UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

URINARY METANEPHRINES IN CATS – DOES COLLECTION METHOD MATTER?

ANA LUÍSA PEREIRA DA SILVA

ORIENTADOR:
Doutor Rodolfo Assis Oliveira Leal

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METANEFRINAS URINÁRIAS EM GATOS – O MÉTODO DE COLHEITA IMPORTA?

Resumo

Atualmente, o conhecimento sobre a aplicabilidade clínica do doseamento das metanefrinas em gatos é limitado. A medição de metanefrinas urinárias constitui uma ferramenta de diagnóstico segura e prática para excluir tumores produtores de catecolaminas. Embora a colheita urinária seja habitualmente realizada por cistocentese, a colheita em ambiente doméstico é uma opção menos invasiva, prática e com menor indução de stress. Visto que o stress é uma preocupação em pacientes felinos, é relevante avaliar o impacto do método de colheita nas concentrações urinárias de metanefrinas. Este estudo teve como objetivo comparar os resultados de amostras colhidas em casa e em hospital veterinário por cistocentese.

Após aprovação ética, realizou-se um estudo observacional de medidas repetidas com gatos saudáveis ou com doença não adrenal estável, submetidos a ecografia abdominal e exames laboratoriais. Os tutores receberam areia não absorvente e colheram urina em casa antes ou após três dias da cistocentese. As amostras foram refrigeradas a 4 °C no máximo por 24h e depois armazenadas a -80 °C até ao envio para laboratório. Todas as amostras foram analisadas em simultâneo para determinação de metanefrinas e normetanefrinas urinárias (uMN e uNMN, respectivamente) por cromatografia líquida com espectrometria de massa em tandem. A análise estatística foi efetuada com recurso ao software IBM SPSS Statistics.

Foram incluídos 19 gatos no estudo. A concentração de uMN livre (mediana [intervalo]) em urina colhida no hospital (690,36 [197,97 – 2228,43] nmol/L) foi significativamente superior à da colhida em casa (324,87 [116,75 – 883,25] nmol/L) (p = 0,003). A concentração de uNMN livre colhida no hospital (1475,41 [475,41 – 5180,33] nmol/L) foi também significativamente superior à da colhida em casa (1267,76 [469,95 – 3459,02] nmol/L) (p = 0,022).

O rácio uMN:creatinina na urina colhida no hospital (41 [17 – 154] µg/g) foi significativamente superior ao da urina colhida em casa (26 [9 – 93] µg/g) (p = 0,015). No entanto, os rácios uNMN:creatinina não apresentaram diferenças significativas entre a urina colhida no hospital (85 [44 – 239] µg/g) e em casa (83 [42 – 209] µg/g) (p = 0,481).

Estes resultados demonstram que o stress ambiental e consequentemente o método de colheita da urina alteram significativamente as concentrações de uMN livres, de uNMN livres e o rácio uMN:creatinina. No entando, o rácio uNMN:creatinina permanece inalterado, revelando-se um indicador diagnóstico fiável e independente do método de colheita.

Palavras-chave: Metanefrinas urinárias; Normetanefrinas urinárias; Gatos; Stress; Cromatografia líquida com espectrometria de massa em tandem.

URINARY METANEPHRINES IN CATS – DOES COLLECTION METHOD MATTER?

Abstract

Knowledge regarding the clinical applicability of metanephrines in cats is scarce. Urinary metanephrine measurements provide a safe and practical diagnostic tool to rule out catecholamine-producing tumors. While urine collection is usually performed via cystocentesis, urine collection at home is a less invasive option, providing a convenient and stress-free method to collect samples. As stress is always a concern in feline patients, it is essential to assess the impact of sample collection method in metanephrine measurements. This study aims to evaluate potential differences between urinary metanephrines collected at home versus by cystocenthesis in the hospital.

After ethical approval, a repeated measures observational study was performed including either healthy cats or cats with stable non-adrenal illness, submitted to an abdominal ultrasound and analytical work-up. Owners were supplied with non-absorbent litter and instructed to either collect urine at home before or at least three days after cystocentesis. Collected samples were refrigerated at 4°C for a maximum of 24h before being stored at -80°C until shipment. All samples were shipped at the same time for urinary metanephrine (uMN) and normetanephrine measurements (uNMN) by liquid chromatography with tandem mass spectrometry. Statistical analysis was preformed using the software IBM SPSS Statistics. Normality was assessed and statistical tests were then chosen accordingly.

A total of 19 cats were included. The free uMN concentration (median [range]) in urine collected at the hospital (690.36 [197.97-2228.43] nmol/L) was significantly higher than in urine collected at home (324.87 [116.75-883.25] nmol/L) (p = 0.003). The free uNMN concentration in urine collected at the hospital (1475.41 [475.41-5180.33] nmol/L) was also significantly higher than in the urine collected at home (1267.76 [469.95-3459.02] nmol/L) (p = 0.022).

The uMN:creatinine ratio in the urine collected at the hospital (41 [17-154] µg/g) was significantly higher than in the urine collected at home (26 [9-93] µg/g) (p = 0.015). However, the uNMN:creatinine ratios in urine collected at the hospital (85 [44-239] µg/g) and at home (83 [42-209] µg/g) did not differ significantly (p = 0.481).

These results show that environmental stress, and consequently the urine collection method, significantly influence free uMN, free uNMN and the uMN:creatinine ratio. However, the uNMN:creatinine ratio remains unchanged, establishing itself as a good diagnostic indicator regardless of collection method.

Keywords: Urinary metanephrines; Urinary normetanephrines; Cats; Stress; liquid chromatography with tandem mass spectrometry.

URINARY METANEPHRINES IN CATS – DOES COLLECTION METHOD MATTER?

Resumo Alargado

Os tumores produtores de catecolaminas, nomeadamente feocromocitomas e paragangliomas, são raros em medicina felina. Contudo, quando presentes, originam quadros endócrinos graves de apresentação clínica variada e frequentemente inespecífica. O diagnóstico destas neoplasias é desafiante devido à sua baixa prevalência, ausência de sinais patognomónicos e dificuldade em confirmar a secreção excessiva de catecolaminas.

As catecolaminas – adrenalina, noradrenalina e dopamina –, são produzidas na medula adrenal a partir do aminoácido tirosina, sendo metabolizadas principalmente pela catecol-O-metiltransferase (COMT) e pela monoamina oxidase (MAO). Este metabolismo origina metanefrinas (MN) e normetanefrinas (NMN), metabolitos mais estáveis do que as catecolaminas. Em medicina humana, a determinação de metanefrinas livres, plasmáticas ou urinárias constitui atualmente o método de referência para o diagnóstico de feocromocitomas e paragangliomas, apresentando elevada sensibilidade e especificidade.

Em medicina veterinária, os dados disponíveis sobre a utilização de metanefrinas são limitados. Estudos em cães sugerem que os rácios de metanefrinas urinárias corrigidas para creatinina podem ser úteis como biomarcadores de diagnóstico. Em gatos, o conhecimento é ainda muito restrito, sendo necessário investigação adicional para compreender se a medição destes metabolitos tem relevância clínica semelhante à observada em humanos e em cães.

A colheita de urina em felinos possui particularidades próprias desta espécie a ter em consideração. A cistocentese, técnica de eleição em contexto hospitalar, permite recolher uma amostra estéril e de elevada qualidade, mas é invasiva e geradora de stress. Este fator é particularmente relevante na espécie felina, onde o stress pode induzir aumentos transitórios significativos na libertação de catecolaminas e respetivos metabolitos. Como alternativa, a colheita em ambiente doméstico com recurso a areias não absorventes constitui uma técnica prática, não invasiva e com menor indução de stress. Assim, é relevante avaliar se o método de colheita influencia de forma significativa as concentrações urinárias de metanefrinas, comprometendo a sua aplicabilidade clínica.

O presente estudo teve como objetivo comparar as concentrações urinárias de metanefrinas (uMN) e normetanefrinas (uNMN) em gatos, entre diferentes métodos de colheita (cistocentese hospitalar versus colheita doméstica). Pretendeu-se ainda avaliar os rácios normalizados para creatinina e determinar se estes poderiam representar parâmetros mais robustos e independentes do método de colheita.

Após aprovação ética, realizou-se um estudo observacional de medidas repetidas incluindo 19 gatos, saudáveis ou com doença não adrenal estável. Os animais foram

submetidos a exame físico completo, ecografia abdominal e exames laboratoriais para excluir doença adrenal. Cada tutor recebeu areia não absorvente (Catrine®) e instruções para recolha de uma amostra de urina em casa, antes ou após três dias da cistocentese hospitalar. As amostras foram refrigeradas a 4 °C por um período máximo de 24 horas, congeladas a -80 °C e posteriormente enviadas em conjunto para análise laboratorial.

A determinação das concentrações de uMN e uNMN foi realizada por cromatografia líquida com espectrometria de massa em tandem (LC-MS/MS), método de elevada sensibilidade e especificidade. Para minimizar a variabilidade analítica, todas as amostras foram processadas simultaneamente. A análise estatística foi efetuada com recurso ao software IBM SPSS Statistics e incluiu testes paramétricos ou não paramétricos conforme a normalidade dos dados, análise de concordância intra-classe (ICC) e gráficos de Bland-Altman para avaliar a consistência entre métodos de colheita.

Os resultados mostraram diferenças estatisticamente significativas entre métodos de colheita. A concentração mediana de uMN livre em urina colhida no hospital foi significativamente superior (p = 0,003) à obtida em urina colhida em casa, (690,36 nmol/L (197,97 – 2228,43) vs 324,87 nmol/L (116,75 – 883,25), respetivamente). De forma semelhante, as concentrações de uNMN livres foram mais elevadas na urina de cistocentese (1475,41 [475,41 – 5180,33] nmol/L) do que na urina colhida em casa (1267,76 [469,95 – 3459,02] nmol/L; p = 0,022).

Aquando da normalização com a creatinina, observou-se que o rácio uMN:creatinina foi significativamente superior nas amostras de hospital (41 [17 – 154] µg/g) em comparação com as colhidas em casa (26 [9 – 93] µg/g; p = 0,015). No entanto, o rácio uNMN:creatinina não apresentou diferenças estatisticamente significativas entre os dois métodos (85 [44 – 239] µg/g no hospital versus 83 [42 – 209] µg/g em casa; p = 0,481).

A análise de concordância intraclasse revelou que a uNMN apresentou boa concordância entre métodos (ICC = 0,715), enquanto o rácio uNMN:creatinina demonstrou concordância moderada (ICC = 0,489). Em contraste, tanto a uMN como o rácio uMN:creatinina apresentaram concordância baixa (ICC = 0,190 e 0,159 respetivamente).

A análise multivariável revelou que o método de colheita influenciou significativamente três das quatro variáveis – uMN, uMN:creatinina e uNMN –, com valores consistentemente superiores nas amostras de cistocentese. O estado de saúde afetou significativamente apenas as concentrações de uMN, com gatos cronicamente doentes a apresentar valores superiores deste metabolito. O sexo influenciou significativamente os rácios de creatinina, com valores superiores nas fêmeas em relação aos machos. A idade não demonstrou influência significativa em qualquer dos parâmetros avaliados.

A comparação com valores previamente descritos por Prego et al. (2023) revelou que 94,7% das amostras do presente estudo se situaram dentro ou abaixo dos intervalos descritos

para gatos saudáveis e muito distantes do valor descrito num caso confirmado de feocromocitoma felino. Esta elevada concordância reforça a consistência dos resultados e a fiabilidade destes intervalos de concentração como representativos de valores não tumorais.

Em síntese, verificou-se que o ambiente hospitalar e a cistocentese influenciam significativamente as concentrações absolutas de metanefrinas urinárias, possivelmente devido ao stress associado ao procedimento e exposição a um ambiente não familiar. Contudo, o rácio uNMN:creatinina manteve-se estável entre métodos, sugerindo que pode constituir um indicador robusto pouco afetado por fatores externos e, consequentemente, mais fiável em contexto clínico. Este resultado é consistente com dados de medicina humana onde a normalização para creatinina contribui para a redução de influência de fatores como a variação de volume urinário, stress ou condições de colheita.

Este é o primeiro estudo a comparar diretamente a influência da cistocentese e da colheita doméstica via areia não absorvente nas metanefrinas urinárias felinas medidas por LC-MS/MS.

No entanto, apresenta algumas limitações, nomeadamente o reduzido tamanho de amostra, a variabilidade no momento de colheita das amostras domésticas e a ausência de gatos com diagnóstico confirmado de feocromocitoma ou paraganglioma. Assim, não foi possível avaliar diretamente a utilidade diagnóstica destas medições em casos clínicos de neoplasias secretoras de catecolaminas.

Estes resultados encorajam a realização de mais estudos, que incluam populações maiores e multicêntricas, protocolos padronizados de colheita doméstica com altura de colheita definida e, crucialmente, casos confirmados de feocromocitoma para estabelecer intervalos de referência formais e limiares diagnósticos. Esta linha de investigação permitirá investigar os mecanismos subjacentes às flutuações hormonais e respostas fisiológicas ao stress, que afetam a secreção de MN e NMN, com vista a clarificar fatores de influência e melhorar a utilidade clínica destes biomarcadores.

O nosso estudo revela assim importantes conclusões com implicações práticas. Confirmámos que o método de colheita deve ser considerado na interpretação dos resultados de metanefrinas urinárias em gatos, e que o stress associado ao método e ao ambiente de colheita é relevante. Em segundo lugar, concluímos que o rácio uNMN:creatinina é o parâmetro mais promissor para futura utilização diagnóstica, podendo assumir-se como biomarcador mais estável e clinicamente relevante na exclusão de tumores produtores de catecolaminas, independentemente do método de colheita utilizado.

Palavras-chave: Metanefrinas urinárias; Normetanefrinas urinárias; Gatos; Stress; Cromatografia líquida com espectrometria de massa em tandem.

