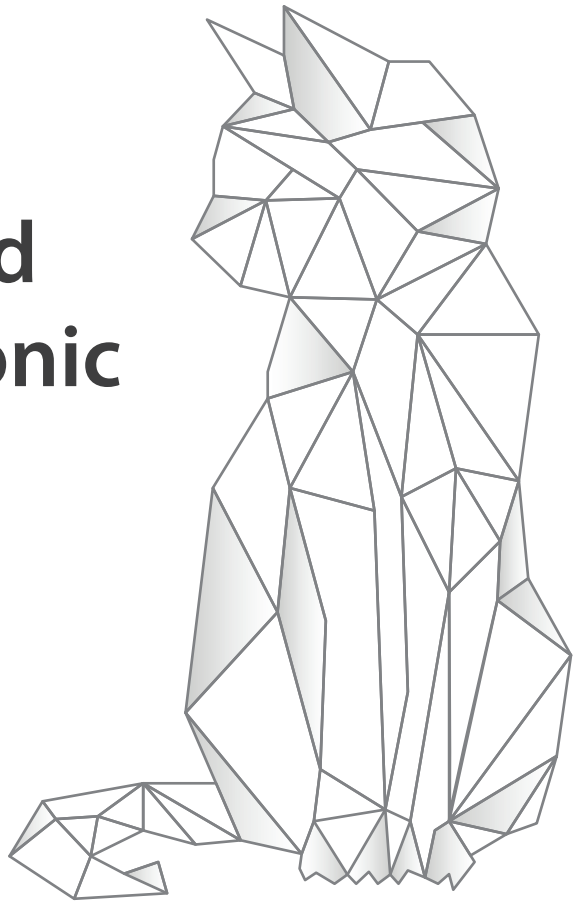
Programa de Doctorado de Investigación en Biomedicina

Tesis Doctoral Internacional

ULPGC
Universidad de
Las Palmas de
Gran Canaria

The role of obesity and diabetes in feline chronic kidney disease



Laura Pérez López | Las Palmas de Gran Canaria 2020

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
ESCUELA DE DOCTORADO

Programa de doctorado de Investigación en Biomedicina

Título de la Tesis

Papel de la obesidad y la diabetes en la nefropatía felina

Tesis Doctoral presentada por Dª Laura del Carmen Pérez López

Dirigida por el Dra. Dª Ana María Wägner

Codirigida por el Dr. D Mauro Boronat

Directora **El Codirector** **La Doctoranda**

Las Palmas de Gran Canaria, a 30 de Mayo de 2020

D.ª ANA M. WÄGNER FAHLIN, PROFESORA TITULAR DEL DEPARTAMENTO DE CIENCIAS MÉDICAS Y QUIRÚRGICAS DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA (ULPGC) Y ESPECIALISTA EN ENDOCRINOLOGÍA Y NUTRICIÓN EN EL COMPLEJO HOSPITALARIO UNIVERSITARIO INSULAR MATERNO-INFANTIL DE GRAN CANARIA.

CERTIFICA,

Que el trabajo de investigación titulado: “The role of obesity and diabetes in feline chronic kidney disease” ha sido realizado por Laura del Carmen Pérez López bajo su dirección y asesoramiento. Y que una vez revisada la presente memoria, la encuentra apta para su defensa ante el tribunal.

Y para que así conste y surta los efectos oportunos, extiende el presente certificado en Las Palmas de Gran Canaria a 14 de Mayo de 2020

D. MAURO BORONAT CORTÉS, PROFESOR TITULAR DEL DEPARTAMENTO DE CIENCIAS MÉDICAS Y QUIRÚRGICAS DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA (ULPGC) Y ESPECIALISTA EN ENDOCRINOLOGÍA Y NUTRICIÓN EN EL COMPLEJO HOSPITALARIO UNIVERSITARIO INSULAR MATERNO-INFANTIL DE GRAN CANARIA.

CERTIFICA,

Que el trabajo de investigación titulado: “The role of obesity and diabetes in feline chronic kidney disease” ha sido realizado por Laura del Carmen Pérez López bajo su dirección y asesoramiento. Y que una vez revisada la presente memoria, la encuentra apta para su defensa ante el tribunal.

Y para que así conste y surta los efectos oportunos, extiende el presente certificado en Las Palmas de Gran Canaria a 14 de Mayo de 2020

University of Zagreb
Faculty of Veterinary Medicine
Animal production and biotechnology
Department of Animal Nutrition and Dietetics

5th June 2020

I, Diana Brozić, PhD, DVM, 83358100535, presently working as Assistant Professor at Department of Animal Nutrition and Dietetics, University of Zagreb, Faculty of Veterinary Medicine, Heinzelova 55, 10000 Zagreb – Croatia declare that:

I have read the PhD thesis "The role of diabetes and obesity in feline chronic kidney disease", by Laura Pérez López.

After assessing the relevance and originality of its contents, I consider them to be of international standard.

Thus, I judge the mentioned PhD to qualify for an International Mention

5th June 2020, Zagreb, Croatia

Assist Prof Diana Brozić, PhD, DVM

I, Norberto Ruiz Suárez with ID card number 78521460N, presently working as educational supervisor at Faculty of Veterinary Medicine (Ghent University), Merelbeke, Belgium, declare that :

I have read the PhD thesis "The role of diabetes and obesity in feline chronic kidney disease", by Laura Pérez López.

After assessing the relevance and originality of its contents, I consider them to be of international standard.

Thus, I judge the mentioned PhD to qualify for an International Mention.

Norberto Ruiz Suárez

Date and Signature

5/06/2020

The role of obesity and diabetes in feline chronic kidney disease

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SUMMARY

Obesity is a problem of epidemic proportions in people and cats. It problem represents the main cause of type 2 diabetes mellitus (T2DM) in both species. Furthermore, in people, obesity and T2DM are considered independent risk factors for the development of chronic kidney disease (CKD). However, in cats it is unknown whether or not obesity or diabetes is associated with CKD. Therefore, the main objective of this research was to assess the association of obesity and diabetes with CKD in cats. Two reviews, one cross-sectional study, and one prospective study were done.

The retrospective study of this thesis showed an association between CKD and diabetes in adult cats. Furthermore, the cross-sectional study suggested that mild chronic hyperglycemia might have an impact on kidney function in this specie.

Overall, this research did not find an association between obesity and CKD in cats. Neither the cross-sectional study nor the prospective study found an association between both disorders; although further analyses with greater sample size and longer follow-up, or histological studies could be of interest.

RESUMEN

La presente tesis se centra en la asociación entre la obesidad, la diabetes y la enfermedad renal crónica (ERC) en los gatos domésticos. En estos animales, al igual que en las personas, la obesidad ha comenzado a ser un problema de proporciones epidémicas. Además, en la especie felina, esta alteración, y el consecuente desarrollo de resistencia a la insulina, son las causas predisponentes de diabetes mellitus tipo 2, como sucede en las personas. Por tanto, el gato se considera un modelo espontáneo de diabetes tipo 2, y se estima que alrededor de un 80% de los gatos con diabetes sufren de este tipo de diabetes. Sin embargo, se desconoce si la obesidad y la diabetes son factores de riesgo de ERC en los gatos, como sucede en la especie humana. Para su estudio, en este proyecto se utilizaron algunos posibles marcadores precoces de daño renal y de distintas alteraciones del metabolismo de la glucosa. La investigación resultante se presenta a través de cinco artículos (2 revisiones y 3 artículos originales):

1) Revisión bibliográfica: Pérez-López L, Boronat M, Melián C, Brito-Casillas Y, Wägner AM. La obesidad felina y humana son problemas endocrinológicos que comparten complicaciones similares. Clin Vet Peq Anim 2018;38(3):155-167

El objetivo de esta revisión bibliográfica fue profundizar en las diferencias y similitudes que presentan las alteraciones resultantes de la obesidad en la especie felina y humana. Esta revisión ofrece una introducción general sobre el objeto de la tesis y sobre la necesidad de estudiar si los gatos con obesidad y diabetes son más propensos a padecer ERC.

En general la revisión señala que además de la diabetes, los gatos también pueden presentar otros desórdenes asociados a la obesidad que comparten características con el síndrome metabólico humano. Sin embargo en la especia felina no se ha descrito la aterosclerosis, la aparición de la hipertensión no parece guardar relación con la obesidad, y el papel de la diabetes y de la obesidad en el desarrollo de la ERC es incierto.

La ausencia de producción de algunas citoquinas pro-inflamatorias en el tejido adiposo y la corta esperanza de vida de esta especie pueden ser algunos de los mecanismos que contribuyen a que en los gatos no se observen dichas alteraciones.

2) Estudio de tipo retrospectivo Pérez-López L, Boronat M, Melián C, Saavedra P, Brito-Casillas Y, Wägner AM. Assessment of the association between diabetes mellitus and chronic kidney disease in adult cats. J Vet Intern Med. 2019;33(5):1921-1925

El objetivo de este estudio fue evaluar, de manera retrospectiva, la asociación entre la diabetes y la enfermedad renal en una población de gatos adultos. Con este fin, se revisaron las historias clínicas de los gatos atendidos durante 2014-2016 en dos centros veterinarios: Clínica Veterinaria Atlántico y Hospital Clínico Veterinario de la ULPGC. Sólo se incluyeron aquellos casos en los que el gato tenía una edad igual o superior a 3 años de edad y en cuyas historias había información suficiente para definir si el gato presentaba o no diabetes y/o ERC. También se descartaron aquellos gatos con sospecha de azotemia pre-renal. En total se incluyeron 561 gatos, de los cuales 67 (11.9%) presentaban ERC y 16 (2.9%) presentaban diabetes. Entre los gatos con diabetes, siete (44%) también presentaba ERC. La ERC y la diabetes fueron diagnosticados a la vez en seis de los siete gatos, mientras que en un gato el

diagnóstico de diabetes precedió al de ERC. En el análisis univariante se observó una asociación de la ERC con la edad ($p<0.001$), la raza mestiza ($p <0.001$), y la diabetes ($p = 0.001$) Para valorar esta asociación independiente entre la diabetes y la ERC, se realizó un análisis de regresión logística multivariante, en el que la asociación se mantuvo significativa para todas las variables. Para evaluar la sensibilidad del análisis, se realizaron otros dos modelos de regresión multivariante (uno incluyendo solo la edad y la diabetes como variables independientes, y otro excluyendo los casos con grados más leves de ERC). Los resultados sugirieron que a una misma edad, un gato con diabetes tiene una probabilidad aproximadamente 4 veces mayor de presentar enfermedad renal que otro gato sin diabetes.

3) Revisión bibliográfica: Pérez-López L, Boronat M, Melián C, Brito-Casillas Y, Wägner A.M. Animal Models and Renal Biomarkers of Diabetic Nephropathy. In: Advances in Experimental Medicine and Biology. Springer, New York, NY. 2020.

Esta revisión se centró en los nuevos avances sobre marcadores bioquímicos y modelos animales de nefropatía diabética. El objetivo principal de su realización fue la búsqueda de la información necesaria para seleccionar los marcadores renales más convenientes a emplear en los estudios posteriores de la presente tesis.

En general, la revisión refleja que aunque tradicionalmente la albuminuria, un indicador de lesión de los glomérulos renales, ha sido considerada como el principal marcador de nefropatía diabética y daño glomerular, actualmente se ha visto que en algunos pacientes con nefropatía diabética podría existir daño tubular previo al daño glomerular. Por tanto, el uso conjunto de la albuminuria junto con diferentes potenciales marcadores tubulares, permitiría identificar el daño en diferentes partes

de la nefrona y podría ayudar a un diagnóstico más precoz de la nefropatía diabética. Con respecto al posible uso de marcadores de daño renal temprano, tal y como ha sucedido con la determinación la cistatina C en humanos, y la determinación de dimetilarginina simétrica (SDMA) en animales de compañía, que se han incorporado a las guías de práctica clínica para el diagnóstico de la ERC ("*KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease*" en humanos, "*International Renal Interest Society*" en animales de compañía). No obstante, aunque son muchos los marcadores que se han estudiado para el diagnóstico precoz de nefropatía diabética, los resultados son contradictorios para la mayoría de ellos. De hecho, esos biomarcadores que han sido identificados como predictores de aparición o progresión de ERC, no parecen ser más beneficiosos que aquéllos tradicionalmente utilizados (creatinina sérica, estimación del filtrado glomerular y albuminuria). También es importante destacar que la mayoría de los estudios realizados hasta la fecha son de carácter transversal en su diseño, y se necesitan más estudios prospectivos y de intervención. De hecho, grandes consorcios como SysKid *("Systems Biology Towards Novel Chronic Kidney Disease Diagnosis and Treatment"*), SUMMIT *("Surrogate markers for micro and macrovascular hard endpoints for innovative diabetes tools"*) y BEAt-DKD (*"Biomarker Enterprise to Attack Diabetic Kidney Disease")* están proporcionando y proporcionarán importantes resultados en un futuro cercano.

4) Estudio de tipo transversal: Pérez-López L, Boronat M, Melián C, Brito-Casillas Y, Wägner AM. Kidney function and glucose metabolism in overweight and obese cats. Veterinary Quarterly. 2020; 40(1):132-139

Este estudio transversal consta de los siguientes objetivos: 1. Evaluar la asociación entre el sobrepeso u obesidad con un conjunto de marcadores renales establecidos y otros potenciales. 2) Evaluar la asociación de dichos marcadores renales con diferentes medidas que evalúan el metabolismo de la glucosa. 3) Comparar la utilidad de la fructosamina frente a la glucemia en ayunas como marcador de alteraciones tempranas del metabolismo de la glucosa. Para cumplir con estos objetivos, se incluyeron gatos adultos sanos que fueron clasificados en dos grupos: gatos con una condición corporal normal y gatos con una condición corporal aumentada (sobrepeso u obesidad). En total, se incluyeron 54 gatos con una edad igual o superior a 5 años; 17 tenían una condición corporal normal y 37 presentaban sobrepeso u obesidad. Se midieron una serie de marcadores renales: creatinina sérica, SDMA, ratio urinario proteínas-creatinina en orina, densidad urinaria, ratio urinario factor de crecimiento transformante beta 1 activo-creatinina en orina y proteína ligadora de retinol en orina. Como marcadores del metabolismo de la glucosa se incluyeron glucemia en ayunas, insulina en ayunas y fructosamina. Además se estimó la sensibilidad a la insulina mediante el uso de fórmulas simplificadas, que han sido previamente estudiadas en gatos: *homeostasis model assessment* (HOMA), *quantative insulin check index* (QUICKI) y ratio de insulina-glucosa en ayuno (I/G). En comparación con los gatos con una condición corporal normal, los gatos con sobrepeso u obesidad presentaron algunas alteraciones que se observan en el síndrome metabólico humano: niveles de triglicéridos más altos, mayores concentraciones de insulina, mayores concentraciones de glucemia basal, mayores valores de HOMA, mayores concentraciones de fructosamina y una disminución del índice QUICKI. Sin embargo, las concentraciones de los marcadores renales no difirieron entre gatos con una condición corporal normal

y gatos con sobrepeso u obesidad. No obstante, los gatos con fructosamina >250 mg/dL presentaban una concentración inferior de SDMA sérico. A partir de estos resultados se ha sugerido la posibilidad de que el SDMA disminuya en estos gatos un estado leve de hiperglucemia debido a un posible estado de hiperfiltración glomerular. Esta hipótesis se sustenta en que la hiperfiltración es una de las primeras alteraciones que se observan en las personas con nefropatía diabética, así como en un estudio previo que observó niveles más bajos de SDMA en gatos con diabetes en comparación con gatos sanos. No obstante, son necesarios más estudios para evaluar adecuadamente la existencia de esta alteración en la especie felina.

Cabe mencionar que, en las personas, aparte de la diabetes, tanto la obesidad como la prediabetes se han considerado factores independientes de riesgo de enfermedad renal. En el gato diferentes estudios apuntan a que existe un estado de prediabetes, si bien no existen unos criterios establecidos para definirla. Aunque se ha sugerido que los gatos con una glucemia basal en ayunas persistentemente >117 mg/dL presentan un estado anormal de la glucosa, no se ha demostrado que este umbral se asocie con un mayor riesgo de desarrollo de diabetes en estos gatos. En este sentido, es necesario establecer criterios diagnósticos de prediabetes, también para evaluar si los animales en este estado pueden sufrir daño renal. La investigación de este tercer estudio muestra que la fructosamina, además de correlacionarse con la condición corporal, tiene una asociación más fuerte con los parámetros que miden la sensibilidad a la insulina en comparación con la glucemia en ayunas. Cuando los gatos fueron clasificados según la concentración de fructosamina (<250 vs ≥250 µmol/L), se observaron diferencias significativas entre grupos para los valores de HOMA y la concentración de insulina en ayunas.

5) Estudio de tipo prospectivo: Pérez-López L, Boronat M, Melián C, Santana A, Brito-Casillas Y, Wägner AM. Overweight and obesity are not associated with loss of kidney function in healthy domestic cats (borrador).

El objetivo de este estudio fue evaluar el efecto del sobrepeso y la obesidad en marcadores de la función renal (SDMA y creatinina). El estudio se realizó de modo prospectivo sobre una cohorte de gatos sanos que fueron evaluados cada 6 meses durante un periodo máximo de 18 meses. Los criterios de inclusión fueron los mismos descritos en el estudio transversal. Se incluyeron un total de 56 gatos pero 16 de ellos no volvieron para seguimiento. De los 40 gatos con seguimiento incluidos en el estudio, siguiendo el "*body condition score system*" en escala de 1-9, 14 gatos tenían un condición corporal = 5 o normal (5 machos, 9 hembras), y 26 gatos tenían un condición corporal >5 (13 machos, 13 hembras). Entre los gatos con una condición corporal >5, 19 tenían una condición corporal comprendida entre 6-7 (sobrepeso), y 7 tenían una condición corporal >7 (obesidad).

La mediana del seguimiento de los gatos de este estudio fue de 12 meses. Después de este periodo de seguimiento, tras el análisis de los datos con un modelo lineal mixto, no se observó variabilidad intra e inter sujeto para las variables SDMA y creatinina. Por tanto, este estudio sugiere que no existe un efecto del sobrepeso u obesidad en la función renal de los gatos. No obstante serían interesantes estudios histopatológicos o estudios que incluyeran un mayor número de gatos con obesidad severa.

En general, los estudios que componen esta tesis, no encontraron una asociación entre la obesidad y la ERC. Sin embargo, los resultados sugieren que la ERC en los gatos

podría estar asociada a la diabetes, y quizás también a la existencia de una hiperglucemia crónica leve.

INTRODUCTION

1. Feline obesity

Obesity has risen to epidemic proportions in people and in their pets. The World Health Organization has reported that, among the worldwide adult population, approximately 39% are overweight and 13% are obese [1]. In a similar manner, it is estimated that around 35-50% of domestic cats are overweight or obese [2]. Moreover, obesity and physical inactivity are the main risk factors for the development of type 2 diabetes (T2DM) in both humans and cats; and as a consequence of the growing problem of obesity, T2DM is also increasing worldwide [2,3]. The main risk factors for feline obesity are summarized in table 1 [2,4-12].

Table 1: Main risk factors for feline obesity

Risk factors of obesity in cats	
Breed predisposition	British Short-Hair, Manx, Persian, Domestic Short hair, others
Age	5-11 years
Gender	Male
Life style	Sedentary and indoor lifestyle (physical inactivity)
Diet	Energy intake

Obesity can lead to insulin resistance in cats; several studies have shown that obese cats have higher insulinemia than their normal-weight counterparts, as well as lower

glucose clearance and effectiveness (i.e. insulin independent glucose uptake), compared to healthy cats [10,13,14]. In fact, it has been estimated that with each kilogram increase in body weight, cats lose around 30% of insulin sensitivity and glucose effectiveness [13]. Peripheral insulin resistance and compensatory hyperinsulinemia can lead to beta cell "exhaustion", resulting in lower insulin secretion and impaired insulin action. People with decreased insulin sensitivity commonly develop increased endogenous liver glucose production, and impaired fasting glucose or impaired glucose tolerance, which could lead to the onset of T2DM [15,16]. Cats with obesity have a fourfold risk of developing diabetes [17]; and around 44% of cats with obesity have impaired glucose tolerance [15]. It should also be highlighted that lower expression of glucose transporter proteins GLUT4 in the muscle is considered one of the first events in the development of diabetes in obese patients; and lower expression of this transporter has also been observed in obese cats compared to lean cats [18].

