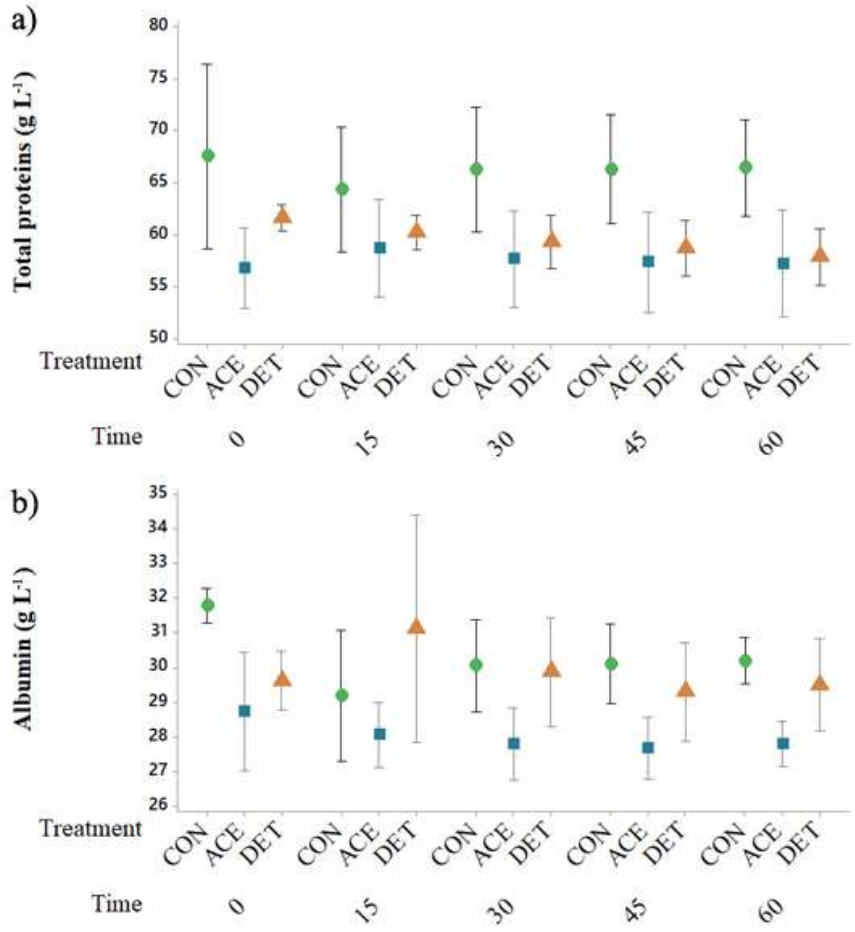

Figure 3.3.1 Confidence interval (95%) plots of mean (a) total serum protein and (b) ALB over time of horses receiving acepromazine maleate (ACE) and detomidine hydrochloride (DET) or no treatment (CON).