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< – less than

≤ – equal or less than

> – higher than

± – more or less

% – percentage

µg/g – microgram per gram

µg/L – microgram per litre

MHz – megahertz

mm – millimetres

nmol/L – nanomole per litre

ºC – degrees Celsius

A-POCUS – abdominal point-of-care ultrasound

AAAD – aromatic-l-amino acid decarboxylase

ACE – angiotensin-converting enzyme

ACTH – adrenocorticotropic hormone

ALT – alanine transaminase

CBC – complete blood count

CH – chronically ill (group)

CI – confidence interval

CKD – chronic kidney disease

COMT – catechol-O-methyltransferase

CPR – cardiopulmonary resuscitation

CRH – corticotropin-releasing hormone

CT – computed tomography

DBH – dopamine-β-hydroxylase

DHEA – dehydroepiandrosterone

DOPA – 3,4-dihydroxyphenylalanine

ECD – electrochemical detection

ELISA – enzyme-linked immunosorbent assay

Epi – epinephrine

EPO – erythropoietin

FIV – feline immunodeficiency virus

GC-MS – gas chromatography-mass spectrometry

GGT – gamma-glutamyl transferase

H – healthy (group)

HPLC – high-performance liquid chromatography

IBD – inflammatory bowel disease

ICC – intraclass correlation coefficients

IQR – interquartile range

IV – intravenous

LC-ECD – liquid chromatography with electrochemical detection

LC-MS/MS – liquid chromatography – tandem mass spectrometry

LoA – limits of agreement

MAO – monoamine oxidase

MN – metanephrine

MRI – magnetic resonance imaging

NE – norepinephrine

NMN – normetanephrine

PCC – pheochromocytomas

PGL – paragangliomas

PPE – personal protective equipment

PNMT – phenylethanolamine-N-methyltransferase

RIA – radioimmunoassay

SD – standard deviation

SDMA – symmetric dimethylarginine

T4 – thyroxine

TH – tyrosine hydroxylase

TPLO – tibial plateau levelling osteotomies

uMN – urinary metanephrine

uNMN – urinary normetanephrine

UPC – urinary protein-to-creatinine

UTI – urinary tract infection

VMA – vanillylmandelic acid

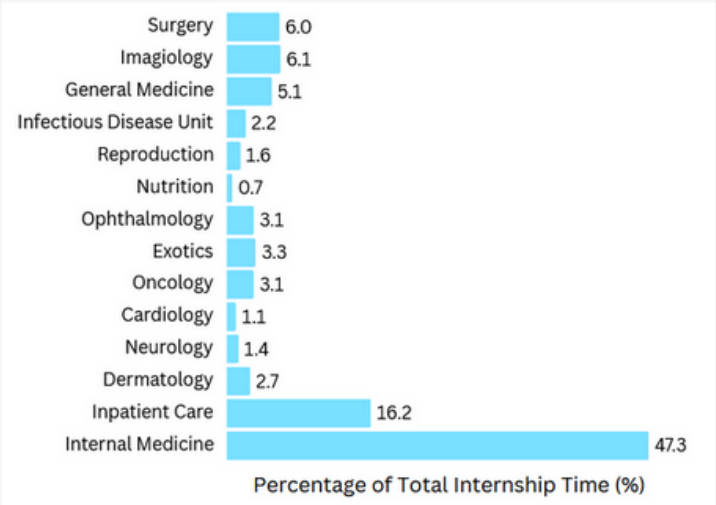
1. Traineeship Report

From December of 2023 to March of 2024, I completed the first half of my curricular internship at the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine, University of Lisbon. During this period, I rotated through several specialty departments for one to three weeks each. Between July and September of 2024, I finished the second half of my internship, focusing exclusively on the internal medicine department. Splitting the internship into two separate periods allowed me to accommodate an externship at the VCA Animal Emergency and Referral Centre in Vancouver, Canada, which will be detailed later in this report.

This internship has been paramount in cementing theoretical knowledge through practical applications and providing invaluable learning experiences beyond the classroom. I had the opportunity to work alongside experienced professionals who demonstrated both deep and continually evolving expertise. They welcomed me into their team and shared their knowledge and stories, creating a unique and supportive learning environment. The doctors encouragement to take initiative, make mistakes, and grow from those experiences resulted in the growth of my own skills and confidence. While specific knowledge may fade over time, the ability to keep learning and adapting will remain a lasting strength throughout my career.

The total duration of my internship was approximately 913 hours across the various departments, as illustrated in Graph 1. The time allocated to each department varied based on the university's schedule for each student, the availability of the departments (mornings, afternoons, or specific days of the week), and the number of appointments scheduled.

Graph 1 – Distribution of time spent across each department.



Surgery

Surgery was my first rotation and lasted for two weeks. Daily responsibilities began with receiving patients scheduled for procedures. Reviewing the schedule and patient medical

history in advance was essential for effective case management. After verifying with the owners that preoperative instructions had been followed, additional considerations were sometimes necessary regarding medication, surgical preparation, or changes in the animal's health status since the last appointment.

Once the patient was admitted, preparations for anaesthesia began. This included selecting and preparing pre-anaesthetic medications, IV (intravenous) catheter placement and preparation of fluid therapy, intubation equipment and the anaesthesia machine, and choosing appropriate induction drugs. Practical skills gained involved peripheral venous catheterization, intubation, drug administration and surgical site preparation including trichotomy and sterilization.

Common surgeries included ovariohisterectomies, orchiectomies, nodulectomies and orthopaedic procedures such as tibial plateau levelling osteotomies (TPLO). Under supervision, I assisted with suturing, applying staples, and had the opportunity to perform an ovariohysterectomy. I also observed or assisted in more complex procedures, including caesarean sections, splenectomies, thoracotomies, mastectomies, hemilaminectomies and ventral slot surgeries. Additional responsibilities included circulating assistant duties and anaesthesia monitoring, which involved managing mechanical ventilation and assisting in two critical cases involving asystole.

Diagnostic Imaging

The diagnostic imaging rotation lasted two weeks and was divided into two separate components – one week in radiology, computed tomography (CT), and magnetic resonance imaging (MRI), and the other in ultrasonography. This experience reinforced the importance of diagnostic imaging in clinical case management.

In radiology, I learned to select appropriate cassettes and adjust exposure settings depending on the patient’s size and the region being imaged. I actively participated in restraining and positioning patients for thoracic, abdominal, and orthopaedic radiographs, applying standard and orthogonal projections. Post-imaging discussions improved my ability to interpret radiographs and identify common pathologies such as pulmonary patterns, abdominal distention, and orthopaedic lesions.

During CT and MRI shifts, I helped prepare patients by placing IV catheters, administering sedation or anaesthetics and intubating when necessary. I also monitored anaesthesia during scans and administered IV contrast agents. Observing the images alongside the radiologist helped me recognise structural abnormalities, tumour invasions and disc compressions.

In the ultrasonography rotation, I practiced patient handling and trichotomy preparation for thoracic and abdominal exams. I observed how organ evaluation was systematically

approached and gained confidence in identifying structures like the liver, spleen, kidneys, and urinary bladder. I also assisted in ultrasound-guided procedures, including fine needle aspirates, percutaneous biopsies and cystocentesis. Learning the principles behind Doppler evaluation and ultrasound artifact interpretation enhanced my diagnostic reasoning and highlighted the utility of ultrasound as both a screening and a targeted diagnostic method. Additionally, I observed an ultrasound exam on a snake, learning about species-specific particularities.

General Medicine

The general medicine rotation lasted two weeks and was one of the most dynamic rotations due to the diversity of cases seen daily. I participated in first-opinion consultations, follow-ups, and urgent care. This department also played an important role in preventive medicine, chronic disease management and addressing acute-onset clinical complaints.

One of the learning opportunities in this rotation was leading the initial patient intake under supervision. I collected a complete anamnesis, performed a basic physical exam and proposed a list of differential diagnoses, which was then reviewed and discussed with the clinician. This allowed me to practice clinical reasoning and decision-making in real-time.

Routine procedures included venous blood collection, IV catheter placement and cystocentesis. I also learned to perform abdominocentesis, thoracocentesis, measure blood pressure and evaluate blood glucose and urine samples on-site. I assisted in emergency cases involving trauma, respiratory distress and intoxications, where rapid decisions and teamwork were essential.

Another important aspect was client communication. I practiced explaining clinical findings, discussing diagnostic plans and setting realistic expectations for treatment and prognosis. By the end of this rotation, I felt more comfortable adapting my communication style to different owners and working under pressure.

Infectious Disease Unit

This one-week rotation took place in the isolation ward, where strict biosecurity protocols were followed. Before entering, I learned proper personal protective equipment (PPE) usage, including disposable gowns, gloves, masks and shoe covers. I observed and contributed to the management of patients with highly contagious diseases such as canine parvovirus, leptospirosis, feline panleukopenia, retroviral infections, and some cases of multidrug-resistant bacterial infections.

Daily duties included medication administration, catheter maintenance, physical exams, temperature and hydration assessments and feeding – both voluntary and assisted (naso-oesophageal or oesophageal tubes). I also participated in disinfection protocols, reinforcing the importance of environmental hygiene in preventing hospital-acquired infections.

Reproduction

Reproduction and neonatology were a week-long rotation, often intertwined with weight control appointments due to logistical constraints.

I had the opportunity to be present for three canine caesarean sections, one during the day and two during night shifts as part of inpatient care. In one case, I assisted the surgeon directly, helping to remove the puppies and their placentas from the uterus and handing them to the neonatal team for resuscitation. In the other two procedures, I was responsible for receiving the newborns, clearing mucus from their airways, stimulating breathing, checking for cleft palates and the presence of an anus, cutting and tying umbilical cords and recording the number and sex of each puppy. I also observed and assisted in semen collections, gaining insight into the practical aspects of male reproductive evaluation.

This rotation highlighted the importance of client education, namely regarding responsible breeding and neonatal care.

Nutrition / Weight Control

The nutrition rotation focused on consultations for obesity management. I learned to assess body condition scores and track weight over time. Appointments involved creating individualized plans for overweight dogs and cats, adjusting caloric intake, evaluating dietary compliance, and discussing appropriate exercise routines with owners. This rotation highlighted the importance of tailored nutrition in disease prevention and recovery, particularly for geriatric and chronically ill patients.

Ophthalmology

This one-week rotation provided a solid foundation in the diagnostic approach to ocular disease. Under guidance, I performed structured eye examinations using appropriate tools and techniques, evaluating palpebral and pupillary reflexes, menace response, dazzle reflex, and performing Schirmer tear tests, fluorescein staining for corneal ulcers, and tonometry to assess intraocular pressure. Fundoscopy and slit-lamp examinations were demonstrated and practiced with supervision, helping me become familiar with common findings in the anterior and posterior segments of the eye. I participated in consultations for conditions such as conjunctivitis, keratoconjunctivitis sicca, entropion, glaucoma, uveitis, and cataracts. Blood sampling was often performed for underlying systemic disease screening.

I also observed and assisted in minor surgical procedures, including eyelid mass removals and tarsorrhaphies, in both small animals and horses, as this department is also responsible for equine ophthalmologic care.

This rotation emphasized how precise ophthalmic work is and highlighted the connection between ocular and systemic diseases.

Exotics

This rotation involved working with a wide range of species including rabbits, guinea pigs, turtles, parrots, snakes and pigeons Species-specific handling and restraint techniques were used to minimize stress and ensure safety. I learned to recognize subtle clinical signs particularly in prey species, and to adapt physical examinations accordingly. I also practiced procedures such as nail clipping and sample collection techniques like blood collection.

Clinical cases included dental disease in rabbits, routine check-ups in pigeons and informing about environmental corrections needed for a new turtle owner. I also observed CT imaging and anaesthesia protocols adapted for small exotic species, including gas induction and temperature management.

Oncology

During this one-week rotation I gained insight into the diagnostic, therapeutic and palliative approaches to neoplastic diseases in small animals. I attended first-opinion, referral and follow-up consultations, as well as chemotherapy sessions.

I participated in detailed physical exams focused on detecting masses, evaluating lymph nodes and monitoring body condition and vital signs. I assisted with pre-chemotherapy complete blood count (CBC) sampling, venous catheter placement and patient monitoring during treatment, and became familiar with tumour-specific protocols including drug selection, dosing, and scheduling.

Discussions with clinicians frequently focused on prognosis communication, quality-of-life assessment and how to guide owners through treatment decisions. This rotation deepened my understanding of the balance between aggressive treatment and palliative care, and the importance of compassion in veterinary oncology.

Cardiology

The one-week cardiology rotation focused on a systematic approach to cardiovascular assessment. Consultations began with detailed anamnesis and auscultation, during which I practiced identifying heart murmurs, arrhythmias, and distinguishing true arrhythmias from vagally mediated respiratory sinus arrhythmia.

During echocardiography, the attending clinician provided real-time explanations and helped identify abnormalities such as valve regurgitation, chamber enlargement and abnormal flow patterns using Doppler techniques. I also interpreted electrocardiogram tracings, identifying arrhythmias like atrial fibrillation, ventricular premature complexes and sinus bradycardia. Common diagnoses included myxomatous mitral valve disease in dogs and hypertrophic cardiomyopathy in cats. I observed the formulation and adjustment of treatment plans using medications such as pimobendan, angiotensin-converting enzyme (ACE) inhibitors, and diuretics, and learned the importance of regular monitoring.

Neurology

Sharing the week with cardiology, the neurology rotation involved cases that required detailed anamnesis, lesion localization and integration of imaging and laboratory results. I was encouraged to perform full neurological examinations, including assessments of cranial nerve function, proprioception, spinal reflexes, and mental status.

Patients presented with a range of neurological signs, and discussions with the neurologist helped understand lesion localization (central or peripheral nervous system) and how to list differential diagnoses and respective treatment options for each case.

I observed and assisted in procedures such as cerebrospinal fluid collection, post-imaging evaluations and blood sample collection. This rotation also reinforced how systemic diseases can present with neurological signs, requiring an integrated diagnostic approach. I particularly appreciated the methodical structure of neurological evaluations and the depth of clinical reasoning involved in reaching accurate diagnoses.

Dermatology

In the dermatology rotation I encountered a wide range of skin and ear conditions, including chronic pruritus, atopic dermatitis, parasitic infestations, and otitis externa.

I assisted in patient history collection and performing dermatological examinations, including quantifying pruritus using standardized scales. I prepared and analysed samples such as skin cytology, ear cytology, trichograms, skin scrapings, and biopsies. I also performed Romanowsky (Diff-Quik) staining and examined samples microscopically. This rotation emphasized the importance of client education for ensuring treatment compliance in chronic dermatologic cases, as well as the significant role of environmental and nutritional factors in managing skin diseases.

Inpatient Care

Inpatient care was a continuous rotation throughout the internship, structured around 12-hour day and night shifts. My responsibilities included administering medications (oral, IV, and subcutaneous), placing and maintaining IV catheters, selecting and supplementing IV fluids, feeding patients (including assisted feeding via nasogastric tubes), monitoring vital signs and performing blood glucose and blood pressure measurements. I also assisted with urinary catheter and nasogastric tube placements, wound care, bandage changes and pain management assessments.

Each shift began with medical rounds where the night team, nurses, and daytime clinicians discussed cases, creating a joint approach and shared perspective on patient progress and treatment planning.

I participated in critical interventions such as cardiopulmonary arrest management and CPR, fluid resuscitation, oxygen therapy, seizure monitoring and treatment, blood transfusions

and end-of-life care and euthanasia. These experiences helped me build confidence under pressure, learn triage principles and understand priorities in emergency medicine. Additionally, I assisted with discharge planning and communicating instructions to owners.