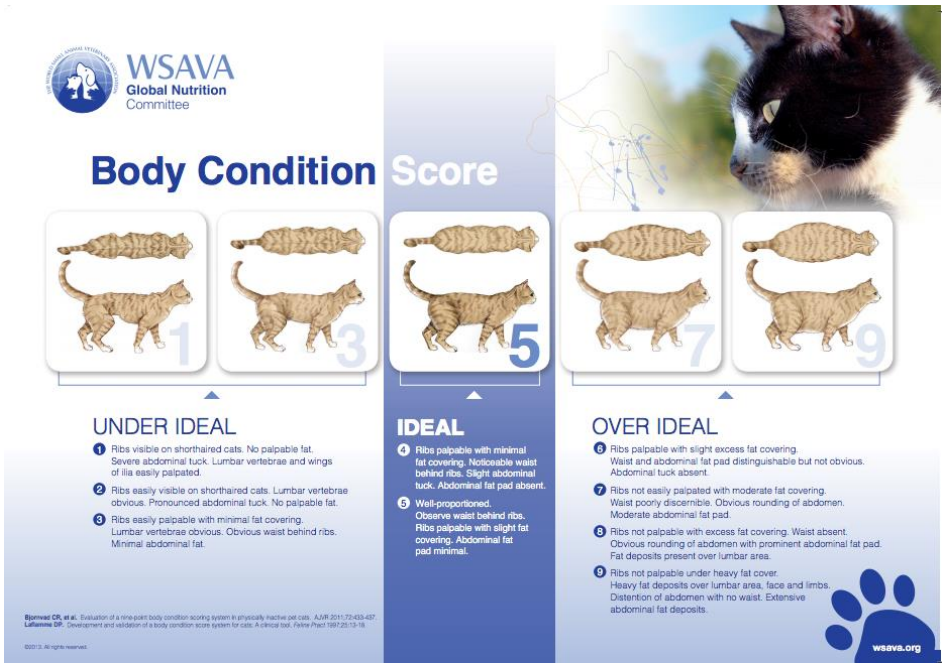
Furthermore, adipose tissue has a role in the development of T2DM in obese people and cats. Adipose tissue participates in the regulation of energy balance, and it also plays an important role as an endocrine organ, secreting fatty acids, renin-angiotensin system (SRA) components and adipokines [2, 19-21]. The latter, mainly leptin and adiponectin, are important to regulate intake, energy expenditure and glucose homeostasis. Leptin suppresses food intake and promotes thermogenesis and weight loss. Adiponectin improves insulin sensitivity, promotes fatty acid oxidation and has a potent anti-inflammatory effect. [2, 19, 22-25]. In the presence of obesity, deregulation of cytokine secretion in the adipose tissue is observed: concentrations of

adiponectin are reduced and concentrations of leptin are increased compared to lean subjects [2,10,19]. People and cats with obesity are considered leptin resistant since the normal physiological response to leptin is not observed [2,10,19]. In the presence of obesity, there is also adipocyte hyperplasia and hypertrophy, as well as an increment of SRA components and chemokine production, such as monocyte chemoattractant protein 1 (MCP-1), which attracts and facilitates the infiltration of monocytes and macrophages. This leads to higher secretion of proinflammatory cytokines within the adipocytes, [19,21,26,27], which impair insulin signaling, and are considered predictive markers of cardiovascular diseases in people [19,28]. In addition, similarly to what is found in people, SRA components have been identified in feline fat deposits [20].

In people, alterations in adipose tissue are associated, not only with insulin resistance and T2DM, but also with other disorders such as dyslipidemia and hypertension. Adipose tissue hypertrophy and subsequent increase in RAS components, together with pro-inflammatory cytokines, contribute to the onset of metabolic syndrome in people [20]. This syndrome is defined as a cluster of disorders, which include abdominal obesity, abnormal glucose metabolism, dyslipidemia and hypertension. It is associated with a threefold risk of coronary heart disease and a fivefold risk of T2DM [29]. Cats with obesity can also develop some alterations similar to the components of the metabolic syndrome, such as insulin resistance, abnormal glucose metabolism and dyslipidemia; however, atherosclerosis has not been described in cats, and hypertension does not seem to be linked to obesity, either [2, 10, 30, 31].

Overweight and obesity can be easily recognized in cats through the body condition score (BCS) system, a semi quantitative method that is carried out through visualization and palpation of the animal (Figure 1) [32-34]. Although other methods such us Dual X-ray absorptiometry (DXA), computed tomography and magnetic resonance imaging, are considered more accurate, these techniques are expensive and require sedation. For that reason, they are mainly reserved for research purposes and are not commonly used in clinical practice.

Figure 1: Body condition score system in cats; from World Small Animal Veterinary Association



Additionally, in a similar manner to the human body mass index, dimensional measurements have been reported for cats. Nonetheless, their performance is cumbersome and has not been successfully adopted in clinical practice. [34-36]. Therefore, the most practical method to evaluate body condition in cats is the BCS

[34,37]. Furthermore, it has been reported as an accurate and reliable method, since it has shown a good correlation with DXA [37]. The BCS classifies cats into a 9-point score ranging from emaciation to obesity. Each point above 5 represents an increment of 10% in body weight (Figure 1 [32,34]).

2. Feline diabetes mellitus

Diabetes is a common endocrine disorder in cats with a prevalence ranging from 0.43-1.24% [38-40]. Criteria for its diagnosis are based on typical clinical signs (polyuria/polydipsia, polyphagia, weight loss), persistent hyperglycaemia (>250 mg/dL), or fructosamine concentration above 400 µmol/L [41, 42].

T2DM is the most common type of diabetes in cats, accounting for around 80% of cats with diabetes [43]. Similarly, in people, T2DM represents around 90-95% of the cases of diabetes [44]. In both species, T2DM is characterized by the presence of insulin resistance and beta cell dysfunction. As in people, obesity and physical inactivity are the main risk factors of T2DM in cats. [43,45] However, not all cats with obesity develop diabetes, and a multifactorial origin is accepted. Genetic factors, gender, increased aged and certain breeds have also been described as predisposing factors for T2DM in cats (table 2 [2, 3, 39, 46-48]).

Table 2. Risk factors for diabetes in cats

Risk factors for diabetes in cats	
Breed predisposition and Genetic factors	• Burmese, Norwegian Forest, Tonkinese • A polymorphism in the melanocortin 4 receptor gene (MC4R:c.92C>T)
Age	>7 years
Gender	Male
Reproductive status	Neutered
Lifestyle	Sedentary and indoor lifestyle (physical inactivity)
Obesity	Obese cats are four more times likely to develop diabetes than cats with a normal weight
Drugs	Progestagens, glucocorticoids
Other diseases	Cushing's syndrome, acromegaly, pancreatic carcinoma, pancreatitis

Despite a similar prevalence of obesity in cats and humans, the prevalence of diabetes is lower in cats of all ages (0.43-1.2%) than in adult humans (8.5%) [38-40,45, 48]. Lower life expectancy of cats (vs humans) has been pointed out as an explanation for this difference [48]. Nonetheless, to make an adequate comparison, the prevalence of diabetes in the adult cat population should be assessed [38-40,45]. Exceptionally, the

prevalence among Burmese cats, one of the breeds with a higher risk of T2DM, is around 10% [39,48], which is consistent with the prevalence observed in people, and supports the suspicion that there is a genetic predisposition in the development of the disease.

Diabetes in cats can also be secondary to disorders causing insulin resistance or beta cell destruction [3, 43, 50, 51]. A few endocrine diseases such as acromegaly or hyperadrenocorticism, can cause insulin resistance and lead to diabetes, as also happens with the administration of drugs, such as progestagens or glucocorticoids [3, 43]. Additionally, pancreatitis might have a role in the development of diabetes or vice versa [50].

Regarding causes of beta cell destruction, we should consider pancreatic carcinoma [44] and immune-mediated beta cell destruction or type 1 diabetes (T1DM), which is considered a rare condition in cats [3, 36]. In contrast, in humans, T1DM represents around 10% of diabetes.

3. Feline chronic kidney disease

Feline chronic kidney disease (CKD) is a very common disorder in aged cats. Its prevalence can be above 30% in cats older than 10 years [52]. Criteria for its diagnosis have been launched by the International Renal Interest Society (IRIS) guidelines. Established markers of CKD include creatinine, urea, urinary protein-creatinine ratio,

urinary specific gravity, and symmetric dimethylarginine (SDMA) [52]. Diagnosis and classification of CKD is mainly based on subsequent measurements of creatinine concentrations, together with urinary specific gravity (USG) and urinary protein to creatinine ratio (UPC). Measurement of systolic blood pressure is also recommended. Recently, SDMA has been incorporated as an earlier marker than creatinine, and values persistently above 14 μg/dl are consistent with a decline in renal function (Table 3) [53].

In people, diabetes is the leading cause of CKD. However, recent studies have suggested that obesity, prediabetes and the metabolic syndrome could also be independently associated with CKD [54-55]. In contrast, the link between diabetes and renal disease is unclear in cats, and discrepancies can be found in the literature [57-62]. Causes of CKD are heterogeneous in cats: urolithiasis, renal lymphoma, infections, hyperthyroidism, vaccines, nephrotoxic substances and genetic causes, such as polycystic disease or renal dysplasia. However, in most of the cases CKD has an unknown origin [52]. Whether obesity, prediabetes or diabetes cause CKD in cats requires further investigation.

Some authors have suggested that kidney damage might not occur in diabetic cats due to the shorter life expectancy of cats compared to people [61]. Nonetheless, cats can be overweight or obese for most of their life [34]. Therefore, chronic exposure to metabolic changes induced by obesity, particularly insulin resistance and impaired fasting glucose, could be sufficient to cause CKD or subclinical CKD in this species.

In people, recent studies focus on the search of novel early biomarkes of CKD, and the use of a combined set of them that could indicate damage from different parts of the nephron and be available for the clinical detection of early diabetic nephropathy [63-65]. One of these biomarkers is Cystatin C, which increases earlier than creatinine in human CKD [65]; and might also predict diabetic nephropathy in patients with T2DM [67, 68]. In cats, however, it has not shown to be a reliable marker of CKD [69]. In regard to the relationship between diabetes and CKD, neither of two retrospective epidemiological studies found a link between both diseases in cats. Nonetheless, a higher prevalence of proteinuria has been reported in cats with diabetes compared to age-matched control cats [59].

The cat in the picture is a domestic shorthair, 10 years old, castrated male cat with severe obesity that developed diabetes mellitus and proteinuria.

Moreover, detection of the early alterations of glucose metabolism (insulin resistance or impaired fasting glucose) in cats, are necessary to elucidate its role in feline kidney

function. Obesity can lead to insulin resistance in cats but not all cats develop diabetes; pancreatic beta cells dysfunction is necessary for its development [3]. Although cats with prediabetes might already show some degree of beta cell dysfunction before the onset of diabetes, and maybe also some degree of impaired renal function, criteria for its diagnosis are not established yet [3]. Some authors have suggested a cut-off of 117mg/dl for impaired fasting glucose [70], but whether cats with impaired fasting glucose are at risk of diabetes is unknown.

Table 3: established markers for CKD in cats and their interpretation following IRIS guidelines

Established markers of CKD	Interpretation
Consecutive measurements of serum or plasma creatinine	Stage 1: creatinine <1.6 mg/dl plus other alterations (proteinuria, decreased USG, abnormal renal imaging findings, increased SDMA)
	Stage 2: ≥1.6-2.8 mg/dl
	Stage 3: ≥2.9-5 mg/dl
	Stage 4: > 5 mg/dl
Consecutive measurements of serum or plasma SDMA*	Stage 1: SDMA persistently >14-18 µg/dl
	Stage 2: SDMA is persistently ≥18-25 µg/dl
	Stage 3: SDMA is persistently >26-38 µg/dl
	Stage 4: SDMA is persistently >38 µg/dl
Substaging by UPC	>0.4 proteinuric
	0.2-0.4 borderline proteinuric
	<0.2 non-proteinuric
Substaging by systolic blood pressure**	≥180 mmHg severely hypertensive
	160-179 mmHg hypertensive
	140-159 mmHg pre-hypertensive
	<140 mmHg normotensive
Urinary specific gravity	1.035-1.060 are values for normal and hydrated cats, although these values does not rule out CKD
	<1.035 requieres further investigation, specially if the animal is dehydrated or presents azotemia

Abbreviations: CKD, chronic kidney disease; SDMA, symmetric dimethylarginine; UPC, urinary protein-creatinine ratio; USG, urinary specific gravity.

*According to IRIS guidelines creatinine prevails over SDMA for interpretation of CKD stages. E.g. if serum or plasma SDMA is persistently >18 µg/dl in a cat whose creatinine is <1.6 mg/dL (IRIS CKD stage 1 based on creatinine)

**Blood pressure should be measured following International Society of Feline Medicine Consensus Guidelines on the Diagnosis and Management of Hypertension in Cats.

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HYPOTHESIS

Diabetes is the main risk factor for CKD in people and recent studies have suggested that obesity, prediabetes and the metabolic syndrome could also be independent risk factors of CKD. As the cat represents a spontaneous animal model of diabetes mellitus and it also shares characteristics of human obesity, we hypothesize that diabetes, prediabetes and obesity could be independent risk factors of CKD in cats. Novel markers of impaired kidney function and early glucose metabolism might help in the search of whether or not these disorders are associated with CKD in cats.

OBJECTIVES

Main objectives:

1. To assess the association of diabetes mellitus and early alterations of glucose metabolism with chronic kidney disease in cats.
2. To assess the association between obesity and chronic kidney disease in cats.

Specific objectives:

1. To assess the association between clinical diabetes and known chronic kidney disease in adult cats.
2. To assess the association between overweight/obesity and markers of kidney damage in adult cats.
3. To assess the association between early markers of abnormal glucose metabolism and markers of kidney damage in adult cats.
4. To compare fructosamine and fasting blood glucose as early markers of abnormal glucose metabolism in adult cats.

RESULTS

To achieve these objectives, this project was structured into three original investigations (one retrospective, one cross-sectional and one prospective) and two reviews:

1. Pérez-López L, Boronat M, Melián C, Brito-Casillas Y, Wägner AM. La obesidad felina y humana son problemas endocrinológicos que comparten complicaciones similares. [Feline and human obesity are endocrine disorders that share similar complications]. Clin Vet Peq Anim 2018;38(3):155-167.
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2. Pérez-López L, Boronat M, Melián C, et al. Assessment of the association between diabetes mellitus and chronic kidney disease in adult cats. J Vet Intern Med. 2019;33(5):1921-1925.
   Journal Impact Factor 2.286
   Rank: 12/141 Q1, Veterinary Sciences

3. Pérez-López L, Boronat M, Melián C, Brito-Casillas Y, Wägner A.M. Animal Models and Renal Biomarkers of Diabetic Nephropathy. In: Advances in Experimental Medicine and Biology. Springer, New York, NY. 2020.
   Do: https://doi.org/10.1007/5584_2020_527
   Journal Impact Factor: 2.126

Rank: 30/87 Q2, Biology Science. 86/136 Q3, Medicine, Research & Experimental Science

4. Pérez-López L, Boronat M, Melián C, Brito-Casillas Y, Wägner AM. Kidney function and glucose metabolism in overweight and obese cats. Veterinary Quarterly. 2020; 40(1):132-139.
   DOI: 10.1080/01652176.2020.1759844
   Journal Impact Factor: 2.340
   Rank: 9/141 Q1, Veterinary Sciences

5. Pérez-López L, Boronat M, Melián C, Santana A, Brito-Casillas Y, Wägner AM. Overweight and obesity are not associated with loss of kidney function in healthy domestic cats (draft).

ARTICLE I

La obesidad felina y humana son problemas endocrinológicos que comparten complicaciones similares

Feline and human obesity are endocrine disorders that share similar complications

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Resumen

La obesidad representa una alteración muy común en la especie felina y, al igual que en la especie humana, se considera el principal factor de riesgo de la *diabetes mellitus* tipo 2. Los gatos también pueden presentar otros desórdenes asociados a la obesidad que comparten características con el síndrome metabólico humano. Sin embargo, en los gatos no se ha descrito la aterosclerosis, la aparición de la hipertensión no parece guardar relación con la obesidad, y el papel de la diabetes y de la obesidad en el desarrollo de la enfermedad renal crónica es incierto. La ausencia de producción de algunas citoquinas proinflamatorias en el tejido adiposo, la disminución de la enzima convertidora de angiotensina 2 en los depósitos de grasa subcutánea observada en gatos obesos y la corta esperanza de vida de esta especie pueden ser algunos de los mecanismos que contribuyen a que en los gatos no se observen dichas alteraciones. Esta revisión aborda la patogénesis de la obesidad en la especie felina, describiendo cada uno de los factores de riesgo derivados de ésta y centrándose en su comparación con la especie humana.

Palabras clave: obesidad, diabetes, enfermedad renal crónica, hipertensión, síndrome metabólico, dislipemia, gatos.
Keywords: obesity, diabetes, chronic kidney disease, hypertension, metabolic syndrome, dyslipidemia, cats.

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Introducción

La obesidad en personas representa un problema de salud de proporciones epidémicas. De acuerdo con las estimaciones más recientes de la Organización Mundial de la Salud, la prevalencia de obesidad en la población adulta en países de la Unión Europea y América oscila entre el 10 y el 30%, mientras que el sobrepeso se sitúa entre el 30 y el 70%.[1,2] Entre sus principales repercusiones, la obesidad favorece el desarrollo simultáneo de distintas alteraciones metabólicas, como la dislipemia, la hipertensión arterial y las alteraciones del metabolismo de la glucosa.[3] El conjunto de estos trastornos se conoce con el nombre de síndrome metabólico y es un factor de riesgo independiente para la aparición de diabetes tipo 2 (DM2) y enfermedad cardiovascular.[3,4] En la actualidad, se calcula que alrededor de 4 millones de personas mueren cada año en el mundo como consecuencia del sobrepeso o la obesidad.[1]

El estilo de vida de las sociedades industrializadas, caracterizado por el sedentarismo y la alimentación basada en el consumo de alimentos hipercalóricos procesados, sumado a factores genéticos predisponentes aún no bien establecidos, es la principal causa del desarrollo de la obesidad y los trastornos de la homeostasis de la glucosa asociados a ella.[5] Como es práctica habitual en la investigación biomédica, los estudios científicos sobre la génesis de la obesidad han utilizado diferentes modelos animales de la enfermedad. Una revisión previa de nuestro grupo discutió el posible uso de modelos animales de obesidad inducida mediante manipulación dietética en varias especies,

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como roedores, gatos, ardillas o simios.[6] Sin embargo, la obesidad es una enfermedad también común en los animales domésticos y es posible encontrar modelos espontáneos de esta enfermedad en perros y gatos. De hecho, el exceso de peso en las mascotas también representa un problema creciente, ya que alrededor de un 35-50% de los gatos domésticos presenta sobrepeso u obesidad.[7] Por estos motivos, y debido a las semejanzas clínicas y fisiopatogénicas entre la diabetes felina y la DM2 humana,[7] la presente revisión se centra en su principal factor de riesgo, la obesidad, ahondando en las diferencias observadas entre ambas especies, y proponiendo al gato doméstico como modelo espontáneo para esta enfermedad.