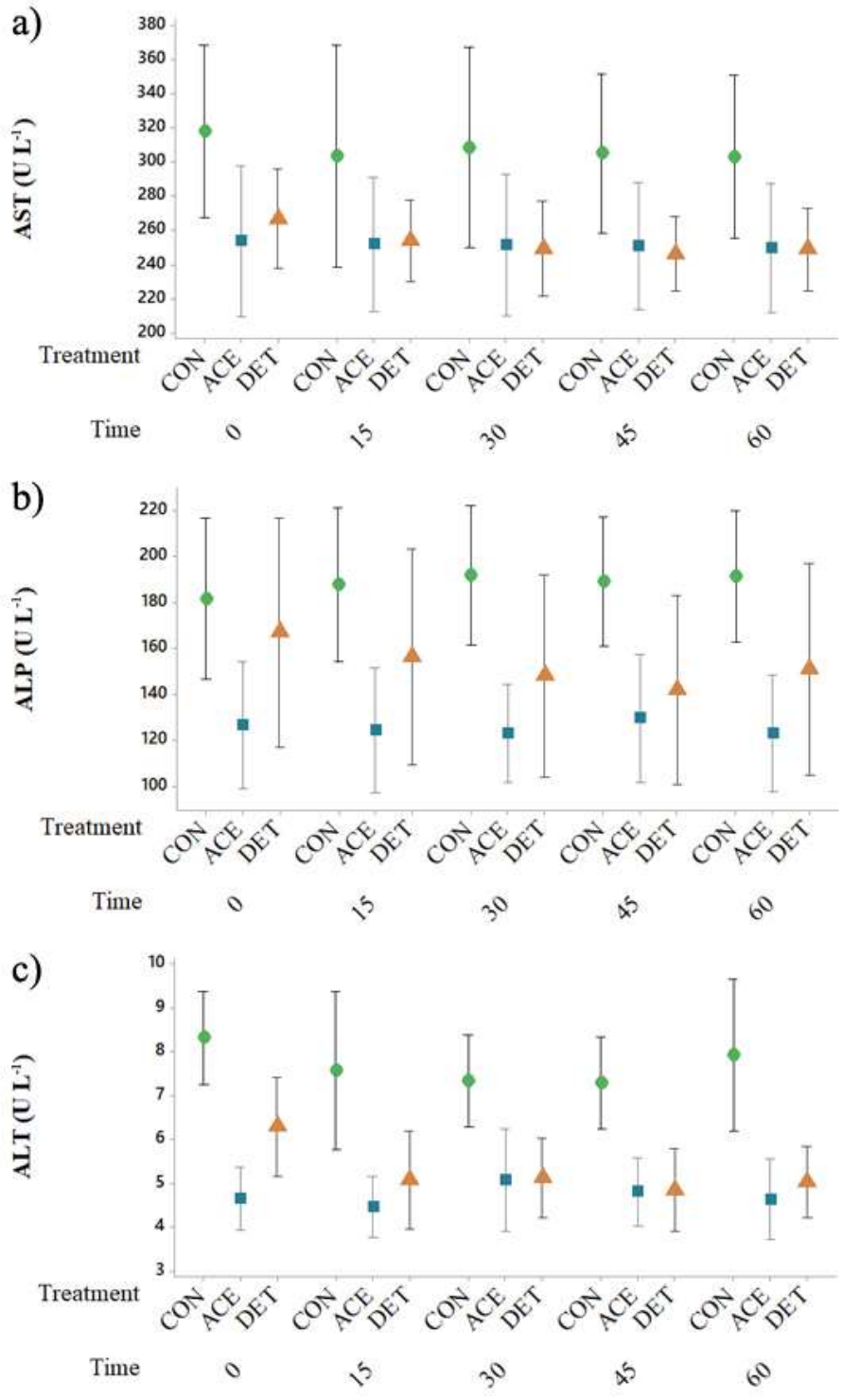

Figure 3.3.2 Confidence interval (95%) plots of mean (a) aspartate aminotransferase, (b) alkaline phosphatase and (c) alanine amino transferase concentrations over time of horses receiving acepromazine maleate (ACE) and detomidine hydrochloride (DET) or no treatment (CON).