This rotation was among the most enriching due to its intensity, emotional complexity, and case variety as well as my personal preference for critical care and emergencies.

Internal Medicine

The internal medicine rotation was the longest part of my internship, providing extensive clinical knowledge and hands-on experience. Over the span of 15 weeks, I worked closely with Professor Dr. Rodolfo Oliveira Leal, the board-certified specialist responsible for the Internal Medicine and referral department, alongside the internal medicine residents Dr. Joana Dias and Dr. Beatriz Mendoza, PhD candidate Dr. Patrícia Marques and head nurse Élia Cosme. This immersive experience allowed me to witness the diagnostic and therapeutic management of a wide range of complex and often chronic conditions.

Each day began with morning rounds focused on hospitalized patients where clinical updates, diagnostic challenges, laboratory and imaging results and treatment adjustments were discussed. Afterwards, I participated in consultations ranging from first-opinion appointments to complex referrals and follow-ups. I conducted thorough anamneses, performed physical examinations and contributed to the differential diagnoses, diagnostic plans and treatment adjustments.

I gained proficiency in procedures such as blood collection, urine sampling via cystocentesis and glucose monitoring with transcutaneous devices. I also assisted in preparing patients for and performing endoscopic procedures including rhinoscopies, bronchoscopies with bronchoalveolar lavage, gastroscopies, colonoscopies and cystoscopies. Additionally, I participated in faecal microbiota transplants, joint aspirations and bone marrow biopsies.

Each case required structured case reporting including problem list formulation, diagnostic reasoning and treatment planning, skills that significantly enhanced my clinical thinking. Regular journal clubs, article presentations and simulated clinical discussions ensured that I also stayed tuned to current literature and evidence-based practice. This rotation solidified my passion for internal medicine and sharpened the analytical skills essential for managing complex, multisystemic cases.

Externship – VCA Canada Animal Emergency and Referral Centre

Between rotations at the Faculty of Veterinary Medicine, I completed an 80-hour externship at the VCA Canada Animal Emergency and Referral Centre in Vancouver, British Columbia. Split between daytime and evening shifts ending at 2 a.m., this opportunity

complemented the academic hospital setting of my internship, highlighting the intensity, speed, and valuable teamwork of a private emergency facility.

My responsibilities at VCA included daily monitoring of hospitalized patients, performing routine physical exams and point-of-care diagnostics such as glucose and blood pressure measurements, as well as A-POCUS (abdominal point-of-care ultrasound) ultrasound screenings. Repeatedly performing this protocol each day improved my anatomical recognition of structures in the ultrasound and significantly improved my confidence performing it.

The hospital operated with a well-coordinated nursing team that formed the foundation of patient care both in hospitalized animals and during emergency triage. Their organization allowed veterinarians to dedicate more time to case interpretation, diagnostics, treatment planning and client communication. This created a workflow in which every team member was ready to respond to the needs of critical patients and an environment of collaboration between professionals, with all members understanding their roles and responsibilities and working proactively to support one another and the patients.

During this externship, I had the opportunity to observe and assist in a wide range of emergency and critical care cases, including: acute neurological deficits in a previously healthy feline patient, later diagnosed with a cerebrovascular accident; hyperosmolar hyperglycaemic syndrome requiring intensive fluid therapy and insulin protocol adjustments; suspected central diabetes insipidus; immune-mediated haemolytic anaemia which required several transfusions and immunosuppressive therapy; suspected serotonin syndrome following amphetamine ingestion; two canine urethral obstructions successfully managed with retrograde urohydropulsion, fluid therapy and urinary catheterization; multiple trauma in cats following high-rise falls; polytrauma in a dog hit by a car; an emergent case of gastrointestinal foreign body obstruction in a canine patient, in which I participated during the surgery.

This externship was a truly enriching experience, both technically and professionally. It reinforced the importance of coordinated teamwork in high-stress clinical settings and exposed me to the management of a wide range of veterinary emergencies. I left this rotation with improved clinical confidence, a deeper understanding of emergency triage and stabilization, and great appreciation for the adaptability and precision required in critical care environments.

2. Literature Review

2.1 Adrenal Glands

The adrenal glands are bilateral organs located in the retroperitoneal space near the cranial pole of each kidney (Adin and Nelson 2017). They are divided into two portions – the cortex and the medulla – which produce different hormones but share a common blood supply (Hinson et al. 2010). The adrenal cortex is divided into three concentric zones, each responsible for synthesizing different steroid hormones. The outermost layer is the zona glomerulosa (Cunningham and Klein 2013), the zona fasciculata is the middle layer of the adrenal cortex (Engelking and Rebar 2012), and lastly the innermost layer is zona reticularis, adjacent to the adrenal medulla (Goff 2015). The medulla is involved in the fight-or-flight response through the production of catecholamines (Cunningham and Klein 2013).

The adrenal cortex and medulla have distinct embryological origins: the cortex arises from the mesoderm (Goff 2015), while the medulla is derived from the neuroectoderm (Cunningham and Klein 2013), which migrate into the developing cortex forming the centre of the gland. This developmental difference justifies their distinct hormonal functions and regulatory mechanisms (Adin and Nelson 2017).

The blood supply to the adrenal glands comes directly from the aorta through the adrenal arteries. These arteries pass through the capsule and form an arteriolar network, or arteriolar plexus, within the cortex (Fitzgerald 2011). Some vessels become medullary arteries (Hinson et al. 2010), which travel through the cortex to supply blood to the adrenal medulla, but numerous sinusoids – small, thin-walled blood vessels – intersect the cortical layers, delivering blood into the medulla (Adin and Nelson 2017). As a result, the adrenal medulla receives most of its blood supply via the cortical sinusoids, with only a small proportion coming directly from the medullary arteries (Fitzgerald 2011). The continuous flow of blood rich in steroid hormones ensures that the cells of the medulla are constantly exposed to the hormonal products of the cortex, highlighting the connection between these two regions (Hinson et al. 2010).

2.2 Adrenal Cortex

The outermost layer of the cortex, the zona glomerulosa, is the thinnest (Cunningham and Klein 2013). Its function is to produce mineralocorticoids, stimulated by the renin-angiotensin-aldosterone system. Mineralocorticoids, such as aldosterone, are involved in the regulation of electrolyte balance and blood pressure (Engelking and Rebar 2012).

The zona fasciculata is the middle zone of the adrenal cortex, responsible for the production of glucocorticoids and controlled mainly by the production of corticotropin-releasing

hormone (CRH) in the hypothalamus and consequent release of adrenocorticotropic hormone (ACTH) from the pituitary gland (Engelking and Rebar 2012). The main glucocorticoid produced is cortisol, which in turn has a negative-feedback effect on the hypothalamus and pituitary gland. Cortisol has many direct and indirect effects, namely the increase of blood glucose, rate of lipolysis, protein catabolism, glomerular filtration rate and water diuresis, the stimulation of hepatic gluconeogenesis, and the inhibition of glucose uptake and metabolism in the peripheral tissues (anti-insulin effect) (Goff 2015). Clinically the most useful effect is the suppression of the inflammatory response through the inhibition of the synthesis of inflammatory mediators such as prostaglandins, thromboxanes and leukotrienes (Cunningham and Klein 2013).

Lastly, the zona reticularis is adjacent to the adrenal medulla and produces both glucocorticoids and androgens, like dehydroepiandrosterone (DHEA) (Goff 2015). The total of glucocorticoids produced here is less significant than that produced by the zona fasciculata. In physiological conditions, the production of androgens is low but some pathological conditions can be responsible for the increase in production of these hormones. Androgen production is stimulated by ACTH, similarly to cortisol (Cunningham and Klein 2013). DHEA is responsible for an appropriate immunocompetence through mechanisms like downregulating the complement cascade and inflammatory cytokines while stimulating lymphocyte proliferation and increasing T cell and NK cell cytotoxicity (Prall and Muehlenbein 2018).

2.3 Adrenal Medulla

The adrenal medulla, as opposed to the cortex, is not clearly divided into sections. It is composed by modified neural tissue (Hinson et al. 2010) and its function is synthesizing and releasing catecholamines into the bloodstream (Cunningham and Klein 2013).

Catecholamines are a group of molecules that contain a catechol structure and a side chain with an amino group (Cunningham and Klein 2013). Norepinephrine (NE), epinephrine (Epi) and dopamine are part of this group, and are released either by the adrenal medulla or by neurons in the central and peripheral nervous systems, functioning as both neurotransmitters and hormones (Hinson et al. 2010).

There are two types of cells responsible for producing different catecholamines, called chromaffin cells, and they are interspersed throughout the medulla. Depending on the type of chromaffin cell, they synthesize Epi or NE, but both are stimulated by the release of acetylcholine (Engelking and Rebar 2012).

Acetylcholine is released by preganglionic sympathetic neurons and binds to specific receptors on the chromaffin cells of the adrenal medulla. These receptors act as ion channels, and when activated cause cell depolarization, ultimately triggering the release of Epi and NE

into the bloodstream (Cunningham and Klein 2013). This stimulus leads to a phenomenon called *stimulus-secretion coupling*, in which chromaffin cells simultaneously release and synthesize more catecholamines, leading to a rapid influx of these hormones in the bloodstream (Fitzgerald 2011).

The adrenal medulla is also responsible for supporting the sympathetic nervous system functions by releasing Epi and NE when the different organs are stimulated directly by sympathetic fibers. This dual mechanism of stimulation enhances the intended response and provides a safety factor, allowing one mechanism to compensate for the other if needed. Moreover, these hormones can stimulate cells that are not directly innervated by sympathetic fibers, increasing the metabolic rate of nearly all body cells, particularly in response to Epi (Hall and Hall 2021). Taking all of this into consideration, the adrenal medulla can be considered a modified sympathetic ganglion, with the chromaffin cells being homologous to postganglionic sympathetic adrenergic neurons (Engelking and Rebar 2012).

2.3.1 Catecholamine Synthesis

Catecholamine synthesis can either begin with the amino acid phenylalanine or tyrosine (Cunningham and Klein 2013), however tyrosine is more often the precursor because it bypasses the need for conversion and is readily available in the bloodstream after being absorbed through the intestine. If phenylalanine is the precursor, it must first be converted into tyrosine in the liver by phenylalanine hydroxylase (Engelking and Rebar 2012). Once formed, tyrosine enters the bloodstream from where it is then taken into the cytosol of chromaffin cells via specific amino acid transporters located on the cell membrane. The first steps in catecholamine synthesis occur in the cytosol and are dependent on the enzyme tyrosine hydroxylase (TH), which converts tyrosine into 3,4-dihydroxyphenylalanine (DOPA) (Zini 2024). TH is the rate-limiting enzyme, meaning its activity controls the overall production rate of catecholamines (Reusch 2015), and is also inhibited by the end products of the pathway in a negative feedback mechanism (Cunningham and Klein 2013). After DOPA is formed, it is converted to dopamine through the enzymatic activity of aromatic-l-amino acid decarboxylase (AAAD) (Engelking and Rebar 2012). Dopamine is then transported into chromaffin granules, where it is converted into NE by dopamine-β-hydroxylase (DBH) (Cunningham and Klein 2013). In cells that produce NE, this marks the end of the synthesis pathway. However, in cells that produce Epi, NE exits the chromaffin granule and re-enters cytosol, where it is converted to Epi by phenylethanolamine-N-methyltransferase (PNMT) (Reusch 2015). Epi is then stored in the chromaffin granule until its release (Cunningham and Klein 2013). The main steps in catecholamine synthesis are outlined in Figure 1.

Both TH and DBH are regulated by various factors, including ACTH, which enhances their activity particularly during stress, promoting an increased synthesis of catecholamines to meet physiological demands (Engelking and Rebar 2012).

The expression of the enzyme PNMT, which is responsible for converting NE to Epi, is regulated by glucocorticoids (Hinson et al. 2010). Due to the close proximity of the adrenal cortex, this regulation contributes to the significantly higher production of Epi when compared to NE in the adrenal medulla, especially during stress (Engelking and Rebar 2012). In cats, it is estimated that NE makes up approximately 40% of total catecholamine production in the adrenal medulla (Fitzgerald and Goldfien 2004). If glucocorticoid production is inhibited, PNMT expression decreases, the adrenal medulla produces mostly NE and shrinks in size (Hinson et al. 2010).

However, it is important to note that most of the NE present in the body does not originate from the adrenal glands. Instead, it is synthesised and released by non-adrenal sympathetic nerve terminals. In humans, up to 93% of all circulating NE is derived from these neuronal sources (Fitzgerald 2011).

2.3.2 Catecholamine Release

The adrenal medulla is stimulated directly by the sympathetic nervous system into releasing hormones in the bloodstream, presenting characteristics of both neuronal and hormonal nature (Cunningham and Klein 2013). This organ's innervation is provided by the splanchnic nerves, which originate from the spinal cord at the D8 to D11 vertebral levels (Hinson et al. 2010). Each preganglionic nerve is capable of inducing the *stimulus-secretion coupling* in a great number of chromaffin cells through the release of acetylcholine, amplifying the sympathetic response many times over (Cunningham and Klein 2013). In turn, acetylcholine release is stimulated mainly by hypoglycaemia and significant decreases in blood glucose, even if within physiological intervals. Acute stimulation of the sympathetic nervous system typically occurs due to events that lead to biochemical, physical, or emotional stress – such as anxiety or apprehension (Engelking and Rebar 2012), but even exercise can lead to an increase in catecholamine secretion (Reusch 2015). Some of the responses to these events also include maintaining blood pressure in cases of blood loss and maintaining body temperature when dealing with cold exposure (Engelking and Rebar 2012; Cunningham and Klein 2013).

When acetylcholine is released by preganglionic sympathetic neurons as a result of these stimuli, it binds to nicotinic acetylcholine receptors (nAChRs) on the chromaffin cells of the adrenal medulla (Hinson et al. 2010). These receptors function as ion channels, and when activated, they allow ions such as sodium (Na^+) and calcium (Ca^{2+}) to pass through the cell

membrane (Cunningham and Klein 2013). The influx of positively charged ions reduces the negative charge inside the cell, causing cell depolarization, which in turn triggers the exocytosis of chromaffin granules containing Epi and NE, releasing these molecules into the bloodstream (Fitzgerald 2011).

Even without a stimulus to the adrenal medulla, there is a constant basal secretion of Epi and NE that contributes to the *sympathetic tone*, the baseline state that allows the sympathetic nervous system to be further stimulated or inhibited according to physiological needs (Hall and Hall 2021). The *sympathetic tone* helps maintain and adjust the heart rate (Cunningham and Klein 2013), and ensures systemic arterioles remain in a partially constricted state, allowing for adjustments in vascular resistance. If sympathetic stimulation is increased, arterioles constrict further, on the other hand, a decrease in stimulation leads to vasodilation (Goff 2015). This regulation is crucial for maintaining blood pressure and overall cardiovascular function, even in the absence of direct sympathetic pathways (Hall and Hall 2021).