Herramientas para definir y valorar el sobrepeso o la obesidad en gatos

La técnica más habitual para valorar el estado nutricional en el ser humano es la medida del índice de masa corporal (IMC). En medicina veterinaria se han realizado evaluaciones dimensionales para establecer un IMC felino,[8] pero no se han establecido valores de normalidad aplicables en la práctica clínica. La absorciometría dual de rayos X (DXA), la tomografía computerizada y la resonancia magnética son los métodos de mayor precisión para la estimación de la composición corporal, tanto en humanos como en pequeños animales. Sin embargo, estas técnicas normalmente son sólo utilizadas en investigación, dado su elevado coste y porque su uso en el ámbito de la veterinaria requiere la anestesia del animal.[9,10]

Por todo ello, el procedimiento más usado en la práctica veterinaria para evaluar el estado nutricional en pequeños animales domésticos es el índice de condición corporal (*Body Condition Score*, BCS), un método semicuantitativo que se realiza mediante la inspección visual y la palpación del animal, clasificándolo en una escala numérica que abarca desde la emaciación hasta la obesidad.[11] Se considera que los animales tienen sobrepeso cuando muestran un incremento de peso del 10% por encima de su peso óptimo, y son considerados obesos si el incremento es superior al 20%.[12] En la escala de 9 puntos del sistema BCS, cada aumento de un punto a partir de los 5 puntos es considerado un incremento de peso de alrededor del 10%.[12,13] Además de su simplicidad, la medida del BCS es precisa y reproducible. Estudios recientes han demostrado que presenta una buena correlación con la masa grasa estimada mediante DXA.[10] Además, se ha comprobado que existe una buena correlación entre las medidas de BCS estimadas por operadores experimentados y aquellas observadas por personas sin experiencia previa en el uso de este método.[6]

Factores de riesgo de la obesidad en la especie felina

Factores genéticos

La obesidad en la especie felina es frecuente en gatos mestizos y en el gato común europeo (pelo largo, medio, corto).[14] Determinados estudios han observado también predisposición de algunas razas tales como el British Short-Hair, Manx, Persa o Bosque de noruega.[14,15] Se sospecha que, al igual que en la especie humana, la obesidad en los gatos puede estar influenciada por factores genéticos.[16] En este sentido, un estudio reciente ha señalado que un polimorfismo del gen receptor 4 de melanocortina (*MC4R*) está asociado con la DM2 en gatos con sobrepeso.[17] En la especie humana, este gen ha demostrado tener una función en el balance de la ingesta energética y sus mutaciones se asocian al desarrollo de obesidad y DM.[18]

Edad

Como ocurre en el ser humano, en los gatos la obesidad también está asociada con la edad, siendo más prevalente entre los 5 y 11 años.[14,19]

Sexo

En la especie felina la esterilización predispone a la obesidad en ambos géneros, si bien se ha observado una mayor predisposición en los machos[20] (Tabla 1), siendo los gatos machos castrados los que presentan un mayor riesgo de desarrollar obesidad, mientras que las hembras enteras son las que presentan menor riesgo.[13] Por otro lado, en la especie humana se ha observado mayor prevalencia de obesidad en las mujeres.[1] La mayoría de los estudios sugieren que, después de la esterilización en la especie felina, se produce un aumento de la ingesta, así como un aumento de los niveles de grelina.[21-23] La grelina es una hormona secretada sobre todo en la mucosa del fundus gástrico, tanto en gatos como en seres humanos, y principalmente lleva a cabo una función orexígena (estimulante del apetito). Durante un largo periodo de tiempo se ha considerado que la ganancia de peso tras la esterilización también

Tabla 1. Factores de riesgo de obesidad en la especie felina

Predisposición racial	British Short-Hair, Manx, Persa o Bosque de noruega, entre otras.
Factores genéticos	*MC4R*
Edad	5-11 años
Género	Esterilizados y machos
Estilo de vida	Sedentarismo, restricción de salir al exterior de la vivienda
Dietas	Ingesta energética

estaba influenciada por una disminución de los requerimientos energéticos. Sin embargo, todos los estudios que recogen este dato estudiaron a los animales entre 5 y 6 meses después de la esterilización. Por el contrario, en otros estudios más recientes se observó que, tras los primeros días después de la intervención, lo que se produce es un aumento de la ingesta sin que disminuya el gasto energético. Por todo ello, no parece que la disminución de los requerimientos energéticos sea la causa principal del aumento de peso inmediatamente después de la esterilización.[23] Recientemente, también se ha observado que, con la esterilización temprana en hembras, en torno a las 19 semanas, se consigue un mejor control de la ingesta energética y del manejo dietético, mientras que esterilizaciones más tardías conducen a ingestas más abundantes y ganancias de peso más abruptas.[24]

De modo similar a los seres humanos, entre el 35-50% de los gatos domésticos sufren sobrepeso u obesidad

Estilo de vida

Los cambios del estilo de vida en la sociedad actual se han visto reflejados en la vida de las mascotas, las cuales con frecuencia no realizan una actividad física adecuada. Algunos estudios demuestran que no existe asociación entre la obesidad de los dueños y sus gatos, pero sí que existe una correlación positiva entre el BCS y la restricción a salir al exterior de la vivienda.[25,26]

Dieta

Al igual que en las personas, parece que el desarrollo de la obesidad en el gato no está tan influenciado por el tipo de dieta como por el total de la ingesta energética.[27-29] Respecto a su composición, las dietas bajas en carbohidratos no parecen efectivas para favorecer la pérdida de peso. De hecho, en gatos alimentados con diferentes aportes de carbohidratos, la mayor disminución de peso sucede en aquellos cuya dieta proporciona una ingesta energética menor, independientemente de la cantidad de carbohidratos.[27]

En cuanto a las dietas ricas en grasas, debido a su mayor densidad calórica, pueden contribuir a una mayor ganancia de peso.[30] Se ha descrito un mayor aumento de peso con dietas altas en grasas frente a dietas altas en carbohidratos.[31,32] Por otro lado, se ha observado que en la especie felina las dietas húmedas pueden contribuir a la disminución de la ingesta energética y del peso corporal.[33]

El papel del tejido adiposo

Al igual que sucede en la especie humana, la obesidad felina conlleva cambios metabólicos en el tejido adiposo que favorecen la resistencia a la insulina en tejidos como el músculo esquelético.[34] El tejido adiposo participa activamente en el balance energético y actúa como un órgano endocrino, principalmente debido a su papel regulador en la liberación de ácidos grasos libres y la secreción de diferentes sustancias proteicas denominadas adipoquinas. Las adipoquinas intervienen en el control de la ingesta, el gasto energético y la homeostasis de la glucosa.[35,36] Como en el ser humano, en la especie felina el incremento de la masa grasa también se correlaciona positivamente con los niveles circulantes de leptina y negativamente con los de adiponectina, las dos principales adipoquinas conocidas.[29] La leptina actúa como un sensor de las reservas corporales de grasa, estimula la sensación de saciedad en el sistema nervioso central y favorece la termogénesis en el tejido adiposo. Por todo ello se acepta que, como en el ser humano, los gatos obesos presentan un estado de resistencia a la leptina.[34-36] En aquellos gatos que pierden peso, se produce una mejora de la sensibilidad a la insulina y una normalización de los niveles de leptina y adiponectina.[29]

Existe, sin embargo, más controversia respecto al diferente papel que pueden desempeñar los procesos inflamatorios del tejido adiposo en el desarrollo de resistencia a la insulina y aterosclerosis en gatos y seres humanos. La expansión y el incremento del tamaño celular de los adipocitos en personas obesas se acompaña de un incremento en la producción de quimiocinas, tales como la proteína quimioatrayente de monocitos 1 (MCP-1), que atraen y facilitan la infiltración de monocitos/macrófagos.[36] Esto conduce a una gran secreción intraadiposa de citoquinas proinflamatorias, como el factor de necrosis tumoral alfa (TNFα) y la interleucina 6 (IL-6). Localmente, estos factores promueven una inflamación tisular que dificulta las acciones de la insulina, mientras que su liberación al torrente circulatorio contribuye a la inflamación y la resistencia a la insulina en el hígado, y a la angiogénesis y la aterosclerosis en el árbol vascular sistémico. Los niveles plasmáticos de IL-6 y otras citoquinas inflamatorias son considerados marcadores predictivos de daño cardiovascular.[36,37] Frente a estas evidencias en humanos, algunos estudios apuntan a que en gatos obesos no se producen cambios en la producción de las citoquinas proinflamatorias como el TNFα y la IL-6,[38] y la enfermedad cardiovascular, la aterosclerosis o la hipertensión arterial no son complicaciones asociadas a la obesidad. No obstante, en un estudio reciente sí se observó una mayor expresión de los niveles de ARNm del TNFα en gatos obesos, así como un incremento de la MCP-1, de la quimiocina atrayente de linfocitos T

(CCL5) y del contenido de linfocitos T CD3+ en el tejido adiposo. Sin embargo, este estudio no pudo demostrar un incremento en los niveles de ARNm de la IL-6 ni en el número de macrófagos en el tejido adiposo.[39]

Por otro lado, respecto a la distribución del tejido adiposo en individuos con obesidad, en personas obesas con alteración del metabolismo de la glucosa se ha observado un desarrollo predominante de la grasa visceral, mientras que en gatos obesos no se observan diferencias entre el tamaño del depósito de grasa subcutánea y visceral.[7] De hecho, algunos estudios sugieren que en estos animales, de forma contraria a la especie humana, es el depósito de grasa subcutánea el que tiene una mayor implicación en las complicaciones derivadas de la obesidad.[39]

Complicaciones de la obesidad

Síndrome metabólico

El síndrome metabólico es un conjunto de alteraciones, tales como la obesidad abdominal, la hipertensión arterial, la resistencia a la insulina y la dislipemia aterogénica, que predisponen a la DM2 y a enfermedades cardiovasculares (Tabla 2). Este síndrome representa un problema creciente a nivel mundial. Su prevalencia en Estados Unidos entre los años 2007 y 2012 ascendía a un 34,2%,[40] y se calcula que a nivel global 425 millones de personas sufren diabetes.[41] Aunque en el gato la obesidad predispone a la insulinorresistencia, la DM2 y a cambios del metabolismo lipídico, la hipertensión arterial y la aterosclerosis no se han asociado a la obesidad o la DM2. De hecho, no se han establecido los criterios para definir el síndrome metabólico en el gato.[7]

Resistencia a la insulina

Varias técnicas utilizadas para evaluar la resistencia a la insulina en seres humanos se han reproducido también en gatos. El *clamp* euglucémico-hiperinsulinémico (CEH) es el método de referencia para evaluar la sensibilidad a la insulina a través de la estimación del índice de sensibilidad a la insulina calculado a partir de modelos matemáticos en función de la eliminación de glucosa.[29] Con el CEH se ha demostrado en gatos que cada kg de exceso de peso reduce la sensibilidad a la insulina y la eficacia de la glucosa en aproximadamente un 30%.[29] Otros métodos comúnmente utilizados para evaluar la sensibilidad a la insulina en las personas, también utilizados en gatos, son la prueba oral de tolerancia a la glucosa (TTOG) y la prueba de tolerancia a la glucosa intravenosa (TTIVG), ambas basadas en fórmulas matemáticas que requieren la medición repetida de glucosa e insulina plasmática tras la administración de una carga de glucosa por vía oral o intravenosa, permitiendo el cálculo de índices para estimar la sensibilidad a la insulina.[42,43] Respecto a la TTOG, algunos autores no la consideran adecuada para valorar la sensibilidad a la insulina en la especie felina, y se necesitan estudios prospectivos para evaluar su utilidad diagnóstica.[43] El índice de modelo de evaluación de la homeostasis (*Homeostatic Model Assessment*, HOMA), que se calcula a partir de la insulina y glucosa basal, es ampliamente usado en humanos y también ha sido definido en gatos, pero en esta especie no se ha realizado una comparación de este parámetro respecto al método de referencia (CEH) y su uso clínico no ha sido validado.[7,44]

Desde el punto de vista fisiopatológico, se sospecha

Tabla 2. Resumen comparativo del desarrollo de la obesidad espontánea y sus complicaciones en la especie felina y humana

	Especie felina	Especie humana
Tejido adiposo	• Igual desarrollo de la grasa subcutánea y visceral. Posiblemente exista una mayor implicación del tejido adiposo subcutáneo • Resistencia a la leptina	• Mayor liberación de citoquinas proinflamatorias • Mayor desarrollo e implicación del tejido adiposo visceral • Resistencia a la leptina
Insulina	• Resistencia a la acción de la insulina y predisposición al desarrollo de la DM2	• Resistencia a la acción de la insulina y predisposición al desarrollo de la DM2
Factores de riesgo cardiovascular	• La obesidad y la DM no parecen estar asociadas a la hipertensión • No desarrollan aterosclerosis • Posible mecanismo protector: disminución de ECA2 en la grasa subcutánea	• Hipertensión • Aterosclerosis
Enfermedad renal	• Su desarrollo asociado a la obesidad y la DM2 es incierto	• El síndrome metabólico es un agente etiológico de la ERC • La DM2 es la principal causa de ERC

ECA2: enzima convertidora de angiotensina 2; DM2: *diabetes mellitus* tipo 2; ERC: enfermedad renal crónica.

la implicación de diferentes órganos en el desarrollo de la resistencia a la insulina. La insulina actúa a nivel hepático disminuyendo la producción endógena de glucosa (PEG), mientras que a nivel periférico favorece la captación de glucosa en el músculo. Se ha señalado que en gatos obesos la resistencia periférica a la insulina podría ser un contribuyente más precoz que los trastornos en la PEG como mecanismo fisiopatogénico de la DM2.[45] En todo caso, la contribución de la resistencia a la insulina en músculo y en hígado podría variar individualmente. En las personas obesas que desarrollan alteración de la glucosa basal como primera manifestación de prediabetes, la resistencia hepática a la insulina y el consecuente aumento en la producción hepática de glucosa podría ser el principal mecanismo en la alteración del metabolismo de la glucosa.[46]

La elevación de los niveles circulantes de ácidos grasos libres parece desempeñar un papel clave en la resistencia a la insulina a nivel muscular, disminuyendo la captación de glucosa e inhibiendo su transporte, lo que conduce a una reducción de la síntesis de glucógeno muscular y de la glucólisis.[47] Los gatos obesos también presentan una elevación de los ácidos grasos libres circulantes, pero contrariamente a la especie humana, sus niveles se suprimen de manera similar a la observada en individuos delgados durante una prueba de CEH. Esto sugiere que los gatos pueden conservar una mayor capacidad para la oxidación y el almacenamiento de ácidos grasos incluso en situaciones de obesidad y resistencia a la insulina.[29,48]

La diabetes felina comparte mecanismos fisiopatológicos de la diabetes tipo 2 del ser humano

Diabetes mellitus

La obesidad y la resistencia a la insulina son las causas predisponentes al desarrollo de diabetes en el 80% de los casos de diabetes felina, que se caracterizan por una disfunción secretora en las células β pancreáticas, tal y como sucede en la DM2 humana. Sin embargo, si bien parece que la prevalencia de la DM2 está aumentando en paralelo a la obesidad,[49] el impacto de la obesidad en la epidemiología de la diabetes es menor en el gato que en el ser humano. Mientras que la prevalencia de obesidad es similar en gatos y seres humanos, la prevalencia de diabetes es mucho más alta en personas (8,5%)[50] que en gatos (1%).[49] Excepcionalmente, la prevalencia entre los gatos Burmeses, una de las razas más predispuestas a la diabetes, se encuentra en torno al 10%, lo que concuerda con la prevalencia observada en la especie humana y apoya la sospecha de que existe una predisposición genética en el desarrollo de la diabetes.[51,52]

Por otro lado, aunque la resistencia a la insulina es un factor predisponente habitual en el desarrollo de la diabetes, para que ésta se manifieste es también necesario que se produzca una disminución de su secreción y una disfunción de las células β pancreáticas. Entre los mecanismos responsables de dicha disfunción, al igual que en humanos, se ha señalado en los gatos con diabetes el depósito de amiloide pancreático. La relación entre la amiloidosis pancreática y la diabetes fue sugerida por O´Brien *et al*[53] en 1985 tras la detección de estos depósitos en el páncreas de gatos diabéticos, pero no en gatos sanos usados como control. Sin embargo, en estudios histopatológicos más recientes se ha encontrado la presencia de depósitos amiloides en la misma proporción en gatos diabéticos y en controles, por lo que el papel de la amiloidosis pancreática en la fisiopatología de la diabetes felina es controvertido.[54] Adicionalmente, también se ha discutido la contribución de la pancreatitis en la diabetes. Si bien un estudio histopatológico reciente no mostró evidencias de que los gatos con diabetes presentaran más hallazgos compatibles con inflamación del páncreas exocrino que los gatos controles, sí se observó que los gatos con diabetes presentaban un mayor número de células acinares, al igual que ocurre en personas con pancreatitis crónica.[55]

También conviene señalar que existen otros tipos de diabetes felina. La diabetes tipo 1 es una condición excepcional en el gato. Su existencia, en realidad, se basa en la descripción de algunos casos con infiltración de linfocitos en los islotes pancreáticos, pero en el gato no se ha demostrado la presencia de anticuerpos anti-insulina o frente a otros antígenos de las células β.[56,57] Otras formas menos comunes de diabetes son las asociadas con otras enfermedades, como acromegalia, hiperadrenocorticismo o carcinoma pancreático.[58] En este sentido, contrariamente a lo que ocurre en la especie humana donde la acromegalia es una causa poco habitual de diabetes, hasta el 25% de los gatos diabéticos pueden tener acromegalia.[59] Respecto al carcinoma pancreático, su asociación con la diabetes no es tan sólida como en humanos. En esta especie, alrededor del 70% de los pacientes con carcinoma pancreático presentan intolerancia a la glucosa o diabetes,[60] mientras que la prevalencia de diabetes en gatos con carcinoma pancreático es de un 14%.[61]

En la especie felina la diabetes se diagnostica cuando se detectan síntomas clínicos compatibles (poliuria y polidipsia, polifagia y pérdida de peso), hiperglucemia persistente (> 250 mg/dl), glucosuria y/o fructosamina elevada (>400 µmol/l).[62] En los gatos se debe tener en cuenta que los valores de glucosa en ayunas pueden estar influenciados por la hiperglucemia de estrés.

Por ello, en muchos casos, para realizar un diagnóstico correcto de diabetes se debe considerar el valor de la fructosamina.[62] No se conocen los procesos implicados en este mecanismo de la hiperglucemia de estrés, pero los gatos carecen de la glucoquinasa (GCK) hepática, y se ha observado que los ratones transgénicos con ausencia de esta enzima también padecen hiperglucemia de estrés y no tienen una tolerancia normal a la glucosa.[63] En humanos, las mutaciones inactivantes en ambas copias de la GCK son causa de diabetes neonatal.[64]

La prueba oral de tolerancia a la glucosa (TTOG) no se utiliza en el diagnóstico de la diabetes en la especie felina. Los resultados de este test en gatos difieren respecto a la especie humana y no existen estudios prospectivos que hayan evaluado su utilidad diagnóstica. Además, los resultados también podrían verse afectados por la hiperglucemia de estrés,[59] e incluso existen algunos resultados contradictorios entre diferentes estudios.[43,63,65]

Por otro lado, aunque existen varios estudios que indican que la hemoglobina glicada (HbA1c) es un buen marcador de los niveles de glucosa durante las 8 semanas previas en gatos,[66] la falta de disponibilidad de un test de HbA1c asequible en veterinaria ha hecho que se use comúnmente la fructosamina para el diagnóstico y control de la diabetes. La fructosamina en gatos es un buen marcador de la glucemia de la semana previa.[66] No obstante, recientemente ha salido al mercado un test más asequible para medir la HbA1c,[62] por lo que es posible que en un futuro próximo su uso se generalice también.