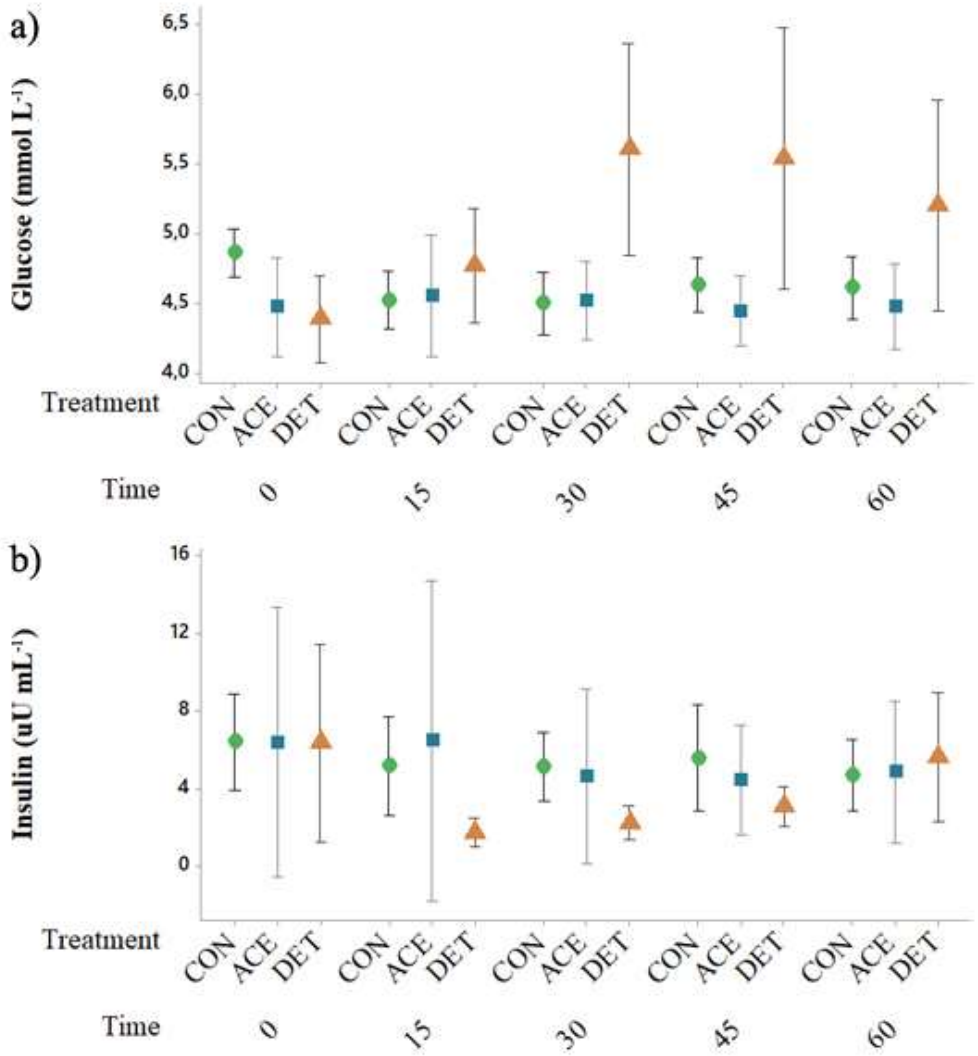

Figure 3.3.3 Confidence interval (95%) plots of mean (a) glucose and (b) insulin concentrations over time of horses receiving acepromazine maleate (ACE) and detomidine hydrochloride (DET) or no treatment (CON).

Chapter 4: Discussion and conclusion

4.1 Discussion

In horses, intravenous administration of acepromazine maleate and detomidine hydrochloride for sedation does alter clinical and haematological variables over time and statistically significant changes occurred within 15 minutes. Drug effects on biochemical variables were mostly statistically insignificant with the exception of the glucose concentration, which increased over time in detomidine hydrochloride-treated horses. Blood samples are used for diagnostic, therapeutic and prognostic purposes and as such the most accurate information available will improve these outcomes and be in the best interests of the equine patient. In most variables, there were changes over time that were within published and locally established reference intervals for horses. However, for some of the variables, even in the control group, values obtained were outside reference intervals. The reference intervals used in this study, to help interpret the clinical relevance of the changes in parameter values over time, were derived from a mixed population of horse breeds. However, our study population was made up mostly of Nooitgedachter horses and their values were within the intervals that were derived from a local mixed breed population (rather than the published reference intervals used) and no breed specific idiosyncratic findings were identified.

We did detect variable values outside of published and laboratory reference intervals. This finding could be because of normal individual variation (http://vetbiologicalvariation.org/database-tables-horse) or due to sample storage and stability, or analysis methods of the measurands and analytes (Braun et al. 2015; Ferrante & Kronfeld 1994; Reimers et al. 1991). The frozen samples that were analysed by the offsite laboratory were stored for up to 90 days before analysis. Previous studies have shown that most of the

analytes analysed from the stored samples remain stable over this time period (Rico et al. 1997; Rendle et al. 2009; Oberg 2012). However, there are mixed opinions regarding the stability of ALT, ALP, CRP, GGT, GLDH, LDH, AST and glucose in frozen samples (Lohni & Thornron 1977; Clark et al. 1990; Thoresen et al. 1995; Cray et al. 2009; Rendle et al. 2009; Rosato et al. 2009; Collicutt 2015; Hillström et al. 2015). The stored samples were analysed in duplicate, as part of the VUT-CPL study not related to the present study (not reported here), on consecutive days and values were found to be within acceptable references intervals, similar to another study which documented no significant changes in these values (Rendle et al. 2009; Cuhadar et al. 2013). Furthermore, the horses were healthy and we did not anticipate values outside of the reference intervals.