Additionally, age and sex have been reported to influence catecholamine metabolism in humans and dogs, as well as the levels of their major metabolites, metanephrine (MN) and normetanephrine (NMN), which will be discussed in further detail below. In humans, multiple studies have reported that plasma MN concentrations tend to be higher in males than in females, and that plasma NMN shows a strong positive correlation with age, particularly after 40 years of age. Some of these sex differences, like in urinary normetanephrine (uNMN), are thought to be largely attributed to differences in body size (Eisenhofer et al. 2013; Eisenhofer et al. 2019). In veterinary medicine, a study in healthy dogs by van den Berg et al. (2023) similarly identified a weak but statistically significant correlation between age and plasma free MN and NMN concentrations, suggesting that comparable influences may exist in non-human species.

2.3.3 Catecholamine Metabolism

Circulating catecholamines consist of Epi and NE released from the adrenal medulla, along with the majority of NE that diffuses into circulation from synapses in the sympathetic nervous system (Fitzgerald and Goldfien 2004). Up to 80% of NE released at synapses is reabsorbed by presynaptic neurons through reuptake mechanisms, while the remaining NE can diffuse into the bloodstream (Engelking and Rebar 2012). These catecholamines circulate in plasma without specific binding proteins, loosely bound to albumin (Hinson et al. 2010).

It was previously thought that the metabolism of catecholamines occurred predominantly in the liver, where 57% of circulating NE and 32% of circulating Epi would be inactivated (Eisenhofer et al. 1995). However, more recent literature indicates that most catecholamine metabolism actually takes place within the same cells in which they were

produced (Reusch 2015). Catecholamines steadily leak from their storage vesicles inside chromaffin cells and are metabolized by cytoplasmic membrane-bound catechol-O-methyltransferase (COMT) (Fitzgerald 2011), which converts Epi into MN and NE into NMN (Boot 2023). These metabolites are then released directly into circulation (Reusch 2015).

The remaining catecholamines that escape this initial metabolism may still be inactivated in the liver, kidneys, or postsynaptic tissues. Alternatively, they may undergo alternative metabolic processes such as conjugation to form sulphates and glucuronides, reuptake by neurons, or remain unchanged in circulation (Engelking and Rebar 2012). Due to these metabolic processes, the half-life of circulating catecholamines is short, lasting approximately 2 minutes for NE and even less for Epi (Cunningham and Klein 2013). After being converted by COMT, MN and NMN are further metabolized into vanillylmandelic acid (VMA) by monoamine oxidase (MAO) (Boot 2023). However, not all MN and NMN are metabolized into VMA in this pathway; a small proportion is also conjugated to sulphates and glucuronides by the liver before re-entering circulation (Engelking and Rebar 2012). Figure 1 illustrates the metabolic degradation pathways that follow catecholamine synthesis and release, indicating where each step occurs within the body.

It is also worth mentioning that other sources of VMA production exist, including norepinephrine and dopamine produced in catecholaminergic neurons. In these neurons, catecholamines undergo a different metabolic process involving the formation of reactive aldehydes, that are converted into different intermediate compounds before VMA is eventually produced in the liver (Eisenhofer et al. 2004). Eventually, both VMA and conjugated MN and NMN are filtered by the kidneys and then excreted in urine (Engelking and Rebar 2012). These rapid metabolic processes ensure that catecholamine levels can be tightly regulated, allowing the body to respond swiftly to physiological demands.

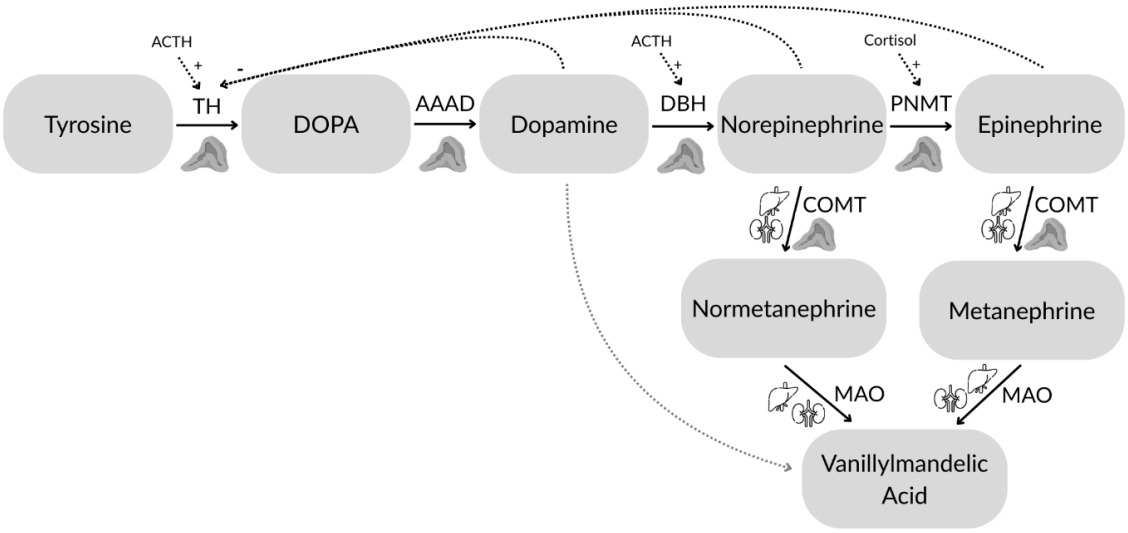


Figure 1 – Catecholamine synthetic and metabolic pathways. In chromaffin cells of the adrenal medulla, tyrosine is first converted into DOPA by TH, and then into dopamine by AAAD. Dopamine enters chromaffin granules, where it is converted into NE by DBH. If the pathway continues, NE is transported back to the cytosol and

converted into Epi by PNMT. NE and Epi are metabolized predominantly within the adrenal medulla itself but can also be released into the bloodstream and metabolized in the liver and kidneys, with other minor pathways also contributing. COMT converts NE and Epi into NMN and MN, respectively, and MAO further metabolizes these intermediates into VMA in the liver and kidneys. The pathway is stimulated by ACTH through increased activity of TH and DBH, while cortisol promotes Epi synthesis by enhancing PNMT activity. Negative feedback regulation occurs via dopamine, NE and Epi, which inhibit TH. VMA can also be derived from dopamine through alternative, less direct pathways not detailed in this figure.

2.3.4 Catecholamine Physiological Effects

Catecholamines primarily regulate metabolism and mediate the body's response to acute stress, known as the fight-or-flight response (Cunningham and Klein 2013). These effects are mediated through adrenergic receptors spread throughout the organism and can be divided and subdivided into different types of receptors. Some control catecholamine release from sympathetic nerve endings and are called alpha (α) receptors, subdivided into α_1 and α_2, whether they affect postsynaptic nerve endings or presynaptic terminals respectively (Fitzgerald 2011). Others are named beta (β) receptors and are subdivided into β_1, that affect mainly the heart, β_2 receptors that affect smooth muscle contraction and intermediary metabolism (Engelking and Rebar 2012) and β_3 that mediates lipolysis and intestinal motility (Fitzgerald 2011). Both Epi and NE can activate all adrenergic receptors, but the responses to either catecholamine vary in intensity. Coupled with the fact that different tissues have different receptor types, the overall response to each catecholamine can be very different (Engelking and Rebar 2012). Epi has a stronger effect in β_2 receptors and thus a bigger influence on the intermediary metabolism (Reusch 2015). NE has a stronger response in α_1 and α_2 receptors, responsible for, but not limited to, decreasing insulin secretion and vasoconstriction (Engelking and Rebar 2012), as well as β_3 receptors (Fitzgerald 2011). Regarding β_1 receptors, both Epi and NE elicit similar responses (Engelking and Rebar 2012).

When the fight-or-flight response is triggered, catecholamines activate catabolic pathways to meet the body's increased energy demands. They have a strong calorigenic effect that accelerates overall metabolic activity (Reusch 2015). This includes processes like hepatic glycogenolysis and gluconeogenesis, both stimulated directly through adrenergic receptors in the liver and indirectly by promoting glucagon release and reducing insulin output in the pancreas (Goff 2015). On top of this glucose increase, catecholamines also stimulate lipolysis, leading to the release of free fatty acids, which, together with glucose, serve as key energy substrates (Cunningham and Klein 2013). In the muscle tissue, Epi stimulates glycogenolysis but due to the absence of the enzyme glucose-6-phosphatase, it does not result in glucose production. Instead, it leads to lactate formation that can enter the bloodstream and be used by the liver as a gluconeogenic substrate (Engelking and Rebar 2012).

The cardiovascular system also undergoes significant changes, with catecholamines inducing vasoconstriction in skin and splanchnic arterioles, while dilating arterioles in skeletal

and cardiac muscles (Cunningham and Klein 2013). This optimizes blood flow to critical areas, accompanied by marked tachycardia and increased cardiac output (Reusch 2015). Catecholamines also stimulate the release of erythropoietin (EPO) from interstitial cells of the inner renal cortex and enhance EPO production (Engelking and Rebar 2012; Hall and Hall 2021). In response to hypovolemic shock, catecholamines promote the release of renin by juxtaglomerular cells of the kidney and the activation of the renin-angiotensin-aldosterone system. This system is responsible for regulating blood pressure and blood volume by promoting vasoconstriction and sodium retention via aldosterone release (Engelking and Rebar 2012).

Furthermore, respiratory function is enhanced through bronchiolar smooth muscle relaxation, increasing both the rate and depth of breathing (Reusch 2015). In parallel, ocular adjustments, such as pupil dilation and ciliary muscle relaxation, improve far vision, further preparing the organism for immediate action (Engelking and Rebar 2012).

2.4 Metanephrines in Daily Practice

2.4.1 Diagnostic Relevance

In a clinical setting, there are various circumstances in which catecholamine levels may be elevated, be it primarily or secondarily, and in response to pathological conditions such as tumours or due to physiological stimuli like exercise or acute stress (Goff 2015). Abnormal production of catecholamines can be due to pheochromocytomas (PCCs), tumours that arise from adrenal chromaffin cells, or paragangliomas (PGLs) that originate from ectopic chromaffin cells that failed to regress during foetal development (Engelking and Rebar 2012). Secondary causes of increased catecholamines include hypertension, chronic kidney disease (Joles and Koomans 2004), stress (Engelking and Rebar 2012), pain (Srithunyarat et al. 2018) and certain medications like insulin and antihypertensives (Boot 2023).

PCCs are rare tumours in both cats and dogs, with only a limited number of cases reported in cats (Wimpole et al. 2010; Prego et al. 2023), making it impossible to estimate their prevalence in the feline population. In humans, adrenal masses are detected in 1–8% of abdominal imaging exams, with prevalence increasing with age. Incidental adrenal tumours (incidentalomas) are found in 0.3–5.1% of abdominal CT scans (Boot 2023), while the incidence of PCCs specifically is estimated at 2 to 8 cases per million people annually. In dogs, PCCs represent approximately 0.01% to 0.1% of all tumours (Reusch 2015). As pets life expectancy increases and veterinary care becomes more accessible, the number of PCC diagnoses in cats may become more common.

Diagnosing PCCs poses several challenges, primarily due to the episodic nature of symptoms and non-specific laboratory abnormalities (Zini 2024). These tumours release

catecholamines in episodic bursts (Goff 2015), often resulting in paroxysmal clinical episodes interspersed with periods of apparent health (Zini 2024). The clinical signs frequently overlap with more common causes, which lowers the pre-test probability for PCC in symptomatic patients (Boot 2023). In dogs, excessive catecholamine release can lead to a broad spectrum of signs including weakness/lethargy, anorexia, vomiting, polyuria/polydipsia (Galac and Korpershoek 2017), tachycardia, hypertension (Goff 2015), panting, collapse, seizures and various degrees of arrhythmia, which in severe cases may cause sudden death (Zini 2024). Although laboratory abnormalities are mostly unhelpful (Kook et al. 2007), mild-to-moderate anaemia, leukocytosis, increased liver enzyme activities, azotaemia, decreased urine specific gravity and proteinuria have been reported in 25 – 50% of affected dogs (Barthez et al. 1997). Regarding imaging techniques, CT is considered the gold standard in dogs and is essential when surgical intervention is planned. However, PCCs in dogs are most often initially evaluated by ultrasonography due to its greater availability in clinical settings (Galac and Korpershoek 2017). Ultrasonographic findings are more commonly associated with advanced tumour stages, and adrenal origin cannot always be confirmed due to tumour size or location. Detection rates may be as low as 50%, and when detected lesions often present with variable echogenicity, which makes it difficult to distinguish PCCs from other types of adrenal or adjacent masses (Barthez et al. 1997). In humans, around 40% of PCC and PGL cases involve a germline mutation, so genetic testing is recommended for all patients as it helps guide follow-up, detect tumour metastasis risks and more (Boot 2023). While not yet routine in dogs, mutations have been found in some canine PCC / PGL cases (Holt et al. 2014), suggesting similar tumour pathways and a possible future role for genetic testing in diagnosis and monitoring.

While PCCs are well documented in humans and increasingly studied in dogs, information on feline PCCs remains limited (Reusch 2015). Plasma and urinary fractionated metanephrines are considered valuable diagnostic aids in feline PCC, with markedly increased concentrations reported in affected cats (Melián and Pérez-López 2019). A comprehensive case report by Prego et al. (2023b) describes the first *in vivo* case of feline PCC in veterinary literature and is one of the few histologically confirmed cases, noting that only six histologically confirmed cases had been reported before. In this report, the diagnostic process included plasma MN and NMN measurements, along with histopathology and immunohistochemical staining for chromogranin A and synaptophysin. The authors highlight that, although rare, PCC should be considered a differential diagnosis for adrenal masses in cats, particularly in the presence of signs like hypertension, polyuria/polydipsia and unexplained weight loss with preserved appetite. In a related study by some of the same authors, they compared plasma and urinary MN and NMN for feline PCC diagnosis. They found no clear advantage of one sample type over the other and emphasized the current lack of species-specific reference

intervals. Their findings suggest that NMN may be a more reliable biomarker than MN in cats (Prego et al. 2023a).

Given the diagnostic challenges posed by the variable clinical presentation (Goff 2015; Zini 2024) and limitations of imaging (Barthez et al. 1997), there has been a growing need for biochemical markers that offer a more reliable and sustained reflection of catecholamine activity for the diagnosis of PCC and PGL (Fitzgerald 2011; Reusch 2015).