Dislipemia

En contra de las evidencias demostradas en el ser humano,[67] en la especie felina no se ha confirmado una asociación entre obesidad y aterosclerosis. Diferentes estudios han comprobado, sin embargo, que los gatos obesos desarrollan alteraciones del metabolismo lipídico similares a las observadas en personas con obesidad, incluyendo hipertrigliceridemia, altos niveles de ácidos grasos libres e incremento de las lipoproteínas de muy baja densidad (VLDL), especialmente de las partículas de VLDL de tamaño medio, ricas en triglicéridos.[68] Respecto a las concentraciones de lipoproteínas de baja densidad (LDL), no se han observado diferencias entre gatos sanos y gatos obesos, aunque los gatos obesos, al igual que las personas con obesidad, presentan una mayor proporción de partículas pequeñas de LDL.[68] También se ha visto una disminución de las concentraciones de lipoproteínas de alta densidad (HDL) en los gatos obesos, con un aumento de las partículas pequeñas de HDL.[68] Dado que todos estos cambios están implicados en el desarrollo de la aterosclerosis en la especie humana, se ha especulado sobre la posibilidad de que existan otros mediadores de la aterosclerosis que se comporten de diferente manera en ambas especies.[68] Entre ellos, se ha sugerido una diferente producción de citoquinas inflamatorias que pueden proteger a los gatos frente al desarrollo de daño vascular.[38]

Hipertensión arterial

En la especie felina se considera que hay hipertensión cuando la presión arterial sistólica supera los 160 mmHg, si bien hay que tener en cuenta el denominado efecto "bata blanca", por el que se produce una elevación temporal de la presión arterial causada por el estrés. Para evitar este efecto se recomienda dejar un periodo de 5-10 minutos de aclimatación en la consulta antes de realizar la medición.[69] El riesgo de padecer hipertensión en esta especie incrementa con la edad.[70,71] La hipertensión en los gatos puede ser idiopática o primaria, cuando no hay otra causa subyacente, lo cual sucede aproximadamente en un 20% de los casos.[69] Puede ser considerada secundaria si existe otra patología de base, siendo dos de las principales la enfermedad renal crónica (ERC) y el hipertiroidismo.[69] Alrededor de un 40% de los gatos con ERC y en torno a un 20% de los gatos hipertiroideos desarrollan hipertensión.[69,71]

Respecto a la obesidad, en esta especie no se ha demostrado su relación con el desarrollo de la hipertensión. Un estudio reciente observó que los gatos que estaban por debajo del peso ideal presentaban niveles de presión arterial menores que aquellos que tenían un peso normal o que tenían sobrepeso. Sin embargo, no se detectó una diferencia significativa de los niveles de presión arterial entre los gatos con un peso ideal y los gatos con sobrepeso.[70]

Tampoco está muy claro el papel de la diabetes en la hipertensión en los gatos, si bien la mayor parte de la literatura al respecto parece apoyar en mayor medida la postura de que no existe una relación entre ambas.[72-75]

La ausencia de hipertensión arterial podría ser un factor de protección de los gatos con obesidad y diabetes frente al desarrollo de enfermedades cardiovasculares. Los mecanismos que pueden explicar estas diferencias con la especie humana aún no se conocen bien, aunque un campo prometedor puede ser el estudio del sistema renina-angiotensina (SRA). Como en los seres humanos, también en los gatos se han identificado componentes del SRA en los depósitos de grasa visceral y subcutánea, aunque sus patrones de expresión génica durante la adipogénesis son diferentes respecto a los de las personas. En un estudio realizado con gatos obesos y sanos, se encontró que los obesos tenían una

disminución de la enzima convertidora de angiotensina 2 (ECA2) en los depósitos de grasa subcutánea.[76] El estudio de la ECA en la especie felina podría ser, por tanto, de gran interés, ya que un bloqueo de la ECA puede disminuir el estrés oxidativo y con ello disminuir la resistencia a la insulina[77] y, además, los inhibidores de esta enzima juegan un papel crucial como antihipertensivos y protectores cardiovasculares.

Enfermedad Renal Crónica (ERC)

La DM2 es la primera causa de ERC en humanos.[78] Sin embargo, en los últimos años se ha acumulado evidencia sobre el posible papel de la obesidad, la resistencia a la insulina y el síndrome metabólico en la génesis de la ERC, incluso sin la existencia de diabetes.[79] La obesidad, además, podría tener un efecto aditivo sobre la diabetes. Un estudio reportó una fuerte asociación entre obesidad y ERC en pacientes con diabetes tipo 1 y DM2.[80] La implicación de la resistencia a la insulina se apoya en estudios epidemiológicos transversales realizados sobre diferentes poblaciones, que han mostrado una correlación entre diferentes marcadores de insulinorresistencia y ERC[81-83] Más aún, varios estudios prospectivos han documentado que el síndrome metabólico es un factor de riesgo independiente para la aparición de ERC y para su progresión cuando ésta ya está presente.[84-86]

De hecho, se ha especulado con la posibilidad de que el síndrome metabólico pueda ser el agente etiológico principal del daño renal en muchos pacientes con ERC de causa desconocida, un grupo de pacientes cada vez más amplio entre la población con ERC.[87]

En la especie felina, la ERC es una patología común, especialmente en los gatos de avanzada edad, donde la prevalencia puede llegar hasta el 30-40%.[88] Su diagnóstico se lleva a cabo teniendo en cuenta los niveles de creatinina, la densidad urinaria y el ratio proteína-creatinina, clasificando la enfermedad en 4 estadios diferentes (creatinina <1,6 mg/dl: IRIS estadio 1; 1,6-2,8 mg/dl: IRIS estadio 2; 2,9-5 mg/dl: IRIS estadio 3; > 5 mg/dl: IRIS estadio 4).[89] Recientemente se ha comenzado a utilizar la dimetilarginina simétrica (SDMA) como marcador de daño renal,[90] de modo que niveles persistentemente por encima de 14 µg/dl indican que puede haber una función renal reducida y niveles ≥ 25 µg/dl indican un estadio 2 de enfermedad renal.[89]

En medicina humana, el diagnóstico y la estadificación de la ERC se realiza principalmente a partir de la tasa de filtración glomerular (TFG), que normalmente se estima a partir de la creatinina o la cistatina C.[91] En la práctica clínica veterinaria no se realizan dichas estimaciones de la TFG, siendo necesarios más estudios para valorar su utilidad clínica.

La etiología de la ERC en la especie felina obedece a diferentes enfermedades, como urolitiasis, linfoma renal, infecciones, hipertiroidismo, vacunas, sustancias nefrotóxicas y causas genéticas, como la enfermedad poliquística o la displasia renal. Sin embargo, la mayoría de los casos con enfermedad renal son de etiología desconocida.[92] A diferencia de lo que sucede en seres humanos, el papel de la obesidad y la diabetes en el desarrollo de la ERC es incierto. En un estudio que comparó los hallazgos histopatológicos en riñones de gatos con y sin diabetes, la expansión de la matriz mesangial, la lesión más común en personas con nefropatía diabética, fue la lesión glomerular más frecuente en los gatos de ambos grupos. Sin embargo, las lesiones tubulointersticiales fueron más comunes que las glomerulares, y una mayor proporción de gatos con diabetes presentaron necrosis tubulointersticial.[93] No obstante, entre los diabéticos, los que presentaban mayor tiempo de supervivencia tenían más lesiones glomerulares y vasculares, sugiriendo que la diabetes podría tener un impacto solo a más largo plazo. Esto ha conducido a postular que la ausencia de ERC relacionada con la diabetes podría deberse a la menor esperanza de vida de los gatos, ya que en la especie humana las lesiones glomerulares a menudo aparecen 15 años después del diagnóstico de la enfermedad.[93]

Hígado graso no alcohólico o lipidosis hepática

El síndrome de hígado graso felino consiste en la acumulación de triglicéridos en los hepatocitos. Se designa como lipidosis hepática felina cuando este acúmulo es mayor del 80%.[94] Tradicionalmente ocurre tras periodos de anorexia, fenómeno que también ha sido descrito en otros carnívoros estrictos, como el hurón doméstico (*Mustela putorius*),[94,95] y su mecanismo no se ha esclarecido, aunque se ha atribuido al descenso en los niveles de glucemia, aumento en la producción de glucagón, lipólisis y aumento de ácidos grasos circulantes, posteriormente depositados en el hígado.[94] Sin embargo, también puede ser debido a enfermedades subyacentes, como la diabetes y la obesidad,[96] sugiriendo un proceso similar a la esteatohepatitis no alcohólica del ser humano.

Enfermedades cardiopulmonares

Se ha observado que en gatos con fallo cardíaco, la mortalidad es mayor en aquellos con mayor peso corporal.[97] Por otro lado, al igual que se ha sugerido en medicina humana,[98] estos mismos estudios indican que la relación entre el peso corporal y el tiempo de supervivencia en los gatos sigue una representación gráfica en forma de U, de modo que la mortalidad es mayor en

animales que presentan extremos más altos y más bajos de peso corporal.[97] En personas con DM2 y ERC, las alteraciones cardiovasculares y las infecciones son las principales causas de muerte.[99] En gatos, un estudio describió que la probabilidad de muerte por fallo cardíaco podría ser también hasta 10 veces superior en animales que presentan DM2.[100] Aunque en dicho estudio no se descartaron causas secundarias que pudiesen estar implicadas en el desencadenamiento de la enfermedad cardíaca, otro estudio, en el que sí se descartó la existencia de acromegalia o hipertiroidismo, confirmó que la DM2 produce efectos negativos sobre la función diastólica de los gatos.[101]

En ambas especies, la restricción calórica es de gran importancia en el tratamiento de la obesidad

En cuanto a la función pulmonar, los gatos obesos presentan una disminución del volumen tidal y del flujo espiratorio e inspiratorio.[102] Sin embargo, en gatos no se han descrito síndromes como el de apnea obstructiva del sueño o el síndrome de hipoventilación por obesidad, que ocurren en las personas obesas.

Enfermedades ortopédicas

En la especie felina aún no se ha descrito si la obesidad puede influir en la densidad mineral ósea, o si los gatos diabéticos presentan o no mayor riesgo de fracturas. Tampoco se conoce la relación entre obesidad y artrosis. Al igual que en humanos, la edad es un factor de riesgo importante y la prevalencia de artrosis en gatos entre diferentes estudios difiere debido a las diferencias de edad entre las poblaciones estudiadas. En conjunto, alrededor de un 16-26% de los gatos padecen enfermedad articular degenerativa y sólo un 14% de los gatos afectados presenta obesidad.[103]

Cáncer

Numerosos estudios epidemiológicos han demostrado una relación entre obesidad y distintos tipos de cáncer.[104,105] Esta relación se ha estudiado también en modelos animales, sobre todo en roedores, en los que se ha visto que la obesidad predispone al desarrollo de tumores.[105] Esta relación se desconoce en animales de compañía, para los cuales sólo se han publicado estudios retrospectivos preliminares en perros.[105]

Tratamiento de la obesidad

En veterinaria el tratamiento de la obesidad está enfocado en la restricción calórica, el incremento de la actividad física y el aumento del gasto energético.[106] Las restricciones calóricas se pueden iniciar reduciendo la ingesta calórica actual en un 10-20%.[30] Con todo, algunos autores también han sugerido que si no es posible estimar la ingesta calórica actual, se puede comenzar administrando la cantidad necesaria para cubrir los requerimientos energéticos en reposo calculados para el peso ideal (RER).[30] Por otro lado, muchos animales con sobrepeso ya tienen una ingesta calórica próxima a dicho objetivo terapéutico y, por eso, en algunos casos se aplican restricciones calóricas más severas, aportando el 80% de los RER.[30] En general, el uso preferente de las dietas terapéuticas formuladas para la pérdida de peso ha sido recomendado por diferentes autores, que sugieren que éstas ofrecen mayores garantías de conseguir el aporte necesario de todos los nutrientes cuando se realizan restricciones calóricas.[13,30] Además, algunos de estos autores apuntan a que todos aquellos animales con un sobrepeso >20% (BCS 8 o 9) pueden beneficiarse de este tipo de dietas.[30] No obstante, no hay evidencia de ensayos clínicos controlados que demuestren un beneficio claro.

La densidad calórica del alimento se puede reducir añadiendo fibra insoluble, agua o aire (disminuyendo la densidad de la croqueta de pienso).[30,33] De la misma forma, en medicina humana se tiene en cuenta la densidad calórica y para el tratamiento dietético de la obesidad, aparte de realizar una restricción calórica, se aconseja consumir una dieta equilibrada en la que se incluyan alimentos con baja densidad calórica, como pueden ser las frutas o las verduras, las cuales también son unos de los principales aportes de vitaminas, minerales y fibra.[107,108]

La AAFCO (*Association of American Feed Control Officials*) recomienda que las dietas felinas bajas en calorías que se usen para el control del peso tengan menos de 3250 kcal/kg en el caso de la comida seca, y menos de 950 kcal/kg en el de las dietas húmedas.[13]

Es importante hacer una estimación adecuada de la cantidad de ingesta y del aporte de calorías diarios, pues, como hemos mencionado, más que el tipo de dieta influye la ingesta calórica y aunque una dieta sea reducida en grasas, puede que no aporte la reducción calórica necesaria.[30] De la misma forma, dietas bajas en carbohidratos también pueden ser muy calóricas.[27]

Se ha planteado que las dietas altas en proteínas podrían ser beneficiosas para la pérdida de peso en la especie felina. Algunos estudios han señalado que las dietas hiperproteicas (45,2-47%) producen un aumento del gasto energético y de la termogénesis.[29] Sin embargo, cuando se han administrado *ad libitum*, se ha observado que los gatos pueden llegar a ganar más peso con las dietas altas en proteínas que con las altas en carbohidratos.[109] No obstante, independientemente del tipo de dieta, la alimentación *ad libitum* predispone a la ganancia de peso.[109]

De cualquier forma, es importante durante la restricción calórica asegurar un buen aporte de todos los nutrientes, poniendo especial atención en el aporte proteico para evitar déficits y pérdida de masa muscular, siendo también importante para ello que la pérdida de peso semanal se mantenga entre el 0,5 y el 2%.[13] Cuando se suministra el 80% de los RER, el Consejo Nacional de Investigación[13] recomienda que el aporte proteico mínimo sea de 89 g/1000 kcal y, para restricciones superiores en las que se ofrece el 60% de los RER, de 104 g/1000 kcal. Los casos que requieran restricciones calóricas mayores deberían consultarse con un veterinario diplomado en nutrición con el fin evitar carencias de nutrientes en la dieta.[13,30]

Los premios o golosinas no deben superar un 10% de la ingesta calórica total cuando se realiza un plan de pérdida de peso.[13] De la misma forma, en personas que siguen un plan de pérdida de peso, se recomienda que la ingesta energética se reduzca a 1200-1500 kcal por día en mujeres y a 1500-1800 kcal por día en hombres.[111] Estas restricciones calóricas consisten en un aporte de calorías de un 60-75% respecto a las recomendaciones para adultos no obesos.[112]

En los gatos diabéticos, una dieta baja en carbohidratos y alta en proteínas parece ser favorable para el control de la enfermedad.[113] Esto no significa que las dietas ricas en carbohidratos sean las causantes de la diabetes felina, si bien existe cierta controversia sobre el efecto postpandrial que producen los carbohidratos sobre la glucosa y la insulina en estos animales que son carnívoros obligados. De-oliveira *et al*,[114] utilizando dietas experimentales con un 35% de carbohidratos, apuntan a que su efecto sobre la glucosa y la insulina postpandrial es menor que el observado en perros y humanos, probablemente debido a una digestión y absorción más lenta de los carbohidratos en la especie felina. Además, los gatos han demostrado tener una digestión postpandrial más prolongada que los perros y los humanos.[115]

Sin embargo, Farrow *et al*,[116] realizando un test de alimentación *ad libitum*, observaron que tras 36 horas de ingesta, el aumento de la glucemia postpandrial era mayor en aquellos gatos alimentados con dietas ricas en carbohidratos (47%). Puede ser que el efecto sobre la glucosa y la insulina varíe con la cantidad de carbohidratos añadidos a la dieta[115] o, como señalan otros autores, que se deba a la presencia de carbohidratos complejos en las dietas comerciales, que son más difíciles de digerir.[28]

En medicina humana, algunos autores han señalado que con dietas bajas en carbohidratos se produce una reducción de la HbA1c en pacientes diabéticos.[117] Sin embargo, otros autores no han considerado que estas dietas consigan un mejor control glucémico de estos pacientes.[117] La recomendación dietética para las personas con diabetes se basa en una dieta equilibrada con restricción calórica a fin de disminuir el peso corporal. En caso de pacientes con otras alteraciones asociadas, como hipertensión o enfermedad renal, se puede estudiar de forma individualizada si conviene realizar algún cambio en los macronutrientes. También parece bastante aceptado evitar en estos pacientes el consumo de alimentos con carbohidratos refinados y azúcares añadidos.[117]

Respecto a los gatos, se ha observado en diferentes estudios que aquellos con una dieta alta en carbohidratos presentan niveles más altos de insulina que aquellos alimentados con una dieta rica en grasas o en proteínas.[109,118] Sin embargo, en otro estudio se detectó una disminución de la sensibilidad a la insulina con una dieta muy baja en carbohidratos (7%) equivalente a un aporte menor que el de las dietas comerciales.[119] Además, en este mismo estudio, los gatos alimentados con una dieta baja en proteínas (28%) mejoraban su sensibilidad a la insulina. Esto podría explicarse por el poder gluconeogénico de los aminoácidos en los carnívoros estrictos. Al comparar estos resultados con los de otros estudios, los autores postularon que el hecho de que se observe una mayor resistencia a la insulina con niveles altos y niveles bajos de carbohidratos en la dieta puede deberse a que el efecto de estos nutrientes sobre la insulina siga una representación gráfica en forma de U.[119] Por el contrario, en 2007 Hoening *et al*[29] observaron que la resistencia a la insulina es mayor en gatos obesos, con independencia del tipo de dieta.[29]

Por otro lado, en gatos alimentados con dietas grasas se ha descrito un aumento de los ácidos grasos libres y del colesterol, además de una respuesta disminuida de la insulina tras un test de tolerancia a la glucosa.[120] En el 2015, Gooding *et al*[31] observaron que la glucosa tras 24 horas en ayunas era mayor en gatos alimentados con dietas altas en grasas que en aquellos alimentados con dietas ricas en carbohidratos.

Por lo tanto, existen varios estudios que establecen diversas teorías en relación con cómo afectan los macronutrientes de la dieta al metabolismo de la glucosa, a la sensibilidad a la insulina o al desarrollo de la obesidad en la especie felina. Sin embargo, parece que, en general, estos estudios conducen a concluir que son las dietas más calóricas las que promueven la obesidad.

Finalmente, el tratamiento farmacológico y quirúrgico de la obesidad felina no se ha desarrollado. Aunque en gatos con diabetes se han evaluado distintos fármacos hipoglucemiantes que favorecen la pérdida ponderal, hay pocos datos sobre sus efectos específicos sobre el peso. En un estudio experimental con gatos

sanos tratados con liraglutida, se observó disminución del apetito y pérdida de peso.[121]

Conclusión

El gato, a diferencia de muchos modelos animales, sufre obesidad de forma espontánea y con el tiempo muchos gatos obesos, al igual que muchas personas con obesidad, desarrollan DM2. Además, esta especie también se considera un modelo animal valioso porque comparte el mismo entorno medioambiental que las personas. Sin embargo, sigue siendo una incógnita en la especie felina la ausencia de aterosclerosis, así como de hipertensión ligada a la obesidad. El estudio en esta especie de los mecanismos implicados como posibles factores de protección frente a estos desórdenes supone un interesante campo de investigación para intentar dilucidar a su vez cuáles son los mecanismos implicados en el síndrome metabólico humano y establecer estrategias de prevención.

Por otro lado, se desconoce la relación que guardan la obesidad y la diabetes en el desarrollo de la ERC en los gatos, pero parece lógico pensar que, al igual que en las personas, ambas enfermedades predispongan al daño renal. Se necesitan nuevos estudios prospectivos para buscar marcadores precoces de daño renal en esta especie y poder llegar a concluir así esta posible relación.

La patogenia de la obesidad en esta especie, al igual que en las personas, tiene un carácter multifactorial en el que participan los factores genéticos, pero también el estilo de vida y los hábitos alimenticios. Educar a los propietarios sobre la importancia de establecer un control de la ingesta calórica de estos animales podría ayudar a la prevención de la obesidad en esta especie, sobre todo cuando son esterilizados. Esta cirugía de esterilización parece que permite un mejor control dietético cuando se realiza en hembras a edades tempranas. Además, convendría realizar nuevos estudios para confirmar si en los gatos machos se puede obtener el mismo beneficio. La esterilización temprana junto con el control de la ingesta podrían suponer un pilar fundamental en la prevención de la obesidad en la especie felina.