The dose of DET and ACE used in our study were equivalent or similar to dose rates reported in comparative studies (Muir et al. 1979; Parry & Anderson 1983; Jochle & Hamm 1986). At T15 both the DET and ACE sedated horses showed sedative effects of the drugs, as expected (Freeman & England 2000; Pequito et al. 2012), the effect of DET peaked at T30. None of the horses were deeply sedated nor did they become recumbent at any point. The horses recovered from the sedative effects of the drugs within 90 minutes, as expected (Jochle & Hamm 1986; Marroum et al. 1994).

The results demonstrate that DET and ACE had a drug effect on a number of haematological measurands; but only few of the biochemical analytes measured in this study and commonly measured when evaluating and managing equine patients. Most notable were the changes in the haematocrit, red cell count, total white cell count, glucose and the lack of change in total serum protein, ALB, AST, ALP, ALT and insulin. Only minor changes were evident in ACTH, cortisol and CRP, these were considered unremarkable.

The DET and ACE groups showed a moderate decrease in haematocrit, as has been previously reported (Daunt et al. 1993; Parry & Anderson 1983; Gasthuys et al. 1990; Wagner et al. 1991; Kullman et al. 2014). We have determined that the red blood cells also decrease over time, which has not been previously reported on in horses. Various theories have been put forward to explain the decrease in haematocrit, such as: capillary shift fluid mechanisms; redistribution of interstitial and intracellular fluids to the intravascular spaces; or the sequestration of the red blood cells in the spleen (Parry & Anderson 1983; Gasthuys et al. 1987; Kullman et al. 2014).

The DET and ACE cause a decrease in sympathetic nervous system activity which may account for the sequestration of red blood cells and platelets in the spleen (Dalton 1972; Parry & Anderson 1983; Wagner et al. 1991; Lemke 2007). The equine spleen has a low dynamic flow within the circulation that is regulated in response to blood pressure and Tablin & Weiss (1983) stated "neuronal influence on contractile reticular cells [placing] blood flow, cell storage, and release under adrenergic control". This electron microscopy study (Tablin & Weiss 1983) revealed rich innervation of the reticular cells and vast red pulp of the spleen with adrenergic nerves. It would stand to reason, then, that the tone of the smooth muscle, capsule and vastly adrenergically innervated reticulum would then be reduced by DET and ACE. This corroborates our findings with regards to the red blood cells, in that we noted a decrease in haematocrit and RCC. However, the platelet concentration and intravascular proteins showed no remarkable changes. The repeated venepuncture may have caused an increased tissue demand for platelets, as each venepuncture would cause a cellular "micro trauma" type response to the puncture. This then may have caused a minor increase in platelet concentration around the venepuncture site which in the face of an overall diminution in platelet concentration in circulation and therefore may account for the relatively unaltered platelet concentration. If there were fluid shifts into the intravascular compartment, then we would have expected the proteins to change in concentration too. It has been determined that the primary

organ for red blood cell sequestration is the spleen (Kullman et al. 2014, De Moor et al. 1978, Perrson et al. 1973a &1973b). However, other organs in which red blood cell sequestration occurs and which cannot be discounted will include the liver and lungs, although their relative contribution is likely to be less, given the importance of the spleen as a reservoir for red blood cells in the horse.

Changes in total WBC have been attributed to margination of white blood cells along vascular walls (Lumsden et al. 1975). The total WBC decreases, which is to be expected with cell margination. Rashid et al. (2017) concluded in their study that sedative combinations (these included ACE and DET) resulted in minimal haematological side effects. Furthermore, they reported a gradual decrease in WBC as well as differential leukocyte count (neutrophils, eosinophils, and monocytes in particular) from 15 minutes following sedation, but that these fluctuations remained within expected reference intervals. The decreases in WBC observed by Rashid et al. (2017) were postulated to be as the result of a marginal lymphocytic pool in the spleen or other reservoirs as a result of the decreased sympathetic activity caused by the sedation

No significant changes can be seen in the neutrophil: lymphocyte ratios. This is indicative of both cell types decreasing proportionally. The effect of DET and ACE on the total WBC remains minor. Our findings are then consistent with the results of these studies (Lumsden et al. 1975, Rashid et al. 2017), the clinical relevance of which has not, to the authors' knowledge, been described in horses and warrants further investigation.