Due to the short half-life of circulating Epi and NE, direct measurement of these catecholamines provides limited diagnostic insight and it is more efficient to measure metabolites that reflect cumulative production over a longer period of time (Srithunyarat et al. 2018). The primary metabolites of catecholamines are VMA, conjugated MN and conjugated NMN (Engelking and Rebar 2012). Since MN and NMN are the earliest metabolites to be produced after the inactivation of catecholamines and are detectable both in the bloodstream and in urine, they are usually the preferred markers of catecholamine production (Srithunyarat et al. 2018). Furthermore, it has been shown that PCCs exhibit continuous intratumoural production and secretion of metanephrines due to cytoplasmic membrane-bound COMT in chromaffin cells (Fitzgerald 2011), in contrast to the episodic secretion of catecholamines (Eisenhofer et al. 2004; Boot 2023). This makes metanephrine measurements more reliable for diagnostic purposes when compared to catecholamine measurements. In addition, metanephrine levels also correlate more strongly with tumour size than catecholamines, making them a more reliable marker (Reusch 2015). In contrast to MN and NMN, VMA has a slower production and originates from both adrenal and neuronal catecholamine sources (Eisenhofer et al. 2004) making it less specific for diagnostic purposes.

When interpreting metanephrine measurements results for PCC diagnosis, some cut-offs have been suggested for both plasma-free and urinary NMN in dogs (Zini 2024). Using a cut-off value equal to 4 times the upper limit of the reference range enables PCC to be detected with excellent specificity but poor sensitivity. Lowering the cut-off value to 2-3 times the upper limit of normal allows for a balance between sensitivity and specificity (Quante et al. 2010).

In humans, various other tests have been used to aid in the diagnosis of PCC. The clonidine suppression test, chromogranin-A measurement in blood and VMA assays in urine are commonly employed, providing valuable information for diagnosis (Fitzgerald 2011). For instance, the urinary VMA-to-creatinine ratio has shown moderate to high sensitivity and specificity in distinguishing dogs with PCC from those without, making it a useful tool in veterinary diagnostics (Zini 2024). Additionally, measuring serum inhibin concentration can help differentiate PCC from adrenocortical tumours, as inhibin is typically undetectable in PCC cases but detectable in adrenocortical tumours, though this is only true in neutered dogs (Zini 2024). While these tests provide helpful insights, they are often used in conjunction with more

advanced techniques like metanephrine liquid chromatography – tandem mass spectrometry (LC-MS/MS) to enhance diagnostic accuracy (Boot 2023).

In human medicine, elevated metanephrine levels have been documented not only in patients with catecholamine-producing tumours but also in those suffering from renal failure or severe illness. In cases of renal insufficiency, impaired clearance of conjugated metanephrines can lead to significantly elevated plasma concentrations, particularly in the deconjugated (free) forms. Free metanephrines are generally less affected and remain within reference intervals in most patients (Eisenhofer et al. 2005). Similarly, critical illness and physiological stress, such as that encountered in intensive care settings, have been associated with increases in plasma and urinary metanephrines due to sustained activation of the sympathetic nervous system (Lenders et al. 2014).

In veterinary medicine, similar illness-related elevations in urinary catecholamines and their metabolites have been observed in dogs, suggesting a comparable origin. A study in critically ill dogs reported significantly elevated urinary Epi, NE, MN and NMN concentrations when compared to the healthy control group (Cameron et al. 2010). Additionally, dogs with hyperadrenocorticism were also shown to have elevated urinary catecholamine and NMN concentrations, further emphasizing the impact of illness on these biomarkers (Quante et al. 2010). Although these influences have not been directly studied in cats, existing evidence supports the hypothesis that chronic illness, renal insufficiency, or stress-related conditions in feline patients may similarly affect catecholamine metabolite concentrations.

2.4.2 Serum versus Urinary Metanephrines

In humans, both serum metanephrines and urinary metanephrines – specifically urinary metanephrine (uMN) and uNMN – are widely used for diagnostic purposes (Eisenhofer et al. 1998; Boot 2023). A large study by Eisenhofer et al. (2004) demonstrated that plasma free metanephrines have a higher diagnostic accuracy (97.9%) compared to urinary free (93.4%) and deconjugated metabolites (92.9%). Specificity is comparable between plasma and urinary free metabolites (94.2%), but slightly lower for deconjugated forms (92.1%). One factor to consider in human diagnostics is the variation in urinary catecholamine production throughout the day, influenced by the circadian cycle (Kawanoa et al. 1990). Specifically, lower amounts of urine catecholamines are produced during the night. To account for this, 24-hour urine collections are recommended in humans (Boot 2023), although this approach is not always feasible in veterinary practices. Recently, a study by Peitzsch et al. (2020) proposed that overnight or first-morning urine collections could serve as a practical alternative to the 24-hour method, offering improved specificity for urinary free metanephrine measurements. Furthermore, metanephrines are excreted in urine predominantly in their sulfur-conjugated

form rather than as free molecules. Laboratory techniques for urinary measurements commonly measure total (free and conjugated) metanephrines, unlike plasma measurements, which typically quantify only the free form (Ahn et al. 2021).

However, several additional considerations must be addressed in veterinary medicine, including the behavioural differences in animals, their varying susceptibility to stress, the stability of biological samples during storage and the availability of specialized facilities capable of measuring these metabolites.

One key challenge is the storage and handling of plasma samples. Plasma catecholamines, in both humans and dogs, are highly susceptible to oxidation and must be preserved by acidification to prevent degradation. Even with acidification, rapid processing is still essential to minimize the loss of these compounds (Srithunyarat et al. 2018). For veterinary settings, where laboratories capable of measuring plasma metanephrines may not be easily accessible, the logistical challenges of sample transport and storage can make this diagnostic method less practical. In contrast, studies indicate that catecholamines remain stable in urine, particularly when samples are acidified, offering a more convenient and reliable alternative for veterinary diagnostics (Boot 2023).

Furthermore, the process of blood sample collection is known to induce stress in cats (Belew et al. 1999), which is a significant concern as stress can elevate catecholamine release and potentially lead to false positives in plasma metanephrine tests. In contrast, urinary samples are typically collected with less stress and may provide a more reliable and accurate reflection of baseline catecholamine levels in animals (Srithunyarat et al. 2018).

One additional advantage of urinary measurements is the ability to normalize analyte concentrations using urinary creatinine. Creatinine is a small, non-protein-bound molecule that is produced at a relatively constant rate via the spontaneous conversion of creatine and phosphocreatine in muscle tissue. Creatine is synthesized by the liver, kidneys and pancreas and is closely tied to an individual's muscle mass, age and sex (Chew and Schenck 2023). Once formed, creatinine is released into the bloodstream and excreted by the kidneys primarily through glomerular filtration, with negligible tubular secretion or reabsorption in dogs and cats (Cunningham and Klein 2013). Because its urinary excretion remains relatively stable over a 24-hour period in a healthy animal (Goff 2015), urinary creatinine is commonly used as a denominator to normalize other urinary solute measurements (Chew and Schenck 2023). This approach accounts for variations in urine concentration, making ratios like the urinary protein-to-creatinine (UPC) ratio more accurate and reliable across different samples (Yadav et al. 2020). Similarly, urinary metanephrine results can be normalized to urinary creatinine concentrations (Zini 2024), as demonstrated in studies such as Srithunyarat et al. 2018. This approach corrects for fluctuations caused by urine concentration and may help compensate for stress-related influences, enhancing the consistency and clinical interpretation of results.

In contrast, plasma or serum metanephrine concentrations cannot be normalized in the same way and are therefore more susceptible to acute fluctuations due to stress or changes in hydration status.

2.4.3 Urinary Metanephrines in Detail

2.4.3.1 Collection Methods

Metanephrine synthesis is influenced by various stressors – emotional, biochemical and physical (Engelking and Rebar 2012) – so minimizing stress during sample collection is crucial for diagnostic accuracy (Srithunyarat et al. 2018). Cats are particularly sensitive to stressful situations, such as being handled during an examination or being exposed to unfamiliar environments (Belew et al. 1999). Studies suggest that clinical examinations using low-stress handling techniques have a lesser impact on cats, particularly when performed at home rather than in a veterinary clinic setting (Nibblett et al. 2015). Consequently, collecting urine samples for metanephrine measurement in a non-stressful home environment may yield more reliable results (Boot 2023). Unlike catecholamines, metanephrines are relatively stable in collected samples, and special preservation is not required if the samples are assayed or frozen within one week of collection (Sasaki et al. 2021).

Various methods of urine collection exist, each with specific advantages and disadvantages depending on the clinical context. Some methods that warrant further discussion include cystocentesis, transurethral catheterization, free catch and post-voiding collection (Chew and Schenck 2023). While each method is discussed in more detail in the following paragraphs, a summary of their respective advantages and disadvantages can be found in Table 1.

Cystocentesis involves inserting a needle through the abdominal wall directly into the bladder, either guided by ultrasonography or using the bladder palpation technique (Bonaparte 2024). This method is ideal for obtaining a sterile sample and preventing contamination from lower urinary tract bacteria or debris and requires the use of sterile syringes and needles (Yadav et al. 2020). To obtain a representative sample, gentle agitation of the bladder before collection is recommended to resuspend settled sediment (Chew and Schenck 2023). The needle should enter the bladder lumen at a 45º angle to promote wall collapse after withdrawal, reducing the risk of urine leakage (Chew and Schenck 2023). While cystocentesis is a practical and accurate method, it has some limitations. It requires a distended bladder for successful collection and is not suitable for animals with coagulopathies (Yadav et al. 2020) due to the risk of internal bleeding from abdominal access. Another concern regarding this technique is the risk of iatrogenic haematuria, which may lead to false-positive blood contamination results. To prevent this, needle movement should be minimized through methods like the “dart-like”

technique that requires the use of only one hand and no finger adjustment to perform the urine collection (Chew and Schenck 2023).

Catheterization is rarely performed in cats as a urine collection procedure. It has various associated risks like trauma to the urinary tract, iatrogenic bacterial urinary tract infections (UTIs) (Yadav et al. 2020), urethral obstruction and in extreme cases urethral rupture (Chew and Schenck 2023). Furthermore, it requires sedation and is a more invasive procedure that is challenging both in male and female cats (Bonaparte 2024). Catheterization is commonly performed in hospitalized patients, and secondarily urine can be collected taking advantage of this situation. However, it can also be considered when cystocentesis is not feasible – such as in cases where the bladder is too small for needle aspiration. Similarly to cystocentesis, the bladder should be agitated before collection and a midstream sample is preferred to obtain the most representative sample (Chew and Schenck 2023).

Finally, both free catch and post-voiding collection are non-invasive voiding sampling methods and serve as alternatives to cystocentesis when sterility is not a primary concern (Chew and Schenck 2023). These methods rely on urine being collected during or after the animal's voluntary urination, which means the sample is in contact with the lower urinary tract where bacteria or debris can be present. As a result, sample contamination is one of the major disadvantages of these techniques (Yadav et al. 2020). Free catch is done by placing a container into the urine stream, preferably midstream for more accurate measurements, which can prove to be quite challenging. In cats with severe polyuria, this method might be successful but is overall unencouraged due to its difficulty. Instead, other options like post-voiding collection are recommended for this species. While post-voiding can consist of planned or unplanned collection of urine from a variety of surfaces or materials (Chew and Schenck 2023), it can also make use of special hydrophobic materials that mimic cats' normal litter box environment (Bonaparte 2024). Despite these improvements, urine collection at home can still be challenging, with studies showing that even among motivated owners, only 64% successfully obtained a sample (Mortier et al. 2023).

Hydrophobic sand litter is one of these options, as studies have shown it does not significantly alter key diagnostic parameters, such as the protein-to-creatinine ratio (UPC), even after 24 hours of exposure. This makes it a suitable method for serial monitoring of cats being treated for proteinuria (Kennils et al. 2022). Another option is non-absorbent plastic beads, such as Catrine® and Katkor®, which have been shown to be a viable choice for diagnostic purposes. Research indicates that Katkor® does not significantly alter phosphate concentrations or urine pH even after 2 hours, making it a reliable alternative to catheterization for repeated sampling (Delport and Fourie 2005). Katkor® is particularly beneficial when repeated urine sampling is required, as it eliminates the need for anaesthesia and reduces stress. By providing a non-invasive alternative to catheterization, it also minimizes the risk of

complications associated with this procedure (Delport and Fourie 2005). Catrine®, the non-absorbent litter used in the present study, has not been previously tested in published research. However, it is similar to Katkor®, suggesting that conclusions may be consistent between the two products.

According to Yadav et al. (2020), urine characteristics fluctuate throughout the day, influencing the suitability of different collection times for specific diagnostic purposes. Morning urine, which remains in the bladder overnight, tends to have a more acidic pH. This acidity provides a better reflection of renal tubular activity and helps preserve renal tubular casts by slowing their breakdown. In contrast, freshly produced urine is more suitable for microbial cultures and cellular morphology assessments, as it improves the recovery of fastidious microorganisms and prevents distortion of cellular components due to prolonged exposure in the bladder. For diagnosing conditions such as urinary tract infections or neoplasia, recently formed urine is recommended, as it ensures better preservation of cellular structures when preparing air-dried smears (Yadav et al. 2020). As previously mentioned, for uMN and uNMN measurements overnight or first-morning urine collection serves as the best alternative to the 24-hour method and is the preferred choice when only a single sample is being collected (Peitzsch et al. 2020).

Table 1 - Overview of urine collection methods: pros ⊕ and cons ⊖ for veterinary use

Collection Method	Sterility	Stress	Feasibility	Diagnostic Reliability
Cystocentesis	⊕ High sterility due to direct bladder access	⊖ Can be stressful; requires expertise	⊖ May not be feasible for all animals, especially small or fractious ones	⊕ Very reliable, minimizes contamination
Transurethral Catheterization	⊕ High sterility if done correctly	⊖ Stressful and invasive	⊖ Requires skilled personnel; may be difficult in certain animals	⊕ Reliable, but prone to minor contamination if incorrect technique
Free Catch	⊖ Lower sterility due to potential contamination from the external genitalia	⊕ Minimal stress as it is non-invasive	⊕ Easy and quick, no special equipment required	⊖ Potential for contamination, less reliable for diagnosing infections
Post-Void Collection	⊖ Contamination risk due to possible contact with external surfaces	⊕ Non-invasive, minimal stress	⊕ Simple to perform, no specialized equipment required	⊖ Can lead to contamination, less reliable for diagnosing infections

2.4.3.2 Laboratory Methodologies: from Humans to Cats

Humans

Most analytical methodologies for measuring urinary metanephrines have been developed and validated in human clinical settings, where MN and NMN are the same chemical compounds as those found in feline physiology. These methodologies are then often extrapolated to veterinary research with species-specific validation. Given the predominance of conjugated metanephrines in urine, most laboratories measure fractionated total metanephrines, including both the free and conjugated forms (Ahn et al. 2021).