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Summary

Obesity is a very common disorder in cats and, as in humans, it is the main risk factor for type 2 diabetes. In addition, feline obesity shares some of the features of human metabolic syndrome. However, atherosclerosis has not been described in cats, and hypertension does not seem to be linked to obesity, either. Furthermore, the role of diabetes and obesity in the development of chronic kidney disease is uncertain. The lack of pro-inflammatory cytokines in the adipose tissue of obese cats, the lower expression of angiotensin II converting enzyme in subcutaneous adipose tissue, and their short life expectancy in comparison to humans could explain some of the differences between human and feline obesity. This review summarizes the pathogenesis of obesity in cats, describing the emergent risks factors associated with this disorder and comparing them with those associated with human obesity.

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Fe de erratas

En relación a la publicación "*La obesidad felina y humana son problemas endocrinológicos que comparten complicaciones similares*", se ha detectado un error en:

1. Tabla 2, en la columna de especie felina: "*Posible mecanismo protector: disminución de la ECA2 en la grasa subcutánea".* Debería decir: disminución de la ECA2 en la grasa subcutánea.

Explicación: La ECA2 interviene en la degradación de la Angiotensina II, su acción es diferente a la ECA, y algunos estudios han indicado que su disminución podría tener un efecto negativo sobre la presión arterial. Por tanto ha habido un error en el concepto y creemos que no es correcto decir que puede haber un posible mecanismo protector, nos gustaría eliminar dicha frase.

2. Mención de la ECA2 en el abstract, por la misma razón del punto anterior, creemos que no debe ser mencionada en el abstract. Donde dice: "*La ausencia de producción de algunas citoquinas proinflamatorias en el tejido adiposo, la disminución de la enzima convertidora de angiotensina 2 en los depósitos de grasa subcutánea observada en gatos obesos y la corta esperanza de vida de esta especie pueden ser algunos de los mecanismos que contribuyen a que en los gatos no se observen dichas alteraciones."* Debería decir: "*La ausencia de producción de algunas citoquinas proinflamatorias en el tejido adiposo, y la corta esperanza de vida de esta especie pueden ser algunos de los mecanismos que contribuyen a que en los gatos no se observen dichas alteraciones"*. Lo mismo sucedería en la versión en inglés del abstract.

Le ruego disculpe las molestia que este error pueda ocasionar.

Atentamente,
Laura Pérez López

ARTICLE II

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STANDARD ARTICLE

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Assessment of the association between diabetes mellitus and chronic kidney disease in adult cats

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Abstract

Background: Diabetes mellitus is the main cause of chronic kidney disease (CKD) in humans. The relationship between the 2 diseases in cats is unclear.

Objective: To assess the association between diabetes and CKD in a population of adult cats.

Animals: Five hundred sixty-one cats that attended 2 veterinary centers in Gran Canaria, Spain, between 2014 and 2016.

Methods: Medical records were retrospectively reviewed. Cats aged 3 years or older, with sufficient data to define whether or not they had diabetes and CKD, were selected. Cats in critical condition, with dehydration or potential causes of prerenal azotemia and those treated with nephrotoxic drugs were excluded. Diagnosis of CKD was established when creatinine concentrations were >2 mg/dL, or serum creatinine 1.6-2 mg/dL and urine specific gravity <1.035, or serum creatinine 1.6-2 mg/dL and urine protein/creatinine ratio >0.4. Factors associated with CKD were identified through multivariate logistic regression analyses.

Results: Sixty-seven (11.9%) cats had CKD and 16 (2.9%) cats had diabetes. Sixty cats without diabetes (11%) and 7 with diabetes (44%) had CKD. Among the latter, both conditions were diagnosed simultaneously in 6 cases, whereas diabetes preceded CKD in the other. Multivariate analysis showed that diabetes was significantly associated with CKD (odds ratio = 4.47; 95% confidence interval, 1.51-13.28; P = .007). Other variables associated with CKD were age and mixed breed.

Conclusions and Clinical Importance: After adjusting for age, this study showed an association between diabetes and CKD in adult cats.

KEYWORDS

creatinine, feline, glucose, nephropathy

1 | INTRODUCTION

Diabetes mellitus is a common disorder in cats, with a prevalence ranging from 0.43% to 1.24%.[1-3] Around 80% of cats with diabetes have type 2 diabetes, characterized by insulin resistance and dysfunction of pancreatic

Abbreviations: BIC, Bayesian information criterion; CI, confidence interval; CKD, chronic kidney disease; IQR, interquartile range; ISFM, International Society of Feline Medicine; OR, odds ratio; UPC, urine protein/creatinine; USG, urine specific gravity.

Correction added on 23 July 2019, after first online publication: in the author byline, "Ana M. Wägner[1,2]" has been corrected to "Ana M. Wägner[1,3]"

beta cells.[4] As in humans, risk factors for type 2 diabetes in cats include obesity, age, and genetic factors.[5-7] In humans, diabetes is the leading cause of chronic kidney disease (CKD).[8] Although the pathophysiological mechanisms of diabetes-related CKD are not fully known, it is accepted that hyperglycemia, advanced glycation products, oxidative stress, inflammatory cytokines, and profibrotic growth factors are involved in renal injury.[8]

Disorders associated with CKD in cats include diseases of the lower urinary tract, renal lymphoma, infections, hyperthyroidism, nephrotoxic drugs, and genetic kidney diseases, although often the cause of CKD is unknown.[9] Although both CKD and type 2 diabetes are common disorders in cats, the relationship between the 2 has not been investigated in depth; indeed, some studies have suggested that diabetes plays little or no role in the development of CKD.[10,11]

The aim of this study was to assess the association between CKD and diabetes in a population of cats attending 2 veterinary centers in the island of Gran Canaria (Spain).

2 | MATERIALS AND METHODS

Clinical records of cats attending at University Veterinary Teaching Hospital and an Endocrinology Clinic in Gran Canaria between 2014 and 2016 were reviewed.

All cats selected were 3 years or older and presented sufficient data to define whether or not they had diabetes and CKD. Given the rarity of diabetes in younger cats, only adult animals were included.[12] Animals without available biochemical data, but with no clinical history of CKD or diabetes, were considered to be free of these diseases. In cases where CKD, diabetes, or both were suspected, but in which the clinical history and laboratory tests were insufficient to establish either diagnosis, the owners were contacted by telephone. When this was not possible, the animals were excluded from the study.

Diabetes was defined by typical clinical signs (polyuria/polydipsia, polyphagia, weight loss), persistent hyperglycemia, or fructosamine >400 mmol/L. Persistent hyperglycemia was defined as blood glucose concentrations above 250 mg/dL for at least 2 weeks.[12,13] Considering the guidelines proposed by the International Society of Feline Medicine (ISFM)[14] and the International Renal Interest Society,[15] CKD was considered in cats with serum creatinine ≥1.6 mg/dL, plus urine specific gravity (USG) <1.035 or urine protein/creatinine (UPC) ratio >0.4. Where USG and UPC ratio were not recorded, criteria to define azotemia used by other investigators were followed,[16,17] and definitive diagnosis of CKD was established only in cases with creatinine values >2 mg/dL. Cats in critical condition, with dehydration or potential causes of prerenal azotemia (trauma, intoxication, fever, infection, urinary obstruction), and those treated with nephrotoxic drugs were excluded.

In addition to age, other data such as sex, breed, and weight were recorded. In cats with CKD and diabetes, the existence of other concurrent diseases was also registered.

2.1 | Statistical analysis

Categorical variables were expressed as frequencies and percentages, and continuous variables as median and interquartile range (IQR = 25th-75th percentile). Percentages were compared using the chi-square (χ^2) test or the Fisher exact test, as appropriate, and medians were compared with Wilcoxon's test for independent data. To identify factors independently associated with CKD, multivariate logistic regression analyses were performed, entering all the variables that showed significant associations with the outcome in univariate analyses. Then, a best subset regression procedure was used to identify the most suitable and parsimonious multivariate logistic model, that is, the 1 with the lowest Bayesian information criterion (BIC), which is a parameter of the goodness of fit of the models, defined as:

$$BIC = -2 \times loglik + (\log N) \times d,$$

with loglik being the log-likelihood, N the sample size, and d the number of variables in the model. The final model was summarized as coefficients (SE), *P*-values (likelihood ratio test), and odds ratios (ORs), which were estimated by confidence intervals (CIs) at 95%. Statistical significance was set at $P < .05$. Data were analyzed using the R package, version 3.3.1 (R Development Core Team, 2016).

3 | RESULTS

A total of 1834 cases were reviewed (1582 from the University Veterinary Teaching Hospital and 252 from the Endocrinology Clinic). Of these, 592 (567 from the University Veterinary Teaching Hospital and 25 from the Endocrinology Clinic) were excluded because of insufficient data about age, CKD, or diabetes, or because they were receiving nephrotoxic drugs. Another 681 cats were excluded (1 with CKD and none with diabetes) because they were under 3 years old.

Of the final population of 561 cats, 67 (11.9%, 35 males, median age 11.4 [8.0-14.5] years) had CKD, and 16 (2.9%, 14 males, median age 11.5 [9.1-14.9] years) had diabetes. The proportion of cats with CKD and diabetes was 12.2% and 2.5% in the University Veterinary Teaching Hospital and 11.1% and 4.3% in the Endocrinology Clinic, respectively. Among cats with CKD, abnormal results of USG or UPC were registered in 21 cases, while diagnosis was exclusively based on serum creatinine in 45. Of these, 29 presented with moderate or severe azotemia, whereas 16 had only slightly increased serum creatinine concentrations (<2.8 mg/dL). No laboratory tests were available in 1 additional case, but the clinical record reported that the cat had been euthanized because of CKD. This information was confirmed by the owner, who also stated that no other diseases, including diabetes, had been reported by the veterinarian. Among the cats with diabetes, persistent hyperglycemia and increased fructosamine, or increased fructosamine were recorded in all cases.

Seven of the diabetic cats (44%), 4 from the University Veterinary Teaching Hospital and 3 from the Endocrinology Clinic, also met the criteria for the diagnosis of CKD and 6 of them (86%) were males. In

TABLE 1 Main laboratory results in cats with chronic kidney disease, diabetes, or both

	DM and no CKD N = 9	No DM and CKD N = 60	DM and CKD N = 7	P*
Glucose, mg/dL	510 (423-610)[a]	128 (100-156)[b]	503 (422-600)[a]	<.001
Creatinine, mg/dL	1.2 (0.9-1.4)[a]	4.1 (2.7-6.2)[b]	2.2 (2.0-2.3)[c]	<.001
Urea, mg/dL	64 (51-84)[a]	175 (104-274)[b]	116 (64-124)[a]	<.001

Data are medians (interquartile range). Different superscripts indicate significant differences ($P < .05$).
Abbreviations: CKD, chronic kidney disease; DM, diabetes mellitus.
*Kruskal-Wallis test.

TABLE 2 Characteristics of the cats according to the presence or absence of chronic kidney disease

	Total N = 561	Chronic kidney disease		P
		No N = 494	Yes N = 67	
Age, y	7.7 (4.7-11.6)	7.0 (4.5-11.0)	11.4 (8.0-14.5)	<.001
Mature (≥7 years)	314 (56.0)	257 (52.0)	57 (85.1)	<.001
Sex, male	279 (49.8)	244 (49.4)	35 (53.0)	.6
Breed				<.001
Domestic Shorthair	426 (80.7)	375 (81.3)	51 (76.1)	
Persian	40 (7.6)	40 (8.7)	0	
Mixed	24 (4.5)	15 (3.3)	9 (13.4)	
Siamese	16 (3.0)	12 (2.6)	4 (6.0)	
Others	22 (4.2)	19 (4.1)	3 (4.5)	
Diabetes mellitus	16 (2.9)	9 (1.8)	7 (10.4)	.001
Veterinary centers				.8
University Veterinary Teaching Hospital	444 (79.1)	390 (78.9)	54 (80.6)	
Endocrinology Clinic	117 (20.9)	104 (21.1)	13 (19.4)	

Qualitative variables are expressed in percentages.

5 of these 7 cats, plasma creatinine concentration was >2 mg/dL, and in the other 2, it was 1.8 mg/dL (1 with increased UPC ratio, and the other with low USG). In 6 of the 7 cats with CKD and diabetes, both diseases were diagnosed simultaneously, while in the other cat, CKD was detected 10 years after diabetes. In 5 of these cases, an additional risk factor that might have contributed to the worsening of renal function was identified (chronic periodontal disease in 2 cats, and pancreatitis, megacolon and primary hyperaldosteronism in 1 cat each).

Plasma creatinine and urea concentrations were higher in cats with CKD without diabetes than in cats with both diseases (Table 1). Table 2 shows the clinical and demographic characteristics of the study population, according to the presence or absence of CKD. Older age, lower weight, mixed breed, and diabetes were significantly associated with CKD. Due to the retrospective nature of the study, it was not possible to determine the breed of the progenitors of the mixed breed cats. In a multivariate logistic regression analysis including the variables that had shown a significant association with CKD in the univariate analysis, diabetes maintained a significant association with CKD (Table 3). To assess the robustness of these results, 2 sensitivity analyses were performed. First, given the limitations regarding the

TABLE 3 Results of multivariate logistic regression analysis adjusted for age for the factors associated with the diagnosis of feline chronic kidney disease shown in Table 1 for a population of 561 adult cats

	Coefficient (SE)	P	OR (95% CI)
(Intercept)	−3.672 (0.364)	<.001	–
Age, per year	0.157 (0.031)	<.001	1.171 (1.103-1.243)
Diabetes mellitus	1.627 (0.559)	.004	5.088 (1.702-15.205)
Mixed breed	1.753 (0.472)	<.001	5.773 (2.291-14.548)

Abbreviations: CI, confidence interval; OR, odds ratio; SE, standard error.

assessment of the origin of the mixed breed cats, a multivariate model including only age and diabetes was created. In this model, the association between diabetes and CKD remained significant (OR for diabetes, 4.47; 95% CI, 1.51-13.28; $P = .007$). Additionally, because the ISFM guidelines recommend the assessment of USG to establish the diagnosis of CKD, the analysis was repeated excluding 16 cats (2 with diabetes) with only slightly increased creatinine concentrations (<2.8 mg/dL), in which no USG or UPC ratio data were available, and excluding the 1 case without a laboratory test recorded. Also in this case, the association

between diabetes and CKD remained significant (OR, 4.63; 95% CI, 1.39-15.37; $P = .01$).

4 | DISCUSSION

In this study assessing 561 adult cats attending 2 veterinary centers, the frequency of CKD was higher among those with diabetes (44%) than among those without diabetes (11%). Multivariate analyses, adjusting for age and breed, showed a significant association between diabetes and CKD. Although these results were obtained from a small population of diabetic cats, they suggest that CKD could be a complication of diabetes in cats as in humans, in whom the prevalence of CKD is 20%-30% in diabetic populations.[19,20] Few studies have assessed the association between CKD and diabetes in cats. One of the strengths of the present study is the use of multivariate logistic regression analyses adjusting for age, because both diseases could arise concurrently in adulthood.

Given the retrospective nature of the study and that no specific procedures were performed to identify the etiology of CKD in diabetic cats, no direct causal relationship between diabetes and CKD can be inferred. The fact that cats with CKD without diabetes had higher concentrations of creatinine and urea might suggest that CKD is milder in cats that also have diabetes or that it is detected earlier and could be due to closer veterinary follow-up.

Our findings are at odds with those of previous studies which have suggested that there is no association between diabetes and CKD in cats. However, few studies have evaluated the presence of diabetes in large populations of cats with CKD. In 2 retrospective epidemiological studies, no relationship was found between diabetes and CKD.[21,22] In the first of these studies, analyzing a large sample, a prior diagnosis of diabetes was associated with a lower risk of CKD in a multivariate logistic regression analysis.[21] The authors stressed that this was an unexpected finding and could not suggest any explanation. The second epidemiological study was a case-control study of 92 cats with CKD and 92 age-matched controls. Although prior diagnosis of diabetes was more frequent in the group with CKD, the differences were not statistically (OR, 2; 95% CI, 0.37-11) significant.[22] Other authors have assessed this issue using biomarkers or renal histopathology. There were no significant differences in cystatin C concentrations between cats with diabetes and healthy cats; however, 39% of the cats with diabetes (and none of the healthy cats) had proteinuria.[10] Similarly, in 66 cats with diabetes and 11 healthy controls, there was a higher prevalence of proteinuria and microalbuminuria in the diabetic cats (70% vs 35% and 70% vs 39%, respectively).[23] Additionally, a postmortem study comparing diabetic cats with controls matched for age, sex, body weight, and breed did not find significant differences between groups.[11] On the basis of the concentrations of creatinine and urea obtained antemortem, 23.3% of cats with diabetes and 31.6% of controls were considered to have CKD.[11] The differences observed between humans and cats might be a consequence of the shorter life expectancy in cats and a shorter time of exposure to diabetes.[11] Renal lesions in people with diabetes are mainly characterized by a disorder of the glomeruli, with basal membrane thickening, increased mesangial matrix and mesangial nodules (Kimmelstiel-Wilson nodules).[24] Patterns of ultrastructural alterations occur in the kidneys of patients with earlier stages of CKD associated with diabetes which differ from those usually considered as characteristic of diabetic nephropathy. Indeed, glomerular lesions are present in only 30% of cases, while the rest of the patients present mainly tubulointerstitial lesions, if any.[24,25] Therefore, the histological lesions observed in cats might be more similar to those described in humans with a shorter exposure to diabetes.

In addition to diabetes, age and breed were also associated with CKD in our population. The prevalence of CKD increases with age, reaching 30%-40% in cats older than 10 years.[9] All the animals included in the present analysis were adult, and 85.1% of those with CKD were over the age of 7 years. The association between mixed breed and CKD is difficult to interpret, but it is possible that it might reflect a specific association conferred by 1 or more breeds overrepresented in mixed breed crosses but not identified in this study. There is an increased risk of CKD in Persian, Abyssinian, and Siamese cats.[9]

The present study has several limitations, deriving mainly from its retrospective nature. The cats seen at the 2 veterinary centers selected are not necessarily representative of the general population. Some cases had to be excluded due to missing data, and it is possible that others with subclinical forms of CKD, diabetes, or both were classified as healthy. Furthermore, the diagnosis of CKD could not be established precisely in some cases in which USG was not available. In this regard, however, the multivariate logistic regression analysis that excluded cases with milder azotemia in which urine was not available was consistent with the analysis performed in the total population. Finally, the diagnosis of mild CKD in cats with diabetes is complicated by the fact that glucosuria could reduce USG.[10] However, most of the cats with CKD and diabetes had serum creatinine concentrations >2 mg/dL, the cutoff value used in many studies to define azotemia in this species.[16,17,26]

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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ARTICLE III

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Animal Models and Renal Biomarkers of Diabetic Nephropathy

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Abstract

Diabetes mellitus (DM) is the first cause of end stage chronic kidney disease (CKD). Animal models of the disease can shed light on the pathogenesis of the diabetic nephropathy (DN) and novel and earlier biomarkers of the condition may help to improve diagnosis and prognosis. This review summarizes the most important features of animal models used in the study of DN and updates the most recent progress in biomarker research.

Keywords

Animal models · Chronic kidney disease · Creatinine · Cystatin C · Diabetes mellitus · Diabetic nephropathy · Early markers · Glomerular filtration rate · Kidney injury molecule-1 · Obesity · Symmetric dimethylarginine

1 Introduction

Diabetic nephropathy (DN) is a common complication of diabetes mellitus (DM) occurring in 20–40% of people with diabetes (Dronavalli et al. 2008). Although cardiovascular diseases are the first cause of death, approximately 10–20% of people with DM die because of kidney failure, and DM is considered the first cause of end stage chronic kidney disease (CKD) (World Health Organization (WHO) 2019). Improved diagnosis and treatment of renal disease has led to better prognosis (Andrésdóttir et al. 2014). Furthermore, detecting the disease at an earlier stage and building on our understanding of the mechanisms of the disease may help to improve diagnosis further.