The reported effect of DET and ACE on TSP concentration range from no effect (Parry & Anderson 1983) to a late decrease after 40 minutes (Serpa et al. 2015) or about an hour (Dalton 1972) with the most significant changes being noted at 2.2 hours (Serpa et al. 2015). We found that the TSP concentration and ALB concentration did not change significantly over time

regardless of DET and ACE during the 60 minutes. The TSP and ALB values fluctuated within published reference intervals. The depression of TSP was greater with ACE than with DET. DET effects on ALB were less predictable than ACE effects. At T15 DET the ALB level was increased above that of T0 and the control, thereafter the ALB was closer to control levels than the decreased ACE ALB. The pathophysiology of a greater reduction in ALB with the use of ACE when compared with the use of DET has not been described previously and contributing factors such as dehydration would be similar in control animals and not be considered applicable in this short time period.

We did not find a relevant change in the liver-associated enzyme activities over the time period studied or among the groups and therefore conclude that this dose of ACE and DET do not cause clinically relevant damage to liver parenchymal or result in liver hypoxia in this short period of time. Our findings were similar to Mostafa et al. (1995) who also reported no significant change in liver-associated enzyme activities in the donkeys that were treated with DET in their study.

It has been noted that a dose dependant hyperglycaemia is expected following sedation with DET and other α-2 agonists (Gasthuys et al. 1987; Clarke 1988; England & Clarke 1996; Serpa et al. 2015). Our study shows an initial relatively low normal serum glucose concentration up to sampling point T30 at which point a moderate increase in glucose concentration is noted in DET treated horses compared to ACE and CON. In conjunction, there is a statistically insignificant hypoinsulinaemia in the DET group at sampling point T15, with lower minimum values from and including T15 to T45.

The changes in the insulin concentrations are not likely to be due to a storage effect (Reimers et al. 1991; Oberg et al. 2012; Carslake et al. 2016) but are clearly observable up to T30 DET. The DET resulted in a less variable range of effect than the ACE on insulin levels. Possible

causes of this may include the direct effects on the beta cells of the pancreas, direct α-related or α-receptor density related effects, or the high inter-variability of insulin reported in many species. Dietary and feed status prior to sampling, along with environmental, physiological and experimental factors should be limited due to the blinded and randomised nature of the study. The recent ECEIM statement regarding equine metabolic syndrome (Durham et al. 2019) details the effect of hormonal factors and their relative contribution to insulin production, the effect of sedation on these hormonal factors does not appear to have been investigated. The Durham group (Durham et al. 2009) likewise stated that a reduced hepatic clearance of insulin may contribute to hyperinsulinaemia. Potentially, the reduced hepatic blood flow effects of ACE may then have a more severe impact on blood insulin levels as the majority of insulin (>70%) is removed via portal blood (Durham et al. 2009). The response has not been well described in horses, in fact comment on and interpretation of insulin results from a single sample in horses is not recommended (Pratt et al. 2009). It is noteworthy that due to a relatively large reference interval for insulin this is not likely to be clinically relevant in healthy equines. However, when monitoring insulin concentrations in starved patients, prior sedation with DET should be noted prior to interpretation of the results.

The study by Clark et al. (1990) revealed significant decreases in plasma glucose concentrations due to storage under a variety of different conditions. Perhaps, the storage of the sample is responsible for an overall lower than anticipated glucose concentration in all of the horses, regardless of the group. This effect could be anticipated from studies where equine glucose concentrations declined from 8 hours at 4°C (Prutton et al. 2015) and the first day at 20-25°C (Rendle et al. 2009), however the effects of longer storage at colder temperatures are not well documented in equine literature. However, we did detect a drug effect between the drugs where there was a marked increase in glucose concentration that borderlines a mild hyperglycaemia in the DET group. Xylazine, a thiazine-derivative α-2 agonist, causes

hyperglycaemia by direct actions on the *beta* cells of the islets of Langerhans of the pancreas, thereby inhibiting insulin release, impairing insulin uptake, or by an inhibition of oxidation of glucose, or through interference with energy metabolism of muscle glycogen (Thurmon et al. 1982; Gasthuys et al. 1987; Nilsfors et al. 1988; Serpa et al. 2015). These studies suggest that DET could cause a similar effect as we have noted. Taking the effects of DET into account, one would have to conclude that DET would not be the sedation drug of choice in a horse in which you would want to monitor blood glucose, but rather ACE should be considered as a sedative instead.