In humans, the main methodologies to have in consideration available when measuring metanephrines are colourimetry, immunoassay (Boot 2023), liquid chromatography with electrochemical detection (LC-ECD) and LC-MS/MS (Fitzgerald 2011).

Colourimetry is no longer commonly used due to its numerous disadvantages, including the inability to differentiate between MN and NMN, lack of sensitivity and specificity and its unsuitability for high-throughput clinical workflows due to labor-intensive protocols. Despite these limitations, it remains an affordable method that doesn't require specialized instruments (Boot 2023).

Immunoassays emerged as an improvement over colourimetry, allowing individual analyte quantification using commercial radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) kits for both plasma and urine (Bílek et al. 2017). However, they have notable drawbacks, such as complicated pre-analytical steps and lack of standardization, leading to significant biases and variability when compared to chromatographic methods like LC-ECD and LC-MS/MS (Boot 2023). In 2018, a study by Srithunyarat et al. evaluated the use of ELISA for measuring urinary NMN and MN concentrations in cats for the first time. While precision for NMN was acceptable, the assay’s measuring range was not ideal for the low concentrations found in feline urine. Measurement of MN was less reliable, showing high variability. Despite these limitations, the study suggested that NMN ELISA testing could be useful for assessing sympathetic activation and diagnosing pheochromocytoma in cats, although further validation is needed.

Compared to previous methods, LC-ECD offers improved specificity and sensitivity, allowing for the separate measurement of MN and NMN in urine following acid hydrolysis (Boot 2023). However, this method is susceptible to interference from various substances, including medications (Fitzgerald 2011) like paracetamol (Davidson 2004) and amoxicillin (Barco et al. 2014), and dietary components. It also has practical challenges such as the need for complex chromatographic optimization and having limited specificity (Boot 2023), which have contributed to it being largely superseded by LC–MS/MS and immunoassay methods (Weismann et al. 2015). A clinical application of LC-ECD was demonstrated by Kook et al. n

2007, who used this technique to measure urinary catecholamines and metanephrines in dogs with suspected pheochromocytoma. They successfully quantified epinephrine, norepinephrine, dopamine, MN and NMN in spot urine samples using LC-ECD. Their study revealed that measurement of these compounds, particularly NMN, could aid in diagnosing pheochromocytoma in dogs, though environmental stress significantly influenced urinary catecholamine levels.

In contrast, LC-MS/MS has become the gold standard for metanephrine analysis, particularly in plasma and increasingly in urine (Lenders et al. 2014). Since its introduction, this technique has seen significant growth due to its faster sample processing compared to conventional high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS). As a result, laboratories can analyse a greater number of samples within the same timeframe (Grebe and Singh 2011). LC-MS/MS offers superior specificity, sensitivity and reduced susceptibility to common interferences, making it more reliable for detecting PCC than previous methods (Boot 2023). Most reference laboratories in the United States use LC-MS/MS for metanephrine assays, achieving a sensitivity of 97% and specificity of about 87% in diagnosing PCC (Fitzgerald 2011). While LC-MS/MS is widely regarded as the most accurate and robust method, it does come with its own challenges, including high initial setup costs, specialized maintenance and variability in calibration approaches between laboratories (Boot 2023). Nevertheless, LC-MS/MS's ability to reduce drug interference, which can be a significant issue for assays using LC-ECD, makes it the preferred method for clinical testing of metanephrines, especially in diagnosing PCC (Fitzgerald 2011).

Cats

Accurate measurement of metanephrines in cats requires following strict sample handling protocols. Plasma is recommended to be collected in EDTA, centrifugated, and frozen prior to analysis, while urine samples were traditionally subjected to an acidification process before HPLC testing (Melián and Pérez-López 2019; Prego et al. 2023a). Acidification was previously considered necessary for LC-MS/MS as well (Prego et al. 2023a). Analytical determination was traditionally performed using HPLC with electrochemical detection (ECD) or LC-MS/MS (Melián and Pérez-López 2019), but LC-MS/MS is now the preferred method due to its increased specificity and sensitivity (Prego et al. 2023).

In a recent study by Prego et al. (2023a), LC-MS/MS was applied to measure plasma and urinary MN and NMN in ten healthy cats and one cat with histologically confirmed PCC. The affected cat displayed markedly increased concentrations, particularly of NMN, compared to healthy controls, highlighting the clinical applicability of these assays. Furthermore, this study also demonstrated that urinary metanephrines remained stable for at least 24 hours at refrigeration temperature without undergoing an acidification process.

3. Urinary metanephrines in cats – does collection method matter?

3.1 Introduction

The measurement of MN and NMN plays a critical role in the diagnosis of catecholamine-producing tumours such as PCCs and PGLs (Engelking and Rebar 2012; Srithunyarat et al. 2018). In human medicine, measurements of catecholamine metabolites are widely recognized as reliable biomarkers due to their high diagnostic sensitivity (Eisenhofer et al. 2004; Reusch 2015). Unlike catecholamines, MN and NMN are continuously produced within chromaffin cells, making them more stable indicators of catecholamine activity (Fitzgerald 2011; Boot 2023). In recent years, interest has grown in adapting this diagnostic approach for veterinary medicine, particularly in feline patients, where PCCs are considered rare but likely under-diagnosed (Reusch 2015; Prego et al. 2023b).

In cats, research about metanephrine measurement is still in its early stages. Studies have begun to establish reference intervals for both metabolites in plasma in healthy cats (Wimpole et al. 2010), and explore the diagnostic value of metanephrines in urine samples (Srithunyarat et al. 2018). However, the impact of urine collection methods on metanephrine concentrations in cats remains unclear. This gap in knowledge is significant, because different collection techniques such as free-catch, cystocentesis, or catheterization may influence the measured values through factors like activation of the sympathetic nervous system due to stress (Boot 2023), but also sample contamination or dilution (Yadav et al. 2020; Chew and Schenck 2023).

Cats are particularly sensitive to environmental stress, and this has direct implications for biochemical testing. The "white-coat effect", in which stress from veterinary visits alters physiological parameters like blood pressure, has been well documented in felines (Belew et al. 1999). In opposition, procedures performed in a home setting have a lesser impact on cats (Nibblett et al. 2015). Since metanephrine production is influenced by stress (Engelking and Rebar 2012), it’s possible that the stress of collection in a hospital setting could artificially elevate urinary levels. In contrast, urine collected in the home environment may better reflect baseline catecholaminergic activity, especially in this stress-prone species.

While studies in humans and dogs have demonstrated that sample handling and collection method can affect metanephrine results (Eisenhofer et al. 2004; Kook et al. 2007; Peitzsch et al. 2020), this topic remains largely unexplored in cats. As such, it is not yet known whether the chosen collection method could compromise the interpretation of metanephrine results in clinical or research settings. Understanding and controlling this potential variability is essential for developing reliable diagnostic protocols for feline patients in veterinary endocrinology.

The objective of this thesis is to investigate whether the stress associated with urine collection methods – specifically in-hospital versus at-home sampling – affects the measurement of urinary metanephrines in cats by LC-MS/MS. This research represents a logical step forward from existing literature, which has so far focused on establishing feasibility and reference intervals rather than stress-related variables. We hypothesize that urinary metanephrines evaluated in urine collected at the hospital will be higher than at home due to the stress effect. By addressing this gap, this study aims to contribute to the refinement of diagnostic tools for catecholamine-producing tumours in cats and contribute to the development of veterinary endocrinology.

3.2 Materials and Methods

Cat Recruitment

This was an observational, cross-sectional study involving repeated measures. Two urine samples were collected per cat – one at home and one in a hospital setting – and were analysed for both MN and NMN. The study included privately owned cats from the Veterinary Teaching Hospital at the Faculty of Veterinary Medicine, University of Lisbon, which were either healthy or diagnosed with stable, non-adrenal illness.

The cats included in this investigation were part of a larger PhD research project ongoing at the clinical department, with some serving as controls for healthy (H) animals and others as controls for chronically ill (CH) animals. Ethical approval for this study was integrated within the ethical protocol of that PhD investigation, which was approved by the local ethical committee (Annexe 1).

All of the participating cats' owners provided informed consent for inclusion, and the animals underwent a health screening prior to enrolment in this study.

Health Screening and Pre-Study Assessment

In order to be included in the study, each cat underwent a complete physical examination, blood and urine analyses, blood pressure measurement and an abdominal ultrasound.

The physical examination began with blood pressure measurement using the oscillometric method (Suntech device), performed first to minimize the effects of stress and obtain more reliable values. This was followed by a standard clinical examination. At the end of the appointment, blood samples were collected, and urine was obtained through cystocentesis.

Blood samples were used to perform a complete blood count, and a biochemistry profile including: alanine transaminase (ALT), gamma-glutamyl transferase (GGT), glucose, inorganic phosphorus, potassium, creatinine, urea, symmetric dimethylarginine (SDMA) and total thyroxine (T4) to evaluate thyroid function. Urine samples were used for urinalysis and to determine the urinary protein-to-creatinine ratio.

Ultrasonographic evaluation was performed by an experienced certificate-holder veterinarian to assess overall abdominal health and to exclude adrenal abnormalities. The equipment used was an Esaote MyLab Omega ultrasound machine, with micro-convex (3-11 MHz) and linear (4-15 MHz) probes. Examinations were conducted in B-mode only. Depth, gain and contrast were adjusted as needed for image quality. Cats were positioned in lateral recumbency, and no sedation was administered.

Cats presenting with signs of acute or unstable illness or abnormal findings on ultrasound were excluded from the study. According to (Pérez-López et al. 2021), adrenal thickness in healthy cats shows weight-dependent variations. Therefore, our study followed the suggested upper limits of 3.9 mm for cats weighing ≤ 4 kg and 4.8 mm for those weighing > 4 kg up to 8 kg. Cats with hypertension or with abnormal results in bloodwork or urinalysis could only be included in the group representing CH animals.

Sample Collection and Analysis

Urine samples were obtained from each cat under two different conditions: one using a post-void collection method in a home setting, and the other via cystocentesis in a hospital setting. This allowed for within-subject comparisons, which was one of the primary objectives of the study.

Owners were instructed to collect the home sample either before the hospital visit or at least three days afterward, to minimize the potential impact of clinical stress on urinary metanephrine levels. For this collection, a non-absorbent litter (Catrine®) was provided. Once collected, samples were then transferred to sterile containers and stored in the owner's refrigerator at 4 °C for a maximum of 24 hours before transport to the hospital.

Cystocentesis, guided by ultrasonography, was performed at the veterinary teaching hospital during a scheduled visit. This procedure was conducted prior to any other diagnostic interventions to reduce the influence of stress on the results.

At the hospital, all urine samples were stored at -80ºC in polypropylene tubes until shipment. Samples were collected over a period of no longer than six months and shipped together in a single batch for simultaneous analysis in order to minimize variability and ensure consistency in data analysis.

Samples were analysed at the external laboratory Algemeen Medisch Laboratorium in Antwerp, Belgium, following validated protocols for veterinary samples previously established for urine metanephrines in cats by Prego et al. (2023a).

Quantification of uMN and uNMN was performed using LC-MS/MS without prior hydrolysis, therefore reflecting the free and unconjugated forms. For each sample, uMN and uNMN concentrations were measured in µg/L, and creatinine ratios were calculated (µg/g). Metanephrine values were then converted to nmol/L for statistical analysis.

Statistical Analysis

Data was collected and recorded in Microsoft Excel version 2504.

All statistical analyses were preformed using the software IBM SPSS version 30.0.1.0. Data was assessed for normality using the Shapiro-Wilk test and visual inspection of Q-Q plots. Based on these results, appropriate parametric or non-parametric tests were selected.

A p-value < 0.05 was considered statistically significant for all tests.

When variables were not normally distributed, they are expressed as the median and interquartile range (IQR). In contrast, if they follow a normal distribution they are expressed as the mean ± standard deviation (SD).

For comparisons between groups, age distributions were calculated through the non-parametric Mann-Whitney U test, and sex distribution was compared using Fisher's Exact Test due to small sample sizes.

To compare values of MN, NMN and their respective creatinine ratios within the same animal and between the two collection methods (cystocentesis and home collection), the Wilcoxon signed-rank test was used due to non-normal distributions. Paired scatterplots were generated to visually support and represent differences, or lack thereof, between paired values from the two collection methods.

Intraclass correlation coefficients (ICC) were calculated using a two-way mixed-effects model for absolute agreement of single measures [ICC(3,1)], comparing metabolite levels measured via cystocentesis and Catrine® samples. This model was selected as each cat was assessed with both collection methods, and the objective was to evaluate absolute agreement between these two specific collection techniques. 95% confidence intervals (CI) were calculated for each ICC.

To visually assess agreement and identify both systematic and proportional bias between the two urine collection methods, Bland-Altman plots were generated for each metabolite. These plots display the mean difference (bias) between cystocentesis and home collection samples, along with the limits of agreement (LoA), calculated as ±1.96 times the SD of the differences. This complements the ICC analysis by revealing whether disagreement

between methods increases with higher analyte concentrations – i.e., whether proportional bias is present.

To assess the existence of confounding factors, linear mixed-effects models were constructed for each variable. Fixed effects included: collection method, health group, sex and age group (adult or senior classification). Random effects grouping factors were specified for the variable “Cat” to account for repeated measures within individual animals. Prior to building the final models, each potential confounding factor was tested individually and a threshold of $p < 0.2$ was used for inclusion, to avoid overlooking potentially meaningful variables that may reach significance in a multivariable context.

3.3 Results

Animal Characteristics and Pre-Study Assessments

A total of 19 cats were included in the study, consisting of 8 males and 11 females. Ages ranged from 7 to 15 years, with a mean of 9.87 ± 2.4 years, resulting in 11 adults and 8 seniors based on the American Animal Hospital Association guidelines (Quimby et al. 2021). Of these, 9 cats belonged to the H group, and 10 to the group representing CH animals. One cat was excluded from the study due to the owner’s inability to collect a urine sample using the provided non-absorbent litter. According to the owner, the cat refused to use the litter and urinated outside the litterbox.

Cats in the H group showed no relevant abnormalities in bloodwork, urinalysis, or adrenal ultrasonography. Incidental findings unrelated to the study objectives were observed in some animals, including one case of myelolipoma and occasional crystalluria. None of the cats in this group were hypertensive, as defined by the latest ACVIM consensus statement on this topic, that considers systolic blood pressure < 160 mmHg to be in the normal range for cats (Acierno et al. 2018).

The CH group presented with abnormalities related primarily with gastrointestinal, renal, or viral conditions. These findings are summarized in Table 2.

The median age of cats in the H group was 8.00 (8.00–9.00) years, consisting of 8 adults and 1 senior. In the CH group, the mean age was 11.00 ± 2.17 years, with 3 adults and 7 seniors. A Mann-Whitney U test revealed a statistically significant difference in age between the two groups ($U = 74.00$, $p = 0.002$, $r = -0.589$).