Recently, many reports have proposed a wide number of markers of CKD, and it has been shown that many of them reflect damage of one specific part of the nephron (Colhoun and Marcovecchio 2018; Domingos et al. 2016; Kim et al. 2013; Kem et al. 2010; Carlsson et al. 2017;

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Colombo et al. 2019a). Albuminuria and microalbuminuria have traditionally been considered as markers of glomerular damage, and they have also been considered the first alterations that can be detected in DN (American Diabetes Association 2004). However, recent studies have shown that some patients with DM have CKD in the absence of microalbuminuria (MacIsaac et al. 2004; Lamacchia et al. 2018; Nauta et al. 2011; Zeni et al. 2017). In addition, the renal tubule could also play an important role in the development of DN (Colombo et al. 2019a). In fact, proteinuria mainly occurs after increased glomerular capillary permeability, but it is also a result of impaired reabsorption by the epithelial cells of the proximal tubule (D'Amico and Bazzi 2003). Thus, the use of tubular markers could also be beneficial for the diagnosis of DN. Indeed, the search for new renal biomarkers could lead to an earlier detection of renal damage. Likewise, animal models are important for improved understanding of the development and progression of DN. This review updates the most recent progress in biomarker research and summarizes the most important features of animal models used in the study of DN.

2 Brief Review of Kidney Anatomy and Physiology

The main functions of the kidney are filtration and excretion of metabolic waste products from the bloodstream, regulation of electrolytes, acidity and blood volume, and contribution to blood cell production (Rayner et al. 2016).

The nephron is the functional unit of the kidney. Each nephron is formed by a glomerulus, a proximal convoluted tubule, loop of Henle, and distal convoluted tubule. The last part of the nephron is the common collecting duct, and is shared by many nephrons (Rayner et al. 2016; National Institute of Diabetes and Digestive and Kidney Disaese (NIDDK) 2019).

The glomerulus is the filtering unit of the nephron. Within the glomerulus, the podocytes are specialized cells lining the outer surfaces of the bed of capillaries, which have interdigitated foot processes that play an important role in the process of filtration. In fact, podocyte damage leads to proteinuria (Pavenstädt 2000) Podocytes are part of the glomerular barrier, and protein barrier passage of a normal kidney is mainly composed by low molecular weight protein. However, after its structural integrity is affected, high molecular weight proteins can pass through the glomerulus (D'Amico and Bazzi 2003).

In regard to the anatomy of the glomerulus, it has two poles: (1) a vascular pole with the afferent and the efferent arterioles; and (2) a urinary pole with the exit to the proximal tubule. The blood is filtered in a specialized capillary network through the glomerular barrier, which yields the filtrated substances into Bowman's capsule space, and then into the renal tubules. The glomerular barrier is composed by five layers: (1) the inner layer is the glycocalyx covering the surface of the endothelial cells; (2) the fenestrated endothelium, (3) the glomerular basement membrane, (4) the slit diaphragm between the foot-processes of the podocytes; and (5) the sub-podocyte space between the slit diaphragm and the podocyte cell body (Rayner et al. 2016).

Another important structure for the process of filtration and for the regulation of blood pressure is the juxtaglomerular apparatus, which is adjacent to the glomerulus. This structure is formed by the macula densa (cells inside the cortical of the thick ascending limb of Henle), mesangial cells, and the terminal parts of the afferent arteriole that include renin-producing cells. Release of renin is stimulated by decreased sodium concentration in the macula densa, systemic volume loss or reduced blood pressure (Peti-Peterdi and Harris 2010; Castrop and Schießl 2014).

3 Pathophysiology of Diabetic Nephropathy

Hyperglycemia, hypertension and obesity are considered risk factors for DN (Mogensen et al. 1983; Câmara et al. 2017; Kanasaki et al. 2013). Therefore, DN is a heterogeneous syndrome that is common in people with type 1 (T1) and type 2 (T2) DM, although in patients with T2DM and

metabolic syndrome, more heterogeneous mechanisms are involved. DN has been classically considered a process with the following sequence of disorders: glomerular hyperfiltration, progression of albuminuria, decline of glomerular filtration rate (GFR) and, finally end stage renal disease (Mogensen et al. 1983); and different pathways that involve hemodynamic, metabolic and inflammatory factors play a role in its development (Table 1).

3.1 Hemodynamic Factors

Glomerular hyperfiltration is considered an alteration of early DN and it has been identified in 10–40% of people with early T1DM, and around 40% of patients with T2DM (Mogensen et al. 1983; Premaratne et al. 2015). However, its role as a leading cause of DN needs further research. In addition, the hyperfiltration mechanism is not well understood yet: it could be the result of a combination of hemodynamic, vasoactive, and tubular factors (Dronavalli et al. 2008; Zeni et al. 2017). Moreover, inhibition of tubuloglomerular feedback could be the main mechanism involved (Zeni et al. 2017). Persistent hyperglycemia produces tubular growth and increased tubular sodium reabsorption, and reduces the delivery of sodium to the macula densa, eliciting the release of renin and the activation of the renin-angiotensin system (RAS). This leads to an inhibition of the tubuloglomerular feedback, causing vasodilation of the afferent arteriole, which increases single-nephron glomerular filtration rate and consequently leads to hyperfiltration (Premaratne et al. 2015; Vallon and Thomson 2012). An implication of the sodium-glucose co-transporter 2 (SGLT2) in this process has been suggested. SGLT2s are expressed in the proximal tubule and their function is the reuptake of glucose and sodium (ratio 1:1), thus their activity is stimulated by the increased glucose filtration in diabetic subjects (Zeni et al. 2017; Premaratne et al. 2015; Hans-Joachim et al. 2016). Vasodilating substances, such as nitric oxide and cyclo-oxygenase 2 derived prostanoids, also take part in the hyperfiltration process (Wolf et al. 2005). Whether they play a key or secondary role in the pathogenesis of hyperfiltration is not well defined. This process, which is associated with increased intraglomerular pressure, could lead to podocyte stress and nephron loss (Hans-Joachim et al. 2016). Additionally, obesity has been considered as an independent risk factor for CKD, and hyperfiltration has also been detected in non-diabetic obese subjects. In addition, the kidney produces components of the RAS that specifically constrain the efferent rather than the afferent arteriole, increasing GFR and glomerular pressure (Yacoub and Campbell 2015). Several studies have demonstrated local production of RAS components in adipose tissue (Sharma and Engeli 2006; Giacchetti et al. 2002) and it has been suggested that RAS could be overactivated in patients with obesity (Sharma and Engeli 2006; Xu et al. 2017). Indeed, a decrease in RAS activity has been reported in obese women after weight loss (Engeli et al. 2005).

Table 1 Main factors involved in the development of diabetic nephropathy

Mechanisms involved in the pathogenesis of diabetic nephropathy	
Hemodynamic factors	Metabolic and inflammatory factors
Vasodilation of the afferent arteriole of the glomerulus	Increased formation of advanced glycation end-products
Increased glomerular filtration rate	Podocyte stress
Implication of RAS, nitric oxide and cyclo-oxygenase 2	TGF-β1 is a profibrotic cytokine that plays an essential role in inflammatory and fibrotic processes
Increased glomerular pressure	Obesity leads to increased leptin concentration, decreased adiponectin concentration, and increased cytokine production

Abbreviations: *RAS* renin angiotensin system, *TGF-β1* transforming growth factor β1

3.2 Metabolic and Inflammatory Factors

Formation of advanced glycation end-products (AGE), resulting from the reduction of sugars, is increased during chronic hyperglycemia. Accumulation of AGE appears to stimulate production of cytokines and renal fibrosis (Forbes and Cooper 2007). Infiltration by inflammatory cells (monocytes, macrophages and lymphocytes) precedes fibrosis, and these inflammatory cells are responsible for the production of reactive oxygen species, inflammatory cytokines and profibrotic cytokines (Kanasaki et al. 2013). Among the latter, transforming growth factor beta 1 (TGF-β1) plays an essential role in inflammation and fibrosis, and together with other cytokines such as connective tissue growth factor, platelet-derived growth factor and fibroblast growth factor 2; TGF-β1 is involved in fibroblast activation (Kanasaki et al. 2013). This produces an increased deposition of extracellular matrix in the interstitial space, as well as glomerular basement membrane thickening, which could lead to podocyte apoptosis and, as a consequence, to increased vascular permeability in the glomerulus (Wolf et al. 2005).

Additionally, in obese people, high leptin and low adiponectin concentrations result in an increased secretion of several adipokines (i.e. tumor necrosis factor-a, interleukin-6, interleukin-18) that promote an inflammatory state, also leading to extracellular matrix accumulation and renal fibrosis (Câmara et al. 2017; Straczkowski et al. 2007; Kern et al. 2001). In obese mice, reduced plasma adiponectin contributes to albuminuria and podocyte disfunction (Sharma et al. 2008).

3.3 Histologic Changes

The main histologic changes observed in kidneys of people with diabetes are located in the glomerulus, and include glomerular sclerosis (Kimmelstiel-Wilson nodules), basement membrane thickening and mesangial expansion. Tubulo-interstitial and arteriolar lesions have been commonly described as late lesions in patients with T1DM. Nevertheless, some patients with T2DM can show tubulo-interstitial and/or arteriolar lesions with preserved glomerular structure (Fioretto and Mauer 2007).

4 Animal Models

4.1 Rodents

4.1.1 Mouse Models

Rodents are the most studied animal models of human DN. They can show spontaneous or induced diabetes (Kachapati et al. 2012; Song et al. 2009), and DN might develop in the course of the disease, or by induction of renal damage by unilateral nephrectomy, or ischemia and reperfusion (Song et al. 2009; Kitada et al. 2016). However, an animal model that develops all of the features of DN is not available (Betz and Conway 2014). Based on the clinical characteristics of DN in humans, the Animal Models of Diabetic Complications Consortium (AMDCC) has published some criteria to define acceptable models of renal disease in mice with diabetes: (1) 50% decline in GFR; (2) 100 fold increase in proteinuria in comparison to matched controls of the same strain, age and gender; (3) presence of pathologic alterations such as mesangial sclerosis, arterial hyalinosis, 50% thickening of the glomerular basement membrane or tubulointerstitial fibrosis. Nonetheless, to date, no animal model fulfills all these criteria (Diabetes Complications Consortium (DiaComp) 2003).

T1DM rodent models include streptozotocin induced DM or spontaneous models due to genetic mutations, such as AKITA and OVE26 mice. T2DM genetic models are leptin deficient (ob/ob mice) or have inactivating mutations in the leptin receptor (db/db mice) (Kitada et al. 2016; Alpers and Hudkins 2011), but there are also models of induced T2DM, usually through a high fat diet (De Francesco et al. 2019; Ingvorsen et al. 2017). Hypertension plays an important role in the progression of human DN, and the deficiency of endothelial nitric oxide synthase (eNOS) through knockout of eNOS genes, has led to accelerated renal damage in db/db

and streptozotocin-treated mice. eNOS$^{-/-}$ C57BL/J^{db} mice can develop T2DM, obesity, hypertension, albuminuria, marked mesangial expansion and mesangiolysis (Nakagawa et al. 2007).

Recently, the black and tan, brachyury (BTBR obese) mouse strain, which is spontaneously insulin resistant, has gained importance in the field of the study of DN. This strain with ob/ob leptin deficiency mutation on BTBR mouse background, has been considered one of the best models of human DN because it rapidly develops pathological changes seen in human DN, such as increased glomerular basement membrane thickness, mesangial sclerosis, focal arteriolar hyalinosis, mesangiolysis, mild interstitial fibrosis and podocyte loss (Hudkins et al. 2010).

4.1.2 Rat Models

The most studied rat models are the Zucker diabetic fatty (ZDF-fa/fa) rat and the Wistar fatty rat (Kitada et al. 2016; Hoshi et al. 2002). Both have an autosomal recessive mutation in the fa gene that encodes the leptin receptor, and both are crossbred with the insulin resistant Wistar Kyoto rats. The ZDF-fa/fa and Wistar fatty rats can develop albuminuria and renal alterations, such as tubular cell damage and tubulointerstitial fibrosis, although nodular glomerular lesions or mesangiolysis have not been observed (Kitada et al. 2016; Hoshi et al. 2002). Another rat strain that is considered a model of DN is The Otsuka Long-Evans Tokushima Fatty (OLETF), in which multiple recessive genes are involved in the development of DM. These rats exhibit mild obesity, hyperinsulinemia with late onset of hyperglycaemia, and can present mesangial matrix expansion, glomerulosclerosis and tubular cell damage (Kawano et al. 1994, 1992).

4.2 Companion Animals

These animals have a great interest as animal models since they develop spontaneous DM and share the human environment (Brito-Casillas et al. 2016). However, it is unclear if dogs and cats with diabetes develop DN.

4.2.1 Cats

Around 80% of cats with DM have T2DM (Nelson and Reusch 2014). As in humans, obesity is a risk factor for DM, which also represents a problem of an epidemic proportion since approximately 35–50% of domestic cats are overweight or obese (Hoenig 2012). Obese cats also show some of the disorders observed in people with the metabolic syndrome; they can present insulin resistance and higher concentrations of very low-density lipoproteins (VLDL), triglycerides and cholesterol (Hoenig 2012; Jordan et al. 2008). Despite these alterations, hypertension has not been linked to feline obesity or DM, and atherosclerosis has not been described either (Jordan et al. 2008; Payne et al. 2017). Lower concentration of angiotensin-converting enzyme 2 has been found in the subcutaneous adipose tissue of overweight or obese cats compared to those with a low body condition score, but whether this could represents a negative mechanism against blood pressure is unknown (Riedel et al. 2006).

Additionally, the link between DM and renal disease is unclear in cats, and discrepancies can be found in the literature. In two retrospective, epidemiological studies, no relationship was found between both diseases (Greene et al. 2014; Barlett et al. 2010). In contrast, higher prevalences of proteinuria and microalbuminuria have been detected in cats with DM compared to age matched controls (Al-Ghazlat et al. 2011) and, recently, in a retrospective study of a population of 561 adult cats, age-adjusted multivariate regression analysis showed an association between both diseases (Pérez-López et al. 2019). Thus, the cat could be a useful model of DN, although prospective studies are still needed for a better understanding and evaluation of the association between feline DM and renal disease.

4.2.2 Dogs

Obesity in dogs is able to induce hyperinsulinemia, to increase blood pressure and to produce glomerular hyperfiltration. Dogs with obesity can develop structural changes in the kidney, such as glomerular basal membrane

thickening and mesangial expansion (Henegar et al. 2001). Insulin sensitivity decreases around 35% in obese dogs. However, obesity does not cause T2DM in these animals (Chandler et al. 2017). Indeed, T1DM is the most commonly recognized form of DM in dogs, although they can also develop DM secondarily to dioestrus, Cushing's syndrome or medications (Nelson and Reusch 2014). Regarding the relationship between DM and CKD, it has not been fully investigated in dogs. However, some studies have shown that markers of vascular resistance (ultrasound renal resistive index and pulsatility index), which are associated with progression of kidney disease and hypertension in humans, are also positively correlated with glycemic status in dogs (Priyanka et al. 2018; Novellas et al. 2010). In a case-control and age-matched study, dogs with alloxan-induced DM, uninephrectomized 4 weeks after the induction of DM, were proposed as a valuable model for the study of DN, since they developed greater glomerular basement membrane thickening and mesangial expansion, compared to controls, already 1 year after DM induction (Steffes et al. 1982).

4.3 Production Animals

Mainly two species could represent this group as animal models of DN: rabbits and swine. However, few studies have been reported on rabbits, and they have been mainly focused on the role of obesity in kidney function (Dwyer et al. 2000; Antic et al. 1999). One study showed an increased hyaluronean content in the renal medulla of rabbits with induced high-fat diet induced obesity (Dwyer et al. 2000). Higher renal medullary hyaluronean content has also been reported in humans and rodent models with DM or with renal alterations (Stridh et al. 2012). In regard to the swine, it is considered a more valuable animal model, since it closely resembles human anatomy and physiology (Zhang and Lerman 2016; Li et al. 2011; Rodríguez-Rodríguez et al. 2020; Spurlock and Gabler 2008). Metabolic syndrome can be induced through high fat diet in the Ossabaw and Iberian swine. These pigs exhibit obesity, insulin resistance, hypertension and dyslipidemia. Regarding renal disease, kidney hypertrophy, increased GFR, renal tubular fibrosis and renal adiposity have all been reported (Zhang and Lerman 2016; Li et al. 2011; Rodríguez-Rodríguez et al. 2020).

Further information in relation to animal models can be found in Table 2.

5 Assessment of Renal Function

Criteria to diagnose CKD are well established in human medicine. However, there are a large number of markers, many still under investigation, that could allow an early detection of impaired renal function. The assortment of markers also represents a variety of mechanisms involved in kidney injury, reflecting damage of different parts of the nephron as well (Fig. 1).

5.1 Routine Evaluation of DN and Gold Standard Methods

In humans, CKD is defined as abnormalities of kidney structure or function, present for more than 3 months, and with implications for health. Patients with altered kidney function present albuminuria and/or a decline in GFR (Levin et al. 2013).

Human DN is diagnosed when urinary albumin to creatinine ratio (ACR) is above 30 mg/g, although some guidelines recommend the use of different ACR thresholds for men (>25 mg/g) and women (>35 mg/g). Despite ACR being the main tool used to diagnose DN, some patients with either T1 or T2DM who have decreased GFR do not show elevated ACR (Gross et al. 2005; Caramori et al. 2003). Therefore, GFR should also be measured in patients with DM to rule out CKD. In fact, estimation of GFR is considered the routine method to evaluate renal function in patients with CKD. The most recent guidelines recommend the interpretation of both albuminuria and GFR for the diagnosis and staging of CKD (Levin et al. 2013).