C-reactive protein is a pentameric acute phase protein that is synthesised by the liver (Roberts et al. 2001) but has not been as extensively studied in horses as the other enzymes in this study. CRP is a component of the innate immune system that can activate the complement system and has pro-inflammatory effects (Takiguchi et al. 1990; Kolb-Bachofen 1991; Yamashita et al. 1991; Roberts et al. 2001; Zabrecky et al. 2015). CRP has been described as both a moderate (Murata et al. 2004) and major (Tugirimana et al. 2011) acute phase protein capable of significant increases with inflammatory stimulation. Such studies have motivated the use of CRP for detection and monitoring of inflammation in horses (Tugirimana et al. 2011) and reported correlations between CRP and band neutrophil counts (positively associated), ALB and platelet concentrations (negatively associated) (Zabrecky et al. 2015). In our study the CRP levels remained low for all groups, and was not significantly altered by sedation, understandably as inflammation was not initiated during the course of the study and sampling was conducted before the 24-hour lag time from stimulus to CRP increase.

4.2 Conclusion

In conclusion, we determined that not only will sedation with detomidine and acepromazine effect clinical variables and haematological measurands, but that these effects can be seen as rapidly as 15 minutes from the time of administration. Biochemical analytes, with the exception of glucose in detomidine sedated horses, did not change in value. Therefore, clinicians are advised to obtain blood samples for diagnostic and therapeutic purposes before the administration of detomidine or acepromazine. When these drugs have been administered, such as during field work or in emergency situations, the effects of the sedation should be taken into consideration when reviewing haematology and serum glucose concentration.

4.3 Future research

This study has shown that changes in haematological and biochemical variables can result sooner than previously estimated, hence further research could be conducted where samples are obtained sooner than 15 minutes after administration to determine how rapid drug effects occur. Furthermore, this study was conducted on healthy horses, the effects of sedation on haematological and biochemical variables is not known when patients are ill or with different disease processes. Additionally, there is a significant lack of recent information regarding the effect of storage on many haematological measurand and biochemistry analyte variables in equids, this may also be an area of future research.

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Addendum

Animal ethics approval certificate V099-16

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**ACTH concentrations in S.A companion equines before and after detomidine or acepromazine sedation**
PROJECT NUMBER	**V099-16**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. D Fisher**

STUDENT NUMBER (where applicable)	**UP_272 696 56**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Equine**	
NUMBER OF ANIMALS	**18**	
Approval period to use animals for research/testing purposes		**August 2016 –August 2017**
SUPERVISOR	**Dr. G Zeiler**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 29 August 2016
CHAIRMAN: UP Animal Ethics Committee	Signature

S420E 15

Owner consent form

Section of Anaesthesiology and Section of Equine Medicine
Department of Companion Animal Clinical Science
University of Pretoria
Dr Gareth Zeiler
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Dr Michael Hewetson
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UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

One day of participation for each season will be required for each horse involved in the study. A good line of communication will be established between the private owner, their veterinarian and the veterinarians conducting the study throughout. The study will provide a definitive diagnosis for equine PPID but will require that no treatment for equine PPID be undertaken until the final samples have been taken in May 2017.

Informed Consent to Provide Horses for the study

I/We

_____ of

_____

Residential Area/Veterinary Practice give permission and informed consent to provide horse(s) with suspected PPID based on clinical signs, namely hypertrichosis to participate in the Section of Anaesthesiology and Section of Equine Internal Medicine Clinical Study with the following conditions:

1. Once the horse(s) are enrolled and have started the study they may not, under any circumstances leave the clinical study until they have completed all stages. The horse(s) will be made available for sampling during the proposed collection dates. If there is a change in appointment time or date that needs to be made for either party, they shall notify the other and reschedule the sampling appointment as soon as possible.
2. The investigating veterinarians will make all the travel arrangements to and from your residence/ stabling facility at our costs.
3. Horses enrolled in the clinical study will undergo a standard, routine venipuncture according to the standards of good veterinary care and practice.
4. The protocols used to induce general sedation being tested in this study are safe and are not expected to cause any unnecessary discomfort, illness or death.
5. The sedation procedure will be monitored by a specialist veterinarian to ensure safety of the horse(s).
6. Difficult, unmanageable horse(s) that cannot be safely handled or where there are no safe handling facilities will not be included in the study.
7. Our veterinary researchers will make every effort to provide the best care and attention for the horse(s) during their sampling periods.
8. **You will not be charged** (billed/invoiced) for any of the work conducted on the horse(s) for the trial, this includes basic clinical examinations, the collection of blood, administration of sedation, analysis of blood and reports therefrom.
9. The veterinarians conducting the study have a right to exclude any horse(s) that do not conform to the requirements (friendly, healthy, good body condition score, clinical evidence of hypertrichosis) of the study participants.
10. The horse(s) cannot be treated for PPID during the study due to a possible change in the study variables. We will provide a definitive diagnosis for equine PPID at no cost to you.
11. Horse(s) may not be vaccinated within 14 days before the study. It is your obligation to inform us if the horse has been vaccinated or undergone any other form of treatment 14 days or less before the study. These horses may be excluded from the study due to potential variables that may alter the results of the study.
12. The undersigned parties further agree that no compensation will be payable to the animal's owner or anybody else and that all research associated costs will be covered by the researcher(s).
13. The undersigned parties further agree that this form would serve to fully indemnify the University of Pretoria and the undersigned researcher(s) against any future claims resulting from the specified procedure by or on behalf of the animal's owner.
14. The undersigned parties further agree that no material of any kind, including data and research findings, obtained or resulting from the procedure, would be passed on to any third party or used for any purpose other than that specified in this form, except with the written consent of the undersigned owner of the animal.

Section of Anaesthesiology and Section of Equine Medicine
Department of Companion Animal Clinical Science
University of Pretoria
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Dr Michael Hewetson
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If you or your veterinary facility consents to assisting our important clinical study that is not harmful to the horse(s) then please indicate the number of horses you will be willing to assist with, their name, identification, and the rightful owner of the horse(s):

_____

_____

_____

Indicating the name(s) here is an obligation to provide the horse(s) for each of the four sampling periods during the duration of the clinical trial.

Signing of the Informed Consent Form

Private Owner or Authorised Person

Name: _____

Signature: _____

Date: _____

Authorised Person involved in Equine ACTH Concentration Study

Name: _____

Signature: _____

Date: _____

Witness

Name: _____

Signature: _____

Date: _____

As a token of appreciation you/your veterinary facility may be mentioned in the acknowledgment section of all scientific articles published from this study:

☐ Yes, please add our/ our veterinary facility's name to the acknowledgement section of all publications written using the data collected from this study.

☐ No, please do not add our/our veterinary facility's name to the acknowledgment section of all publications written using the data collected from this study.

Study participant information form

Section of Anaesthesiology and Section of Equine Medicine
Department of Companion Animal Clinical Science
University of Pretoria
Dr Gareth Zeiler — Gareth.Zeiler@up.ac.za — (012) 529 8002
Dr Michael Hewetson — Michael.Hewetson@up.ac.za — (012) 529 8493

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Equine ACTH Concentration Study

Patient Information Form

This form must be completed by a person who has the authority to consent to the use of the horse or pony or the private owner in the ACTH and sedation research conducted from the Onderstepoort Veterinary Academic Hospital, overseen by the Companion Animal Clinical Studies Department, Section of Anaesthesiology.

Owner _____

Of (address)

_____

_____

Contact number: _____

Name of Horse/Pony _____

Age _____

Physical Description:

_____

Weight _____

Girth Measurement _____

Medical History

_____

_____

_____

_____

Diet

_____

_____

General Management and Use

_____

_____

_____

_____

Information for study

Participant number: _____

Clinical evaluation

_____

_____

Collection dates and times

_____

_____

_____

_____

_____

Page 1 of 1

Data collection forms

3.1 Day 1 Assessment of candidates

Pony #	UP ID	physical ID	weight	Girth measurements	T	P	R	Overall Clinical Health
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								

This recording sheet fors part of a study being held at Onderstepoort, University of Pretoria, 2016
If lost PLEASE CONTACT Diana Fisher 078 6060 818

3.2 Sedation with Detomidine @ 0.01mg/kg

Pony #	UP ID	weight	Dose of Detomidine	0 min			15 min			30 min			45 min			60 min		
				SDS	P	R	SDS	P	R	SDS	P	R	SDS	P	R	SDS	P	R
1																		
2																		
3																		
4																		
5																		
6																		