Sex distribution between groups was also evaluated. The H group included 3 males and 6 females, while the CH group included 5 males and 5 females. A two-sided Fisher’s Exact Test indicated no significant difference in sex distribution between groups ($p = 0.342$).

Table 2 – Relevant diagnoses, imaging and laboratory findings in chronically ill cats

Name	Age (years)	Diagnosis(es)	Relevant Imaging Findings	Relevant Lab Findings
Case 1	11	FIV+; Chronic diarrhea	Diffuse intestinal thickening; Lymphadenopathy	Struvite crystalluria
Case 2	8	CKD	Renal infarcts; Enlarged colic lymph node	↑Urea; ↑Creatinine; ↑Phosphorus; ↑SDMA
Case 3	11	Nasal polyp; Chronic rhinitis	-	-
Case 4	13	CKD	Typical signs of CKD	↑Creatinine
Case 5	7	FIV+	Diffuse intestinal thickening; Hyperechoic kidneys	Proteinuria; Hypertension
Case 6	11	Struvite crystalluria; IBD	Delayed gastric emptying	Struvite crystalluria
Case 7	11	CKD	CKD signs	↑Creatinine; Proteinuria
Case 8	14.5	CKD; Suspected ear tumour	Renal sclerosis; Pancreatic cysts; Hepatic myelolipoma	↑Urea; ↑Phosphorus
Case 9	15	Pancreatitis, CKD	Gastric wall thickening	-
Case 10	9	FIV+, ataxia, toxoplasmosis	Mesenteric lymphadenopathy	-

Legend: CKD – chronic kidney disease; FIV – feline immunodeficiency virus; IBD – inflammatory bowel disease; SDMA – symmetric dimethylarginine.

uMN and uNMN Measurements by LC-MS/MS

Urine samples were successfully collected from 19 cats, each providing one sample at home (Catrine®) and one in the hospital (Cystocentesis). A total of 38 samples were sent for LC-MS/MS analysis. Descriptive statistics for uMN and uNMN concentrations and respective urinary creatinine ratios are presented in Table 3.

Table 3 - Descriptive statistics of uMN, uNMN, and creatinine ratios

Metabolite	Collection Method	Mean ± SD / Median (IQR)
uMN (nmol/L)	Cystocentesis	690.355 (342.640 - 982.234)
uMN (nmol/L)	Catrine®	358.269 ± 192.836
uMN/creatinine (ug/g)	Cystocentesis	41.0 (27.5 - 64.5)
uMN/creatinine (ug/g)	Catrine®	26.0 (16.5 - 30.5)
uNMN (nmol/L)	Cystocentesis	1475.410 (1161.202 - 2030.055)
uNMN (nmol/L)	Catrine®	1410.124 ± 757.333
uNMN/creatinine (ug/g)	Cystocentesis	85.0 (70.0 - 125.5)
uNMN/creatinine (ug/g)	Catrine®	83.0 (72.0 - 110.0)

To evaluate whether there was a statistically significant difference between home and hospital urine collection methods, samples were analysed using the Wilcoxon signed-rank test (Table 4). Paired scatterplots were generated to provide visual representations of differences between pairs (Figure 2).

For three of the four variables – uMN (nmol/L), uMN/creatinine (µg/g) and uNMN (nmol/L) –, values were significantly higher in cystocentesis samples compared to Catrine® samples. These results align with the previously reported means and medians and can also be visually confirmed in the paired scatterplots in Figure 2. Only uNMN/creatinine (µg/g) did not show a statistically significant difference between the two methods, despite a slightly higher median in cystocentesis.

Table 4 - Wilcoxon signed-rank test comparing cystocentesis and Catrine® samples

	Wilcoxon signed-rank test		
Cystocentesis vs Catrine®	W	z	p
uMN (nmol/L)	170.000	3.018	0.001
uMN/creatinine (ug/g)	155.500	2.435	0.016
uNMN (nmol/L)	152.000	2.294	0.020
uNMN/creatinine (ug/g)	112.500	0.704	0.494

Some individual cats did present higher concentrations in their Catrine® samples, as can be observed in Figure 2, but this variation was not statistically significant and did not affect the overall result.

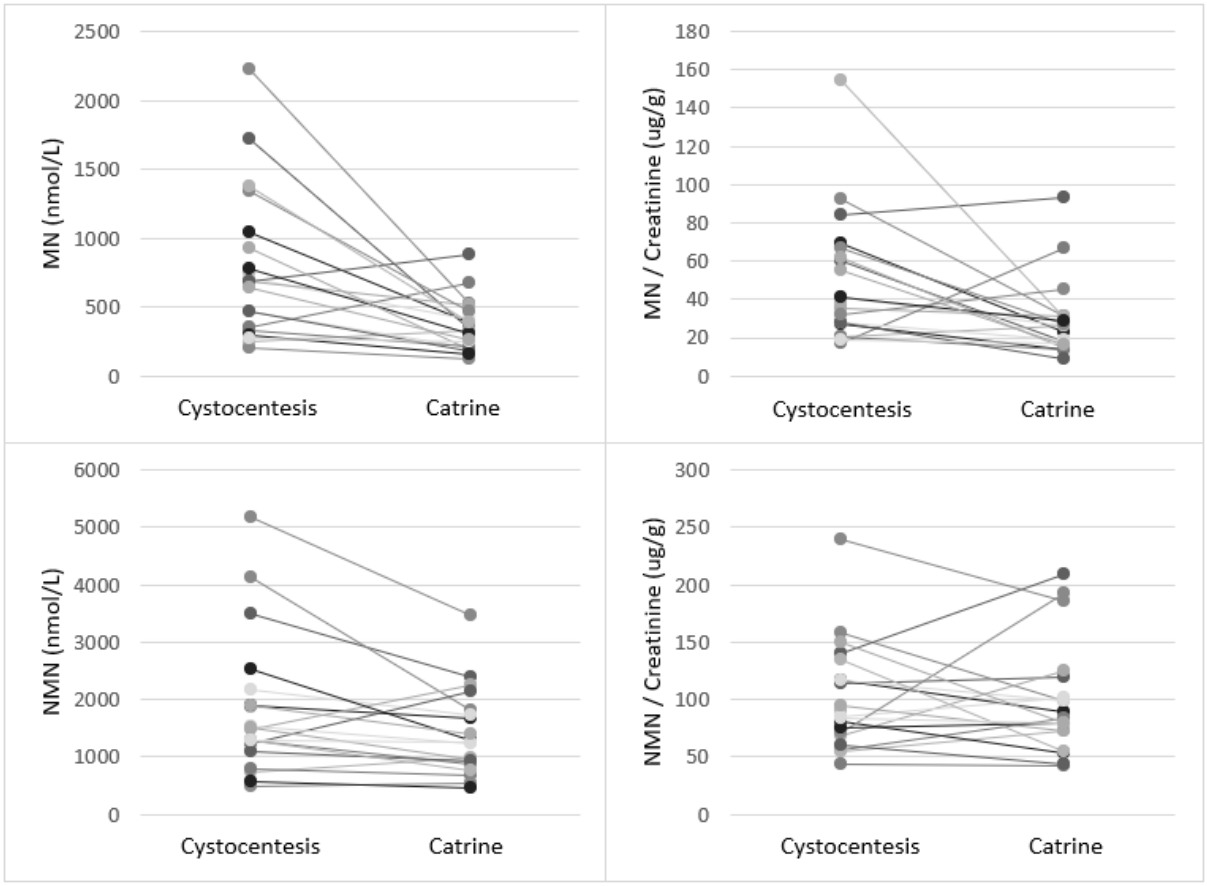


Figure 2 – Paired scatterplots for each metabolite measured, comparing hospital (cystocentesis) to home collected (Catrine®) samples. The top left graphic shows uMN (nmol/L), top right shows MN / creatinine ratio (ug/g), bottom left shows uNMN (nmol/L) and bottom right NMN / creatinine ratio (ug/g).

To assess reliability and agreement between collection methods despite the observed systematic differences, ICCs were calculated (Table 6). NMN showed good agreement (ICC = 0.715), while NMN/creatinine had moderate agreement (ICC = 0.489). In contrast, both MN and MN/creatinine demonstrated poor agreement (ICC = 0.190 and ICC = 0.159, respectively).

Table 6 - ICC values assessing agreement between urine collection methods

Intraclass correlation coefficient

Metabolite	ICC(3,1)	95% CI
uMN (nmol/L)	0.190	-0.139 to 0.481
uMN/creatinine (ug/g)	0.159	-0.169 to 0.456
uNMN (nmol/L)	0.715	0.512 to 0.842
uNMN/creatinine (ug/g)	0.489	0.200 to 0.700

These ICC values were consistent with the generated Bland-Altman plots (Figure 3), which revealed a consistent positive bias across all concentrations, indicating higher values in

cystocentesis samples when compared to home-collected ones. The mean differences and LoA for each metabolite are summarized in Table 7.

Visual inspection of the plots suggested proportional bias, especially for uMN and uNMN, with increasing variability at higher concentrations, indicating that discrepancies between methods tend to be more relevant as metanephrine values rise.

Table 7 - Bland-Altman analysis of metabolite concentrations between collection methods

Metabolite	Mean Difference (Bias)	Limits of Agreement (LoA)
uMN (nmol/L)	418.915	-611.029 to 1448.860
uMN/creatinine (ug/g)	21.105	-49.964 to 92.174
uNMN (nmol/L)	405.522	-1115.908 to 1926.952
uNMN/creatinine (ug/g)	3.526	-93.545 to 100.597

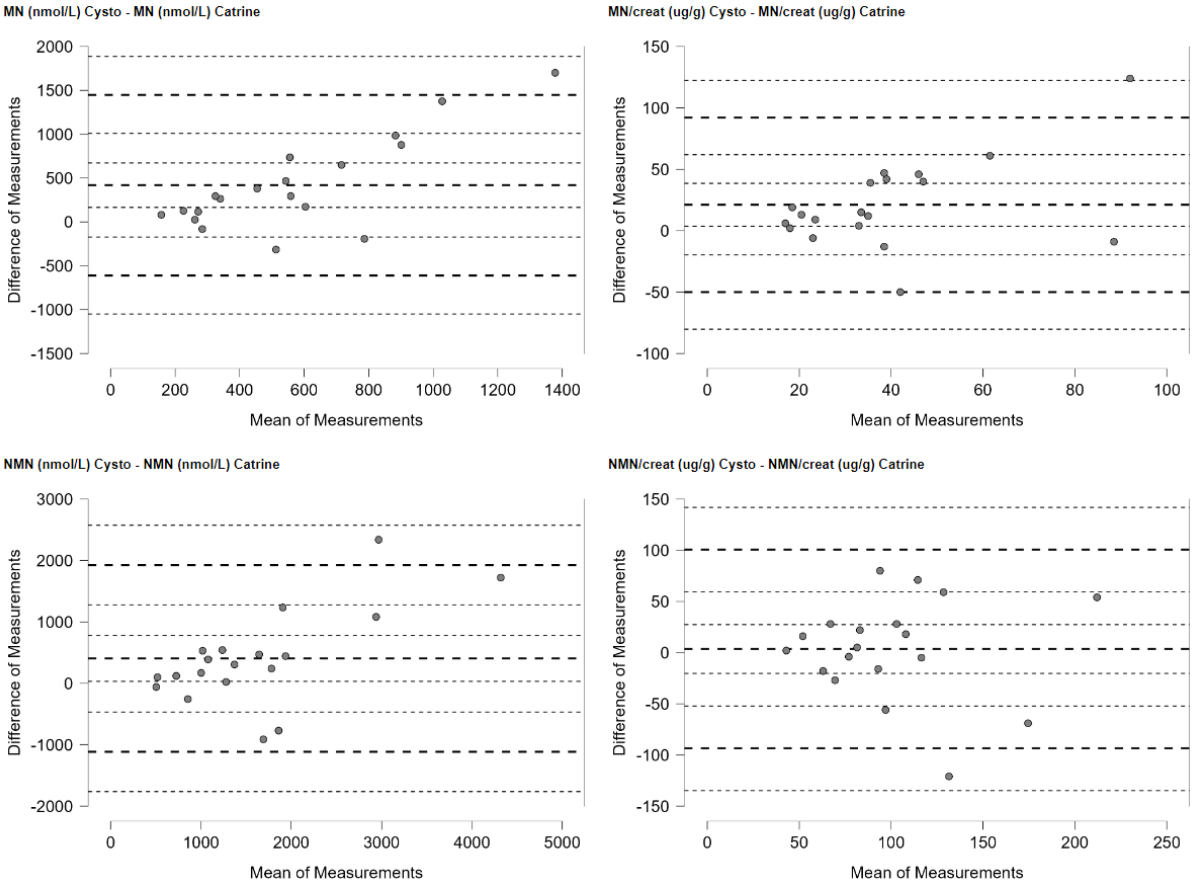


Figure 3 – Bland-Altman plots comparing hospital (cysto) to home collected (Catrine®) urine samples for each metabolite. Plots display the mean difference (bias) and LoA (±1.96 SD) as bold dashed lines, allowing for visualization of both systematic and proportional differences between collection methods.

Assessing Confounding Factors

To evaluate the influence of potential confounding factors on urinary catecholamine metabolite levels, a multiple regression analysis was conducted for each metabolite. The fixed effects variables considered were urine collection method, health group, sex and age group. Prior to building the full model, each variable was tested individually in a linear mixed-effects model to assess its relevance. Variables with a *p*-value < 0.2 were selected for inclusion in the final multivariable model to ensure potentially meaningful confounding factors were not prematurely excluded.

The final model results, including fixed-effect estimates, can be found in Table 8. The collection method significantly affected three of the four dependent variables – uMN, uMN/creatinine and uNMN – with *p*-values ranging from 0.003 to 0.035. In all cases, cystocentesis samples showed higher values compared to Catrine® samples. Regarding health group, there was a significant effect only in uMN levels ($p = 0.019$) where CH group cats had significantly higher values, while no significant effects were observed for the other metabolites. Sex significantly influenced uMN/creatinine ($p = 0.005$) and uNMN/creatinine ($p = 0.013$) values, with females showing higher values than males. As for age group, no statistically significant influence was detected in any of the models.

Table 8 - Summary of fixed-effect estimates from the final linear mixed-effects models.