Table 2 Animal models of diabetic nephropathy

	Animal models of induced diabetes with development of DN			Animal models of spontaneous diabetes with development of DN			Spontaneous animal model of diabetes and unclear development of DN		
	Animal model	Advantages	Disadvantages	Animal model	Advantages	Disadvantages	Animal model	Advantages	Disadvantages
Models of T1DM	Streptozotocin treated C57BL/6 mouse	The most studied strain	Less susceptibility to develop renal injury than other strains	AKITA mouse.	Early pathological changes of DN	Strain-dependent susceptibility	Diabetic dog	Gene-environment interaction	Development of DN is still unclear
		Easy to breed and long life span						Environmental factors shared with humans	
	Streptozotocin treated DBA/2 mouse	More susceptible to renal injury than C57BL/6 mouse	Tubulointerstitial fibrosis does not occur	OVE26 mouse	To study advanced DN	Poor viability			
Models of T2DM	C57BL/6 mouse on high fat diet	Develops metabolic syndrome (increased SBP and lipids)	Inter-individual phenotypic variability of the amount of food intake after receiving a high fat diet	eNOS $^{-/-}$/db/db mouse	Development of glomerular lesions characteristic of advanced DN	Few studies, possible difficulties with breeding	Diabetic cat	Similar mechanisms of human T2DM, with environmental factors and polygenic interactions	Development of DN is still unclear
		Renal injury could be due to renal lipid accumulation	Phenotype more pronounced in males						
	Zucker diabetic fatty (ZDF-fa/fa) rat	Development of tubulointerstitial fibrosis	Does not develop features of advanced DN	BTBR ob/ob mice	Rapidly resembles alterations of advanced human DN	Modest interstitial fibrosis			
	Ossabaw pig on high fat diet	Develops metabolic syndrome, increased GFR, renal tubular fibrosis, renal adiposity	Expensive and specialized husbandry						

Abbreviations: *IgG* immunoglobulin G; kidney injury molecule -1, *NGAl* neutrophil gelatinase-associated lipocalin, *SDMA* symmetric dimethylarginine, *VEGF* vascular endothelial growth factor, *RBP4* retinol binding protein 4, *suPAR* soluble urokinase type plasminogen activator receptor, *TGFβ1* transforming growth factor β-1

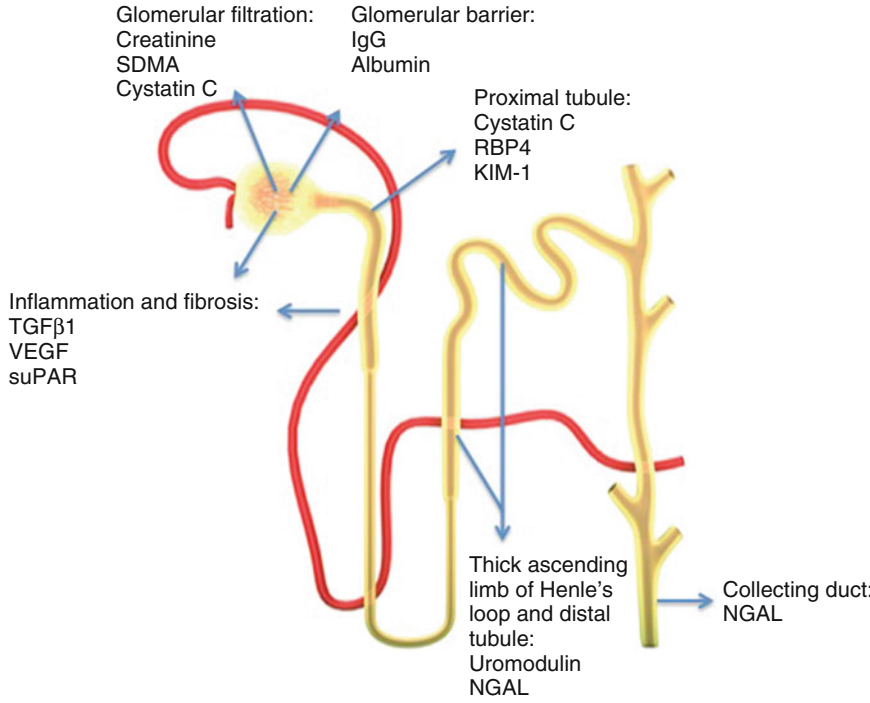

Fig. 1 Classification of markers of renal damage according to part of the nephron involved

The gold standard for assessing GFR is the plasma or urinary clearance of an exogenous filtration marker, such as inulin (Stevens and Levey 2009), Cr-EDTA, ^{125}I-iothalamate or iohexol. However, direct GFR measurement is difficult to perform and is time-consuming; it requires the injection of a suitable marker and several urine sample collections (Stevens and Levey 2009; Levey et al. 2003). Alternatively, equations for estimation of GFR, generally based on serum creatinine levels, are used in clinical practice, and their use is recommended in conjunction with other markers of renal function, such as cystatin C (Levin et al. 2013). However, early alterations of DN are usually associated with hyperfiltration, and these equations are less precise at higher values of GFR, and tend to underestimate GFR in the hyperfiltration state (Levin et al. 2013; Tuttle et al. 2014). Among available formulas, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) has been suggested to be the best one to evaluate early renal impairment in patients with DM, normoalbuminuria and hyperfiltration (Lovrenčić et al. 2012). Recent studies suggest that the CKD-EPI cystatin C based equations for estimation of GFR, are those that best fit the GFR measurement in patients with DM. However, in general, it is considered that GFR equations show high variability in people with DM (Cheuiche et al. 2019).

As stated above, the development of DN is unclear in companion animals, but diagnosis of CKD is based on serum creatinine or symmetric dimethylarginine (SDMA) concentration together with urinary protein-creatinine ratio (International Renal Interest Society (IRIS) 2015a, 2017), and there is still a lack of standardization in the methods of GFR measurement and their interpretation (International Renal Interest Society (IRIS) 2015b). Only one study proposed a method to estimate GFR in cats. It was based on serum creatinine and it was adjusted for a marker of muscle mass. However, this formula did not prove to be a reliable estimation of GFR (Finch et al. 2018).

5.2 Indirect Markers of Glomerular Filtration Rate

5.2.1 Serum Creatinine

Creatinine is a break-down product of creatine phosphate in muscle tissue (113 Daltons). It is not metabolized and is entirely cleared by the kidneys with minimal reabsorption by the renal tubules (Ferguson and Waikar 2012; Kavarikova 2018). Thus, blood creatinine concentration increases when GFR declines. However, in the early stage of CKD, subtle changes in GFR do not alter creatinine concentration. In humans, other markers seems to correlate better with GFR than creatinine (El-khoury et al. 2016) and, in dogs, its concentrations increase only when around 50% of renal function has been lost (Hokamp and Nabity 2016; Nabity et al. 2015). In addition, both in humans and companion animals, creatinine concentration depends on lean body mass, and it has high inter-individual variability (Delanaye et al. 2017; Hall et al. 2014a, 2015). Other factors, such as hydration and blood volume status, urinary obstructions or urinary infections, can also affect creatinine concentration (Hokamp and Nabity 2016; Blantz 1998).

5.2.2 Symmetric Dimethylarginine

SDMA is a catabolic product of arginine-methylated proteins (202 daltons), which is mainly excreted through the kidneys and is not reabsorbed or secreted by the tubules (Nabity et al. 2015; McDermott 1976). Thus, SDMA concentration is affected by GFR in inverse linear relationship in humans and in companion animals (Pelander et al. 2019; Hall et al. 2014b; Relford et al. 2016). Studies in companion animals have shown that, in contrast to creatinine, SDMA is not affected by muscle mass (International Renal Interest Society (IRIS) 2015a, 2017). On the other hand, human studies have found no correlation or an inverse correlation between SDMA and body mass index (Schepers et al. 2011; Schewedhelm et al. 2011; Potočnjak et al. 2018). Furthermore, discrepancies in the levels of SDMA in patients with DM have been reported among different studies. The offspring cohort study from the Framingham Heart Study did not find a relationship between SDMA and insulin resistance (Schewedhelm et al. 2011). Moreover, an inverse correlation between SDMA and glycosylated hemoglobin or fructosamine levels has been reported in T2DM patients, i.e. those with poor glycemic control had lower SDMA concentration (Can et al. 2011). In another study, SDMA was increased in patients with T2DM and microalbuminuria, and this marker predicted impaired renal function and cardiovascular disease in this population (Zobel et al. 2017). Additionally, SDMA has been positively associated with proteinuria and inversely associated with GFR in patients with CKD and T2DM, and SDMA to asymmetric dimethylarginine (ADMA) ratio was one of the strongest predictive markers of renal function decline (Looker et al. 2015). ADMA is another byproduct of the proteolytic breakdown of nuclear proteins. It is an inhibitor of NOS and is considered to play a role in endothelial dysfunction (Sibal et al. 2010).

Interestingly, another study showed that young patients with T1DM and microalbuminuria and high GFR, had lower SDMA concentrations compared to normoalbuminuric T1DM patients; and over time, microalbuminuric patients showed an increment in SDMA concentration, probably reflecting a decline in GFR (Marcovecchio et al. 2010). Therefore, the lower levels of SDMA observed in patients with DM might be explained by hyperfiltration occurring in the early stages of DN. However, this requires further investigation, as other explanations have been suggested (Zsuga et al. 2007; Closs et al. 1997; Simmons et al. 1996; Siroen et al. 2005; Nijveldt et al. 2003).

In cats with DM, lower levels of SDMA have also been observed. However, in this species, further research on the relationship between DM and CKD is still needed (Langhorn et al. 2018).

In relation to the inverse association observed between SDMA and GFR, one study in humans, comparing SDMA to the renal clearance of an exogenous molecule (^{125}I – sodium iothalamate), showed that SDMA had a stronger inverse correlation with GFR than creatinine (El-khoury et al. 2016). In contrast, in dogs, the inverse correlation

between SDMA and GFR determined through iohexol clearance was similar to the correlation observed between creatinine and GFR. However, SDMA was considered an earlier marker of GFR than creatinine, as SDMA was able to detect a decrease in renal function <20% on average, whereas creatinine increased when renal function was reduced by 50% (Hall et al. 2016). Similarly, in cats, the inverse correlation between SDMA and GFR measured by iohexol clearance was similar to the correlation observed between creatinine and GFR, but the sensitivity to detect impaired renal function was higher using SDMA (100 vs 17%) (Hall et al. 2014b). The established International Renal Interest Society guidelines consider that SDMA persistently >14 ng/ml is consistent with CKD (International Renal Interest Society (IRIS) 2015a). This cut-off (>14 ng/ml) of SDMA is able to detect a decrease of 24% from the median of GFR established for healthy cats, with a sensitivity of 91% (Hall et al. 2014b).

5.3 Markers of Glomerular Damage

5.3.1 Urine Albumin

Albumin is an intermediate molecular weight protein (69 KD) and its urinary concentration could increase with moderate disorders affecting the permeability of the glomerular barrier (D'Amico and Bazzi 2003). The initial alterations of the glomerular barrier usually involve a loss of restriction to passage of negatively charged proteins (especially albumin). Albuminuria can also occur when the tubular cells are damaged and tubular reabsorption is impaired, although intense albuminuria is usually glomerular in origin (Nauta et al. 2011; D'Amico and Bazzi 2003). The evaluation of a single albumin measurement is not recommended because it can vary during the day. Therefore, the measurement of urinary ACR in a random or first morning sample, or through a 24 h urine collection, with a measurement of creatinine, is advised (Basi et al. 2008). In addition, diabetic rodent models that develop albuminuria are considered particularly useful for the study of human DN (Diabetes Complications Consortium (DiaComp) 2003). In rats, microalbuminuria has been associated with reduced albumin reabsorption in the proximal tubule in early DN (Tojo et al. 2001). Some studies have used ACR to study renal function in dogs (Tvari-jonaviciute 2013). However, in clinical practice of small domestic animals, the protein creatinine ratio is used instead of albumin. Although clinical interpretation of albuminuria is not well established in dogs and cats, borderline protein-creatinine ratio between 0.2 and 0.5 mg/mg in dogs, and between 0.2 and 0.4 mg/mg in cats, are suggestive of microalbuminuria, and its monitorization is recommended (International Renal Interest Society (IRIS) 2017). It should also be highlighted that albuminuria is not specific for kidney function and it could appear in the presence of non-renal diseases (i.e., hyperadrenocorticism, urinary tract infections or neoplasms) (Kivarikova 2015).

5.3.2 Urine Immunoglobulin G

Immunoglobulin (IgG) is a high molecular weight protein (160 kDa) that is involved in antibody-mediated immunity. Due to its size, this protein cannot pass through an intact glomerular barrier. Therefore, urine detection of IgG reflects glomerular damage (D'Amico and Bazzi 2003). IgG has been associated with albuminuria in patients with DM (Carlsson et al. 2017), but few studies have investigated IgG in animals. In dogs, urine detection of IgG has been associated with X-linked hereditary nephropathy even before the onset of proteinuria. The authors hypothesized that in dogs some degree of alteration of the glomerular basement membrane could allow the passage of proteins not detected by the usual assays to measure proteinuria (Nabity et al. 2012). Similarly, in humans, IgG has been detected in normoalbuminuric patients with T2DM, and it has been considered a predictive marker of albuminuria (Narita et al. 2006).

5.4 Markers of Tubular Damage

5.4.1 Serum and Urine Cystatin C

Cystatin C is a low molecular weight protein (13 kDa) that is considered as a marker of both

GFR and proximal tubular damage. Cystatin C is freely filtered by the glomerulus, and it is almost entirely reabsorbed in the proximal tubule by megalin-mediated endocytosis (Mussap and Plebani 2004; Kaseda et al. 2007). Its inverse correlation with GFR is better than that observed between creatinine and GFR (El-khoury et al. 2016). In addition, studies in animal models have suggested that cystatin C could reflect impaired kidney function earlier than creatinine (Song et al. 2009; Togashi and Miyamoto 2013). For example, after ischaemia-reperfusion injury, partial unilateral nephrectomy and bilateral nephrectomy, serum cystatin C concentration increased before creatinine in BALB/c mice models (Song et al. 2009). Another report observed that, in comparison to Zucker diabetic lean rats, Zucker Diabetic Fatty rats showed elevations of urinary cystatin C concentration, along with other renal biomarkers of kidney injury (β2-microglobulin, clusterin, mu-glutathione S-transferase and kidney injury molecule-1 (KIM-1)), but not of serum creatinine, before the appearance of kidney histopathological changes (Togashi and Miyamoto 2013). Additionally, in this study, the authors observed that immunohistochemical cystatin C expression was predominantly localized in the proximal tubules of the renal cortex, supporting a role of tubular damage in the development of kidney injury due to obesity (Togashi and Miyamoto 2013).

Furthermore, some advantages of cystatin C as a marker of CKD compared to creatinine should be highlighted. In humans, it is less affected by muscle mass than creatinine and it has lower inter-individual variability (Stevens et al. 2009). However, it should be taken into account that the concentration of cystatin C is subjected to changes in people with thyroid disorders or glucocorticoid treatment (Risch and Huber 2002; Fricker et al. 2003).

In human medicine, clinical practice guidelines for the evaluation and management of CKD recommend the measurement of cystatin C, especially in those patients in whom estimated GFR, based on serum creatinine, might be expected to be less accurate, or in those patients with early stages of CKD (estimated GFR between 45–59 ml/min/1.73 m^2), who do not have other markers of kidney damage (Levin et al. 2013). Additionally, cystatin C might be a predictor of impaired renal function in patients with T2DM and, although its concentration can reach higher levels in macroalbuminuric patients, it is independently associated with GFR, and an increase in serum and urine cystatin C concentration has been observed in subjects with DM, normoalbuminuria and decreased GFR (Kim et al. 2013; Jeon et al. 2011). Thus, cystatin C might predict DN in the early stages of impaired renal function in patients with T2DM (Kim et al. 2013; Jeon et al. 2011).

In contrast, in veterinary medicine, the use of cystatin C does not seem so advantageous. In cats, serum cystatin C was not found to be a reliable marker of GFR, and in regard to urinary cystatin C, one study showed higher concentrations of this marker in cats with CKD compared to healthy cats, although, urinary Cystatin C was below the detection limit of the assay in some of the cats with CKD (Ghys et al. 2016; Williams and Archer 2016). In addition, one study reported lower cystatin C concentration in cats with DM compared to healthy cats (Paepe et al. 2015), although in this species it is not clear whether CKD could occur secondarily to DM (Greene et al. 2014; Barlett et al. 2010; Al-Ghazlat et al. 2011; Pérez-López et al. 2019; Zini et al. 2014).

In a similar manner, in dogs, serum cystatin C does not seem to be superior to creatinine, either, and the sensitivity of cystatin C to detect CKD has been considered similar to SDMA and creatinine, whereas its specificity was lower (Pelander et al. 2019; Almy et al. 2002; Marynissen et al. 2016).

5.4.2 Retinol-Binding Protein 4 (RBP4)

Retinol-binding protein is a low molecular weight protein (21 kDa) that acts as the transport protein for retinol in plasma. It is produced in the liver and circulates in plasma bound to transthyretin (TTR), a protein with a molecular weight of 54 kDa that is too large to pass through the glomerular barrier. However, around 4–5% of serum RBP4 circulates freely and can pass through this barrier, to be then reabsorbed by

tubular epithelial cells (Christensen et al. 1999). When tubular damage occurs, reabsorption of retinol is decreased, with subsequent loss of RBP4 into the urine (Zeni et al. 2017). Urinary RBP4 has been considered as a predictive marker of CKD in humans and of microalbuminuria in patients with T2DM (Domingos et al. 2016; Park et al. 2014). However, one study suggested that serum RBP4 does not seem to be a better marker than creatinine or cystatin C to detect GFR impairment (Donadio et al. 2001). On the other hand, serum retinol has also been considered a marker of insulin resistance and cardiovascular risk factors in humans and animal models (Park et al. 2014; Cabré et al. 2007; Mohapatra et al. 2011; Graham et al. 2006; Yang et al. 2005; Akbay et al. 2010), and it could be interesting for the study of early renal alterations in patients with metabolic syndrome. Mouse models have demonstrated that transgenic overexpression of human RBP4, or injection of recombinant RBP4, leads to insulin resistance (Yang et al. 2005), although a few reports disagree on this association (Von Eynatten et al. 2007; Henze et al. 2008).

In dogs and cats, higher urinary RBP4 concentration has been observed in animals with CKD compared to healthy animals (van Hoek et al. 2008; Chakar et al. 2017).

5.4.3 Uromodulin (Tamm-Horsfall Protein)

Uromodulin is a 100 kDa protein synthetized by the epithelial cells of the thick ascending limb of Henle's loop and the distal convoluted tubule (Hokamp and Nabity 2016). Therefore, in healthy individuals it is normal to find uromodulin in urine samples, whereas in patients with tubular damage its urinary concentration is low or even absent (Chakraborty et al. 2004; Steubl et al. 2016). Correlation between uromodulin and GFR has only been assessed through equations of estimated GFR, and conflicting results have been reported. Whereas urinary uromodulin is positively correlated to GFR, the correlation between GFR and serum uromodulin has been reported as positive or negative, depending on the study (Möllsten and Torffvit 2010; Prajczer et al. 2010; Fedak and Kuźniewski 2016; Wiromrat et al. 2019). Nonetheless, several reports have observed that plasma or serum uromodulin concentrations are lower in patients with nephropathy. Uromodulin could be an indicator of renal damage in people with and without DM (Chakraborty et al. 2004; Steubl et al. 2016; Möllsten and Torffvit 2010). Kidneys of patients with very low levels of serum and urinary uromodulin show more tubular atrophy and decreased concentration is present at the earliest stages of CKD (Prajczer et al. 2010). However, in dogs, uromodulin has been considered a progression marker rather than an early marker of CKD (Chakar et al. 2017).

Additionally, studies in uromodulin knockout mice showed that it has a protective role against urinary tract infections and renal stone formation (Rampoldi et al. 2011; Bates et al. 2004; Liu et al. 2010). However, in humans, mutations in the gene encoding uromodulin lead to a rare autosomal dominant disease, which causes tubulointerstitial damage, but these patients do not show increased rates of urinary tract infections or renal stone formation (Rampoldi et al. 2011).

5.4.4 Neutrophil Gelatinase-Associated Lipocalin (NGAL)

NGAL is a low molecular weight protein of 25 kDa belonging to the lipocalin protein superfamily that is produced in the renal tubules (thick ascending limb of Henle's loop, distal tubule, and collecting duct) after inflammation or tissue injury (Singer et al. 2013). Its concentration may also be increased in case of impaired proximal tubular reabsorption (Singer et al. 2013).

In patients with DM, urinary NGAL shows an inverse correlation with GFR and a positive correlation with albuminuria (Kem et al. 2010; Fu et al. 2012a; Nielsen et al. 2011; Vijay et al. 2018). In addition, NGAL concentrations are higher in normoalbuminuric patients with DM, compared to non-diabetic control subjects, which might suggest that tubular damage could be one of the earliest alterations in patients with DN (Nauta et al. 2011; Vijay et al. 2018). Moreover, higher levels of urinary NAGL have been observed in T2DM diabetic patients with

glomerular hyperfiltration compared to T2DM with normal GFR and control subjects (Fu et al. 2012b).

In addition, NGAL has been proposed as a useful indicator of acute kidney injury in human medicine (Singer et al. 2013; Haase et al. 2011) and most studies in animal models focus on its utility to detect acute kidney injury. In dogs, plasma and urinary NGAL are able to distinguish dogs with CKD from those with acute kidney injury (Steinbach et al. 2014). In contrast, in cats with CKD, this marker did not increase until the cats reached an advanced stage of the disease (Wang et al. 2017). Additionally, it should be highlighted that NGAL concentration could be influenced by other conditions, such as urinary tract infections, different types of neoplasms, pre-eclampsia and obstructive pulmonary disease (Giasson et al. 2011; Fjaertoft et al. 2005; Keatings and Barnes 1997; Bolignano et al. 2010).