SDS scoring system for sedation and ataxia

SEDATION	0	No sedation. Animal is alert, with normal posture and response to contact with assessor. Normal objection to intervention
	1	Mild sedation. Low head carriage, relaxed facial muscles and pendulous lower lip. Some response to intervention
	2	Moderate sedation. Head lowered towards ground and swaying of hind legs. Slight response to intervention
	3	Marked sedation. Attempts to or becomes recumbent. No response to intervention

ATAXIA	0	No ataxia. Animal stands and walks normally; is able to turn lightly
	1	Mild ataxia. Animal able to walk, but some lack of limb control
	2	Moderate ataxia. Animal can walk only with support, staggers but saves itself from falling
	3	Marked ataxia. Animal is unable to walk without danger of falling, staggers, falls if turned

This recording sheet fors part of a study being held at Onderstepoort, University of Pretoria, 2016
If lost PLEASE CONTACT Diana Fisher 078 6060 818

3.3 Sedation with Acepromazine @ 0.02mg/kg

Pony #	UP ID	weight	Dose of ACP	0 min			15 min			30 min			45 min			60 min		
				SDS	P	R	SDS	P	R	SDS	P	R	SDS	P	R	SDS	P	R
1																		
2																		
3																		
4																		
5																		
6																		

SDS scoring system for sedation and ataxia

SEDATION	0	No sedation. Animal is alert, with normal posture and response to contact with assessor. Normal objection to intervention
	1	Mild sedation. Low head carriage, relaxed facial muscles and pendulous lower lip. Some response to intervention
	2	Moderate sedation. Head lowered towards ground and swaying of hind legs. Slight response to intervention
	3	Marked sedation. Attempts to or becomes recumbent. No response to intervention

ATAXIA	0	No ataxia. Animal stands and walks normally; is able to turn lightly
	1	Mild ataxia. Animal able to walk, but some lack of limb control
	2	Moderate ataxia. Animal can walk only with support, staggers but saves itself from falling
	3	Marked ataxia. Animal is unable to walk without danger of falling, staggers, falls if turned

This recording sheet fors part of a study being held at Onderstepoort, University of Pretoria, 2016
If lost PLEASE CONTACT Diana Fisher 078 6060 818

3.4 Individual monitoring sheet for pony number _____

SDS scoring system for sedation and ataxia

SEDATION	0	No sedation. Animal is alert, with normal posture and response to contact with assessor. Normal objection to intervention
	1	Mild sedation. Low head carriage, relaxed facial muscles and pendulous lower lip. Some response to intervention
	2	Moderate sedation. Head lowered towards ground and swaying of hind legs. Slight response to intervention
	3	Marked sedation. Attempts to or becomes recumbent. No response to intervention
ATAXIA	0	No ataxia. Animal stands and walks normally; is able to turn lightly
	1	Mild ataxia. Animal able to walk, but some lack of limb control
	2	Moderate ataxia. Animal can walk only with support, staggers but saves itself from falling
	3	Marked ataxia. Animal is unable to walk without danger of falling, staggers, falls if turned

sedation time

0 min	**SDS sedation**	
	SDS ataxia	
	P	
	R	
15 min	**SDS sedation**	
	SDS ataxia	
	P	
	R	
30 min	**SDS sedation**	
	SDS ataxia	
	P	
	R	
45 min	**SDS sedation**	
	SDS ataxia	
	P	
	R	
60 min	**SDS sedation**	
	SDS ataxia	
	P	
	R	
	Recovery time	
	P	
	R	

This recording sheet forms part of a study being held at Onderstepoort, University of Pretoria, 2018.
If lost PLEASE CONTACT Diana Fisher 078 6060 818 or hand in at Onderstepoort. THANK YOU

Presentations and publications arising from the study

Presentations:

EVENT	VENUE	DATE	TITLE	TYPE
SAEVA	Kruger National Park	20 February 2020	Haematology and biochemistry effects of acepromazine or detomidine standing sedation in horses	Oral

Publications:

Fisher D, Rautenbach Y, Hewetson M, Zeiler GE

Haematological and biochemical effects of acepromazine or detomidine standing sedation in horses.

To be submitted to: Equine Veterinary Education