Dependent Variable	Fixed Effect	Estimate	SE	t	p-value
uMN (nmol/L)	Method (Cystocentesis)	209.46	60.28	3.47	0.003
	Sex (Male)	-77.69	62.78	-1.24	0.234
	Group (Chronic)	162.22	62.08	2.61	0.019
uMN/creatinine (ug/g)	Method (Cystocentesis)	10.55	4.11	2.57	0.015
	Sex (Male)	-12.36	4.16	-2.97	0.005
uNMN (nmol/L)	Method (Cystocentesis)	202.76	89.04	2.28	0.035
	Group (Chronic)	493.58	239.15	2.06	0.056
	Age Group (Senior)	42.97	241.86	0.18	0.861
uNMN/creatinine (ug/g)	Sex (Male)	-21.12	7.61	-2.78	0.013
	Group (Chronic)	14.21	7.52	1.89	0.077

Comparison with Previously Reported Values

Although reference intervals for urinary metanephrines in cats are not yet well established, a study by Prego et al. (2023a) proposed the uNMN/creatinine ratios as the most promising urinary biomarker. In that study, ten clinically healthy cats had a median value of

139 µg/g (IQR: 77), corresponding to a range of 100.5 - 177.5 µg/g. No overlap was observed between the healthy cats and one cat diagnosed with pheochromocytoma, whose value was 1262 µg/g (Prego et al. 2023a).

In our H group, 5/9 (55.5%) cystocentesis samples and 2/9 (22.2%) home-collected samples fell within the previously reported range. One sample from each method (11.1%) exceeded the upper limit (239 µg/g via cystocentesis and 209 µg/g via Catrine®), while 3/9 (33.3%) cystocentesis and 5/9 (55.5%) home-collected samples were below the range.

Among CH cats, 2/10 (20%) cystocentesis samples and 1/10 (10%) home-collected samples were within the range, and none exceeded it. Most CH samples were below the range, 8/10 (80%) in both cystocentesis and home-collected samples.

3.4 Discussion

This study explored whether urine collection method influences the measurement of urinary metanephrines in cats, an area that remains largely under-investigated in feline medicine. To the author's knowledge, this is the first study to directly compare in-hospital (via cystocentesis) and at-home (via Catrine® litter) urine samples in cats using LC-MS/MS.

Our results showed that cystocentesis samples yielded significantly higher values for uMN, uMN/creatinine, and uNMN when compared to Catrine® samples. This difference was particularly marked in CH cats, whereas in H cats, the collection method did not significantly impact the measured values. These findings suggest that urine collection method, and thus environmental stress, may influence metanephrine concentrations, especially in cats with underlying diseases. This effect can be explained by the stress associated with clinical procedures such as restraint, transport, exposure to an unfamiliar environment and cystocentesis itself, all of which can trigger acute catecholamine release via activation of the sympathetic nervous system (Belew et al. 1999; Engelking and Rebar 2012). Cats are especially sensitive to stress (Nibblett et al. 2015), and this physiological response may significantly alter catecholamine metabolite levels (Srithunyarat et al. 2018). The potential contribution of the chronic illness itself to elevated values is considered in detail further below. It's important to note that although both uMN and uNMN were significantly influenced by collection method, the uNMN/creatinine ratio remained relatively stable. This suggests that, despite variability in absolute concentrations, this ratio may serve as a more reliable diagnostic marker, less affected by acute stress or sampling conditions. Similar findings have been reported in prior studies, where catecholamine metabolite ratios, especially uNMN/creatinine, demonstrated greater consistency and diagnostic value than absolute concentrations (Srithunyarat et al. 2018). These results support the potential use of uNMN/creatinine as a reliable tool in feline PCC diagnostic protocols.

In the ICC results, uNMN exhibited the strongest agreement between collection methods, suggesting that its concentrations may be relatively consistent across sampling conditions. In contrast, uNMN/creatinine showed only moderate agreement, while both uMN and uMN/creatinine showed poor agreement, with wide confidence intervals suggesting high variability and potential systematic differences between methods. These findings were supported by the Bland-Altman analysis, which revealed a consistent positive bias across all analytes, indicating that cystocentesis samples consistently yielded higher values than home-collected ones. The plots also showed evidence of proportional bias for both uMN and uNMN, with discrepancies between methods increasing as concentrations rose. This suggests that while the two methods produce similar results at low analyte levels, the divergence becomes more pronounced as values rise, particularly for uMN and uNMN. Together, these two findings emphasize that uNMN is the most stable metabolite across collection methods and appears to be less influenced by external factors such as environmental stress. Furthermore, the uNMN/creatinine ratio demonstrated reduced proportional bias at higher concentrations, supporting its potential as a more reliable marker in clinical settings.

In this study, a multivariable linear regression model showed that health status significantly influenced uMN concentrations, with CH cats presenting higher values than H ones. This finding partially aligns with previous observations in both human and veterinary medicine where illness, particularly in systemic or critical conditions, has been associated with elevated catecholamine and metanephrine levels (Cameron et al. 2010; Quante et al. 2010; Lenders et al. 2014). However, in contrast to those earlier studies, no significant effects were observed for uNMN or either creatinine ratios. Given that several cats in the CH group had laboratory or imaging findings consistent with CKD, one possible explanation is that, as described by Eisenhofer et al. (2005), free metanephrines, such as those measured in this study, are generally less affected by renal insufficiency and often remain within reference intervals. Another contributing factor may relate to the characteristics of the CH group itself. The included cats had to be clinically stable, with well-managed chronic conditions, which may not have triggered a sufficiently strong or sustained sympathetic activation to significantly alter all metabolite levels.

Sex significantly influenced creatinine ratios, with females exhibiting higher concentrations of both uMN/creatinine and uNMN/creatinine when compared to males. This finding contrasts with human evidence, where males typically show higher absolute plasma MN levels (Eisenhofer et al. 2013; Eisenhofer et al. 2019). In cats, sex-related individual variation in urinary metanephrine levels, as well as the uncharacterized relationship between body size, creatinine excretion and catecholamine concentrations may help explain these conflicting results.

In contrast, age group had no significant effect on any of the measured metabolites, suggesting that within the studied population, age is unlikely to be a major confounding factor in urinary metanephrine analysis. This differs from findings in humans, where plasma NMN concentrations increase progressively with age, especially after the age of 40 (Eisenhofer et al. 2013; Eisenhofer et al. 2019), and from data in dogs showing weak but statistically significant age-related increases in plasma free MN and NMN (van den Berg et al. 2023). The absence of a similar pattern in the present may be attributable to a relatively narrow age range of the study population, species-specific differences, or the use of urinary rather than plasma measurements.

When compared to the study by Prego et al. (2023a), one of the few investigations to the author's knowledge exploring urinary metanephrines in cats using LC-MS/MS, none of the urinary uNMN/creatinine values in our study, whether from H or CH cats, approached the level reported in their feline pheochromocytoma case. Additionally, when compared to the distribution of uNMN/creatinine values observed in healthy cats from the same study, 94.7% of samples in our cohort fell within or below that range. Only one sample from each collection method slightly exceeded the previously reported upper limit, and both remained far below the pheochromocytoma value. This high level of agreement supports the consistency of findings across studies and strengthens the reliability of these concentration ranges as representative of non-tumoral values, despite differences in study populations and collection methods. Taken together, these observations reinforce the potential of the uNMN/creatinine ratio as a useful biomarker for feline adrenal disease, particularly when markedly elevated. However, further studies including confirmed cases of pheochromocytoma are needed to establish formal reference intervals, define diagnostic cut-offs, and validate the clinical utility of this biomarker.

A primary limitation of this study was the timing and conditions under which the Catrine® urine samples were collected. While the paired samples allowed direct comparisons between methods, logistical constraints meant that owners could collect the Catrine® sample either before the hospital visit or at least three days afterward. As a result, the timing of at-home sampling was not standardized relatively to cystocentesis, and Catrine® samples may have been affected by factors such as post-hospital visit stress, or changes in clinical status or medication administration over those three days. Although median values were lower in Catrine® samples, some individuals exhibited higher values in this method than those obtained via cystocentesis. This suggests that stress-related or physiological variation at the time of collection may have introduced confounding effects. Additionally, one owner was unable to collect urine using the special Catrine® litter, resulting in the exclusion of that cat from the study. This highlights a practical limitation of at-home collection methods, where owner compliance and the cat's behaviour and tolerance of the collection method can impact successful sample

collection and introduce bias. Future studies should aim for tighter control of collection timing, ideally obtaining home samples prior to hospital visits under standardized conditions.

Another limitation is the small sample size which makes it difficult to extrapolate these results beyond the study sample and reduces the statistical ability to detect more subtle effects, such as potential interactions between sex, age, health status, and collection method. While certain trends were observed, the population size was not sufficient to draw firm conclusions.

This study highlights the influence of urine collection methods on the interpretation of catecholamine metabolite concentrations in cats, emphasizing the importance of standardized methodology and consistency in both research and clinical settings. The observed differences in uMN, uNMN and their respective creatinine ratios based on collection technique suggest that variations in sampling could lead to inaccurate conclusions (Mortier et al. 2023). From a clinical standpoint, establishing consistent protocols for urine sampling could improve the accuracy of diagnosis, disease monitoring, and treatment decisions in feline endocrinology. This is particularly relevant given the growing interest in urinary metanephrines as diagnostic biomarkers in cats (Wimpole et al. 2010; Prego et al. 2023).

Future research should aim to validate these findings through larger, multi-center studies that account for variability in both clinical populations and laboratory practices. Special emphasis should be placed on developing and standardizing home collection protocols, which would facilitate sample acquisition in a stress-minimized environment and improve owner compliance (Srithunyarat et al. 2018). When comparing different collection methods, the timing of sampling should also be tightly controlled in order to establish the least conflicting protocol and enable meaningful comparisons without introducing confounding factors such as stress (Belew et al. 1999). Additionally, studies including cats with confirmed pheochromocytoma are imperative to define diagnostic thresholds and assess test performance (Wimpole et al. 2010). Further investigation into the mechanisms underlying hormonal fluctuations and stress-related physiological responses affecting MN and NMN secretion would also help clarify potential confounding factors and enhance the clinical utility of these biomarkers.

3.5 Conclusion

This is the first study to directly compare the influence of cystocentesis and at-home post-void urine collection via Catrine® on feline urinary metanephrines measured by LC-MS/MS. Our investigation provides valuable insight into pre-analytical factors that may affect diagnostic accuracy in feline endocrinology.

This study's findings demonstrate that urine collection method and associated environmental stress significantly influence free urinary metanephrine concentrations, particularly uMN, uNMN and the uMN/creatinine ratio. However, the uNMN/creatinine ratio

remained stable across methods, suggesting its robustness as a biomarker for feline adrenal diseases regardless of collection method.

The observation that higher metanephrine levels in cystocentesis samples, especially in cats with chronic conditions, highlight stress as a confounding factor and highlights the need for standardized, minimally stressful collection protocols.

Although sample size was limited, these results align with previous feline studies and support urinary normetanephrine as a promising diagnostic biomarker. Future research involving larger, multi-center populations, standardized home collection protocols and confirmed cases of pheochromocytoma will be essential to establish formal reference intervals, diagnostic thresholds, and to validate the clinical utility of these biomarkers in cats.

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5. Annexes

Annexe 1 – Local Ethical Committee (Comissão de Ética para a Investigação e Ensino) approval of the PhD research project under which this thesis was conducted.

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FACULDADE DE MEDICINA VETERINÁRIA
Comissão de Ética para a Investigação e Ensino (CEIE)

Exmo. Senhor
Professor Doutor Rodolfo Leal
Faculdade de Medicina Veterinária

Lisboa, 19 de abril de 2023

Assunto: Avaliação projeto de investigação – N/Refª 017/2023

Vimos pela presente informar V.Exa. que a CEIE, após ter avaliado as atividades que envolvem manipulação de animais, no âmbito do projeto de investigação "Unravelling the crosstalk between kidney disease and the hypothalamic-pituitary adrenal axis in cats" considerou que estão salvaguardados os princípios éticos e de bem-estar animal exigidos pela legislação vigente e pelo código de boas práticas, pelo que aprovou a execução do protocolo experimental nas instalações e serviços da FMV, conforme requerido por V.Exa.

Com os melhores cumprimentos,

Graça Ferreira Dias
Coordenadora da Comissão de Ética para a Investigação e Ensino

Avenida da Universidade Técnica – Pólo Universitário do Alto da Ajuda – 1300-477 – PORTUGAL
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Annexe 2 – Acceptance of Oral Communication at the European Congress of Veterinary Internal Medicine for Companion Animals (ECVIM-CA) 2025

Urinary Metanephrines in Cats - Does Collection Method Matter?

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Knowledge regarding the clinical applicability of metanephrines in cats is scarce. Urinary metanephrine measurements provide a safe and practical diagnostic tool to rule out catecholamine-producing tumors. While urine collection is usually performed via cystocentesis, urine collection at home is a less invasive option, providing a convenient and stress-free method to collect samples. As stress is always a concern in feline patients, it is essential to assess the impact of sample collection method in metanephrine measurements. This study aims to evaluate potential differences between urinary metanephrines collected at home versus by cystocenthesis in the hospital.

After ethical approval, a repeated measures observational study was performed including either healthy cats or cats with stable non-adrenal illness. Owners were supplied with non-absorbent litter and instructed to either collect urine at home before or at least three days after bringing the cat to a veterinary teaching hospital for cystocentesis. Cats were also submitted to an abdominal ultrasound and analytical work-up. Collected samples were refrigerated at 4°C for a maximum of 24h before being stored at -80°C until shipment. All samples were shipped at the same time for urinary metanephrine (uMN) and normetanephrine measurements (uNMN) by liquid chromatography with tandem mass spectrometry. Statistical analysis was preformed using the software IBM SPSS Statistics. Normality was assessed and statistical tests were then chosen accordingly.

A total of 19 cats were included. The free uMN concentration (median [range]) in urine collected at the hospital (690.36 [197.97–2228.43] nmol/L) was significantly higher (p=0.003) than in urine collected at home (324.87 [116.75–883.25] nmol/L). The free uNMN concentration in urine collected at the hospital (1475.41 [475.41–5180.33] nmol/L) was also significantly higher (p=0.022) than in the urine collected at home (1267.76 [469.95–3459.02] nmol/L).

The uMN:creatinine ratio in the urine collected at the hospital (41 [17–154] µg/g) was significantly higher (p=0.015) than in the urine collected at home (26 [9–93] µg/g). However, the uNMN:creatinine ratios in urine collected at the hospital (85 [44–239] µg/g) and at home (83 [42–209] µg/g) did not differ significantly (p=0.481).

These results show that, while environmental stress seems to significantly alter free uMN, uNMN concentrations and the uMN:creatinine ratio, the uNMN:creatinine ratio remains unchanged. This suggests stress and, subsequently, the urine sample method, can indeed affect uNMN and uMN concentrations but that the uNMN:creatinine ratio remains a good diagnostic indicator, regardless of collection method.

Disclosures

Sonic Healthcare Benelux (Antwerpen, Belgium) offered a discount for the analyses performed.

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Speaker Information

(click the speaker's name to view other papers and abstracts submitted by this speaker)

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