5.4.5 Kidney Injury Molecule -1 (KIM-1)

KIM-1 is a type 1 transmembrane glycoprotein (90 kDa) located in the proximal tubules and, after tubular injury, its concentration rises before serum creatinine, whereas it is not detected in the urine of humans or other species without kidney damage (Moresco et al. 2018). KIM-1 is a well-known marker of acute kidney injury, and some studies suggest that it might be a good marker for CKD since its concentrations are high in humans with low GFR or albuminuria. Additionally, in humans with T2DM and normoalbuminuria or mild albuminuria, an increment of the urinary concentration of this marker has been observed (De Carvalho et al. 2016). Its ability to predict DN requires further investigation since discrepancies have been observed (Colombo et al. 2019a; Nauta et al. 2011). Two longitudinal studies showed that, the use of KIM-1 in T2DM, together with pro b-type natriuretic peptide or beta 2 microglobulin, GFR and albuminuria, seemed to improve prediction of kidney function decline (Kammer et al. 2019; Colombo et al. 2019b). In contrast, another study reported that KIM-1 was not associated with albuminuria (Nauta et al. 2011), and, in a longitudinal, multicenter study in T1DM, it did not improve the prediction of progression of DN compared to albuminuria and estimated GFR, either (Panduru et al. 2015). In another large study, from an extensive set of biomarkers, KIM-1 and CD27 antigen combined, were the most important predictors of DN progression, although their predictive power did not improve that of historical estimated GFR and albuminuria. (Colombo et al. 2019a). It should also be highlighted that other conditions such as sepsis and urinary tract disease can increase urinary KIM-1 (Moresco et al. 2018).

In animal models, higher concentrations of this marker have been observed in a rat model of T2DM (*Otsuka Long-Evans Tokushima Fatty rats*) than in healthy rats (*Otsuka Long-Evans Tokushima rats*), and an increase of its concentration was observed prior to the development of hyperfiltration and prior to the increment of serum creatinine concentration (Hosohata et al. 2014).

5.5 Markers of Fibrosis and Inflammation

5.5.1 Transforming Growth Factor-β1 (TGFβ1)

TGFβ1 is a cytokine and pro-fibrotic mediator of kidney damage. It is secreted in an inactivated form associated with the large latent complex (LLC) and it has a biological effect only after it is liberated from the LLC as active TGFβ1, a process which takes part in the extracellular matrix (August and Suthanthiran 2003; Lawson et al. 2016; Sureshbabu et al. 2016; Hinz 2015). In kidneys, increased production of active TGFβ1 leads to interstitial fibrosis, mesangial matrix expansion and glomerular membrane thickening, and, as a consequence, kidney damage and reduction in GFR (August and Suthanthiran 2003; Sureshbabu et al. 2016; Ziyadeh 2004). Animal models have shown that TGFβ1 could also participate in podocyte detachment or apoptosis, stimulating podocyte expression of VEGF that

acts in an autocrine loop, leading to increased production of 3(IV) collagen, which probably contributes to the thickening of the glomerular basement membrane (Chen et al. 2004). According to an *in vitro* study, TGFβ1 could also cause oxidative stress in podocytes by itself (Lee et al. 2003). As a consequence of podocyte injury, proteinuria can occur (Nagata 2016).

In vitro studies have also demonstrated that high glucose concentration stimulates TGFβ1 secretion and activation, and this marker has been proposed as an important mediator of DN in animal models (Ziyadeh 2004; Rocco et al. 1992; Hoffman et al. 1998). Also, in humans, high levels of serum and urinary TGFβ1 have been observed in a systematic review that included T2DM patients, showing a positive correlation with albuminuria (Qiao et al. 2017). Treatment with angiotensin-converting enzyme inhibitors has been demonstrated to decrease urinary ACR and plasma TGFβ1, and the latter has been put forward as a mechanism mediating their nephroprotective effects (Andrésdóttir et al. 2014). Moreover, in the db/db mouse, treatment with a neutralizing anti-TGFβ1 antibody avoids the progression of diabetic renal hypertrophy, mesangial matrix expansion, and the development of renal insufficiency, albeit in the absence of a significant reduction in albuminuria (Ziyadeh et al. 2000).

In companion animals, this marker has been studied to investigate DN in cats, where an increment of urinary activated TGFβ1:creatinine ratio precedes the onset of azotemia by 6 months (Lawson et al. 2016).

5.5.2 Vascular Endothelial Growth Factor (VEGF)

VEGF, also named vasopermeability factor, is a homodimeric glycoprotein with different isoforms and heparin-binding properties. VEGF promotes permeability, has mitogenic functions in endothelial cells and is an important angiogenic factor (Khamaisi et al. 2003; Neufeld et al. 1999). In the kidney, reduction of oxygen delivery contributes to inflammation pathways and is the main stimulus for VEGF expression. (Mayer 2011; Ramakrishnan et al. 2014).

In regard to DN, although *in vitro* studies have demonstrated that chronic hyperglycemia is able to increase the production of the VEGF protein and VEGF mRNA expression (Cha et al. 2000; Williams et al. 1997), in humans with DM it is uncertain whether VEGF levels increase or decrease with DN. Down-regulation of VEGF-A mRNA expression has been observed in renal biopsies of patients with DM, and its lower expression was associated with podocyte loss (Baelde et al. 2007). However, two other studies have shown higher concentrations of plasma and urinary VEGF in people with T1DM and T2DM, respectively (Hovind et al. 2000; Kim et al. 2004), and higher urinary VEGF was found in those with advanced DN. Indeed, intervention studies also show conflicting results: both VEGF inhibition and VEGF administration improve kidney function (Schrijvers et al. 2005; Kang et al. 2001).

Additionally, there is scarce information on the role of VEGF in kidney function in companion animals, in which the study of VEGF has been focused on its role in tumor angiogenesis (Millanta et al. 2002; Clifford et al. 2001; Platt et al. 2006). One study detected lower concentrations of urinary VEGF-A creatinine ratio in cats with CKD compared to healthy cats (Habenicht et al. 2013).

5.5.3 Soluble Urokinase Type Plasminogen Activator Receptor (suPAR)

Soluble urokinase-type plasminogen activator receptor (suPAR) is the circulating form of membrane protein urokinase receptor (uPAR), which is a glycosyl-phosphatidylinositol–anchored three-domain membrane protein that is expressed on podocytes and other cells (immunologically active cells and endothelial cells). Both suPAR and uPAR regulate cell adhesion and migration (Salim et al. 2016). Increased levels of plasma or serum suPAR are considered independent risk factors of cardiovascular diseases and CKD. suPAR seems to be a reliable marker of early

CKD since its concentration increases before a decline in GFR is identified (Salim et al. 2016). A cohort study showed that, in patients with T1DM, suPAR predicts cardiovascular events and a decline in GFR, although it was not correlated with albuminuria (Curovic et al. 2019). In contrast, a cross-sectional study showed a positive correlation between suPAR concentration and albuminuria in patients with T1DM (Theilade et al. 2015). Likewise, in patients with T2DM, suPAR concentration showed a positive correlation with albuminuria. Indeed, it was associated with an increased risk of new-onset microalbuminuria in subjects at risk for T2DM (Guthoff et al. 2017). In transgenic mice models, it has also been shown that increased uPAR activity in podocytes leads to proteinuria (Wei et al. 2008).

5.6 Panels of Candidate Biomarkers, Proteomic and Metabolomic Approaches

"Omics" approaches have been used to search for novel biomarkers and different aspects of the pathophysiology of kidney damage (Colhoun and Marcovecchio 2018; Carlsson et al. 2017; Abbiss et al. 2019; Darshi et al. 2016). Each individual biomarker of kidney disease represents a specific pathway. Depending on these pathways, some renal markers can be correlated with others. Therefore, the selection of panels of biomarkers that have low correlation with each other could potentially be beneficial for the prediction of impaired kidney function (Colhoun and Marcovecchio 2018). For example, a set of 297 biomarkers was evaluated in a recent prospective study in two different cohorts of patients with T1DM, and only two biomarkers, CD27, a member of the tumor necrosis factor receptor superfamily, and KIM-1 were considered to give predictive information of DN. This yielded similar predictive power than using historical estimated GFR and albuminuria (Colombo et al. 2019a). Similarly, another study examined 42 biomarkers in 840 serum samples of patients with T2DM and found that prediction of the decline in renal function could be improved by the use of two single markers: KIM-1 and β2 microglobulin (Colombo et al. 2019b). In contrast, in one study in patients with T1DM, KIM-1 did not seem to predict progression of CKD independently of albuminuria (Panduru et al. 2015) (Table 3).

6 Conclusions

DN is a common complication in patients with DM and research in animal models can be useful to fully understand the mechanisms underlying its development and progression. However, since various renal markers are not equally useful in all species, further studies are required in humans. The differences and similarities between people and animals with DN could also bring the opportunity to investigate new treatments for DN in humans. Although rodent models are the most studied, other animals, such as dogs and cats, could provide important information, since they share the human environment.

Regarding the potential use of markers of early kidney damage, cystatin C in humans, and SDMA in companion animals, have already been incorporated to the guidelines for the Evaluation and Management of CKD and of the International Renal Interest Society, respectively. Nonetheless, many other markers have been proposed, but results are conflicting for most of them. In fact, those biomarkers that have been identified as predictors of CKD or its progression, do not seem to add to established diagnostic tools. It is also important to highlight that most studies performed to date are cross-sectional in their design. More prospective and intervention studies are needed to replicate reported findings and to assess the predictive value of novel biomarkers of DN. Indeed, large consortia such as the SysKid (Systems Biology Towards Novel Chronic

Table 3 Epidemiological studies that provide information for the diagnosis of kidney disease or DN in both humans and animal models (histological, immunochemistry or intervention studies were not included)

Marker	Species	Author (reference)	N	Study design	Main results	Potential clinical application
Plasma SDMA and cystatin C	Humans	El-khoury et al. (2016)	40 patients who had clinical indication for measuring GFR	Cross-sectional	SDMA and cystatin C are highly and inversely correlated with GFR, more than creatinine	SDMA and cystatin C could detect an earlier decline in GFR
Plasma SDMA and ADMA	Humans	Zobel et al. (2017)	200 patients with T2DM	Longitudinal	Higher SDMA was associated with incident cardiovascular disease, and deterioration in renal function	SDMA could be a marker of DN and cardiovascular disease in patients with T2DM
Serum SDMA	Dogs	Hall et al. (2016)	19 dogs with CKD and 20 control dogs	Retrospective	SDMA detected CKD earlier than creatinine	SDMA should also be used to evaluate kidney function in dogs
Serum SDMA	Cats	Hall et al. (2014)	21 cats with CKD and 21 healthy control cats	Retrospective	SDMA detects CKD earlier than creatinine	SDMA should also be used to evaluate kidney function in cats
Serum SDMA	Cats	Langhorn et al. (2018)	17 with CKD, 40 with HCM, 17 with DM, and 20 healthy controls	Cross-sectional	Cats with DM had significantly lower SDMA concentrations than controls	SDMA is probabli not a useful marker of DN in cats; and further research about DN is still needed in cats
Serum cystatin C	BALB/c mice	Song et al. (2009)	23 partial nephrectomy 6 ischaemia reperfusion injury model 8 controls	Cross-sectional	Cystatin C increases before creatinine in mice with kidney damage. CysC levels show an earlier and sharper increase than creatinine after bilateral nephrectomy	Cystatin C could be a more precise marker compared to creatinine
Urinary cystatin C	Humans	Jeon et al. (2011)	335 T2DM patients with normoalbuminuria (n = 210), those with microalbuminuria (n = 83) and those with macroalbuminuria (n = 42)	Retrospective	Cystatin C was independently associated with GFR, and was increased in people with diabetes, normoalbuminuria and decreased GFR	Might predict early stages of DN in T2DM patients
Serum and urinary Cystatin C	Cats	Ghys et al. (2016)	49 cats with CKD and 41 healthy cats	Cross-sectional	Sensitivity and specificity to detect CKD were 22 and 100% for Cystatin C and 83 and 93% for creatinine	Cystatin C should not be used to evaluated kidney function in cats

Serum SDMA and Cystatin C	Dogs	Pelander et al. (2019)	30 healthy dogs and 67 dogs with diagnosis or suspicion of CKD	Cross-sectional	Creatinine and SDMA were similar to detect reduced GFR, whereas cystatin C was inferior	SDMA should be measured together with creatinine as it might add information of kidney function in dogs
Urinary Cystatin C- creatinine ratio and nonalbumin protein – creatinine ratio	Humans	Kim et al. (2013)	237 T2DM patients	Longitudinal	After adjusting for several clinical factors, both urinary Cystatin C- creatinine ratio and non albuminuric protein – creatinine ratio had significant associations with the decline of the estimated glomerular filtration rate (eGFR)	Cystatin C could be a useful marker of renal function decline in patients with T2DM
β2-microglobulin, calbindin, clusterin, EGF, GST-α, GST-μ, KIM-1, NGAL, osteopontin, TIMP-1, and VEGF	Zucker diabetic fatty rats	Togashi and Miyamoto (2013)	5 Male Zucker diabetic fatty rats (ZDF/CrlCrlj-Leptfa/fa) and 5 Male nondiabetic lean rats (ZDF/CrlCrlj-Lept?/+	Cross-sectional	Urinary levels of cystatin C, β2-microglobulin, clusterin, GST-μ, KIM-1 were increased before the development of histophatological changes consistent with DN	Cystatin C, β2-microglobulin, clusterin, GST-μ, KIM-1 could be used as markers of DN in mice models of DN
Urinary RBP4	Humans	Domingos et al. (2016)	454 participants with stages 3 and 4 CKD	Cross-sectional	A logistic regression model showed an inverse association between CKD-EPI eGFR and urinary retinol binding protein	Urinary retinol binding protein might be a promising marker of chronic kidney disease progression
Urinary RBP4	Humans	Park et al. (2014)	471 type 2 diabetes patients, 143 with impaired glucose tolerance and 75 controls	Cross-sectional	Urinary RBP4 concentration was higher in insulin resistant patients, and it was highly associated with microalbuminuria (odds ratio 2.6, 95% CI 1.6–4.2),	Kidney function of diabetic patients with higher levels of urinary RBP4 should be evaluated closely, and it could be useful in the management and stratifications of insulin resistant patients. Further investigation is needed
Serum RBP4, CysC, b2M	Humans	Donadio et al. (2001)	110 patients with various kidney diseases	Cross-sectional	Serum concentrations of CysC, b2M and RBP4 increase with the reduction of GFR ROC analysis showed that diagnostic accuracy of CysC and b2M was similar to	Cys, b2M and RBP4 do not seems more reliable markers to detect a decline in kidney function compared to creatinine

(continued)

Table 3 (continued)

Marker	Species	Author (reference)	N	Study design	Main results	Potential clinical application
					creatinine and better than RBP4	
Plasma RBP4	Humans	Cabré et al. (2007)	165 T2DM patients	Cross-sectional	Patients with moderate renal dysfunction (MDRD-GFR <60 mL min^{-1} 1.73 m^{-2} had higher plasma RBP4 than those with normal renal function	RBP4 might predict early decline in GFR in patients with DN
					Albuminuria was not associated with RBP4	
Serum RBP4	Humans	Akbay et al. (2010)	53 T2DM patients and 30 controls	Cross-sectional	Logistic regression analysis showed that microalbuminuria is associated with increased serum RBP4 concentration	RBP4 might predict early DN
Serum RBP4	Cats	van Hoek et al. (2008)	10 cats with CKD, 10 cats with hyperthyroidism and 10 healthy cats	Cross-sectional	Cats with CKD and hyperthyroidism had higher concentration of RBP4 than healthy cats	RBP4 should be investigated as a marker of impaired kidney function in cats
Urinary and serum uromodulin	Humans	Prajczer et al. (2010)	77 patients with CKD and 14 healthy subjects	Cross-sectional	Urinary uromodulin was positively correlated with GFR and negatively correlated with serum creatinine. Patients with lower uromodulin values showed higher degree of tubular atrophy (assessed through biopsy)	Urinary uromodulin might be a early marker of tubular damage
Serum uromodulin	Humans	Wiromrat et al. (2019)	179 T1DM adolescents patients and 61 control subjects	Cross-sectional	Lower levels of serum uromodulin are associated with albumin excretion	Serum uromodulin should be assessed as a marker of DN
Urinary uromodulin	Humans	Möllsten and Torffvit (2010)	301 patients with T1DM, 164 with normoalbuminuria, 91 with microalbuminuria and 46 with macroalbuminuria	Cross-sectional	Patients with albuminuria had lower uromodulin concentrations	Urinary uromodulin might reflect tubular damage in patients with T1DM

Serum uromodulin	Humans	Fedak and Kuźniewski (2016)	170 patients with CKD and 30 healthy subjects	Cross-sectional	Serum uromodulin was inversely correlated with other renal markers and positively correlated with estimated GFR	Serum uromodulin might be assessed as an early marker of CKD
Urinary albumin excretion ratio (AER), N-acetyl-β-D-glucosaminidase, and the advanced glycosylation end-products (AGEs) pentosidine and AGE-fluorescence	Humans	Kem et al. (2010)	55 T1DM patietns with and 110 without macroalbuminuria 91 T1DM patients with and 178 without microalbuminuria	Retrospective case-control	N-acetyl-β-D-glucosaminidase independently is associated with both macroalbuminuria and microalbuminuria. Other markers did not independently predict macro or microalbuminuria.	Tubular damage occurs in patients with T1DM and measurement of urinary albumin excretion ratio and urinary N-acetyl-β-D-glucosaminidase might predict early impaired kidney function
Plasma, urinary NGAL, and urinary NGAL-to-creatinine ratio (UNCR)	Cats	Wang et al. (2017)	80 cats with CKD and 18 healthy cats	Longitudinal	NGAL values were statistically different between healthy cats and cats with stage 3 or 4 CKD, however, no statistical differents were found between healthy cats and cats with those with stage 2 CKD	Plasma NGAL cannot distinguish CKD in cats Urinary NGAL does not detect early stages of CKD in cats
Plasma NGAL and UNCR	Dogs	Steinbach et al. (2014)	17 dogs with CKD 48 dogs with AKI and 18 controls subjects	Cross-sectional	Plasma NGAL concentration and UNCR was significantly higher in dogs with AKI or CKD compared to healthy dogs. In addition, these markers were higher in dogs with AKI compared with dogs with CKD	Although NGAL is an established marker for AKI, it might also be useful to distinguish dogs with CKD from healthy dogs, although further research is needed
Urinary NAGL, NAG and KIM-1	Human	Fu et al. (2012b)	101 T2DM patients 28 control subjects	Cross-sectional	All marker showed higher levels in patients with DM. NGAL and NAG were positively correlated with albuminuria. NGAL showed significant differences between micro and macroalbuminuric patients KIM-1 was not associated with albuminuria	NAGL and KIM-1 could be early markers of DN. Those patients with glomerular hyperfiltration and higher levels of urinary NAGL or KIM-1, should be closely monitored

(continued)

Table 3 (continued)

Marker	Species	Author (reference)	N	Study design	Main results	Potential clinical application
Urinary KIM-1 and NGAL	Humans	de Carvalho et al. (2016)	117 T2DM patients	Cross-sectional	Both markers were observed in patients with T2DM with normal or mild albuminuria, and they were independently associated with albuminuria	Tubular markers could help in the early detection of DN
Urinary KIM-1, NGAL and vanin-1	Rats	Hosohata et al. (2014)	8 male spontaneous type 2 diabetic OLETF rats and 8 male non-diabetic Long-Evans	Cross-sectional	Urinary KIM-1 was more sensitive than albumin to detect DN	KIM-1 detect early tubular damage
			Tokushima Otsuka (LETO) rats			
Urinary KIM-1	Humans	Panduru et al. (2015)	1573 T1DM patients	Longitudinal multicenter study	Mendelian randomization (MR) approach suggested a causal link between increased urinary KIM-1 and decreased GFR. KIM-1 did not predict progression of albuminuria	Further studies to evaluate KIM-1 as an early marker of DN could be interesting
Urinary active TGFβ1: creatinine ratio	Cats	Lawson et al. (2016)	6 non-azotaemic cats that developed azotaemia within 24 months; 6 cats and with renal azotaemia at baseline; and 6 non-azotemic cats	Longitudinal	Increased active TGFβ1: creatinine ratio was observed 6 month before the development of azotemia	Urinary active TGFβ1: creatinine ratio might be an early marker of CKD in cats, this marker should be assessed in a larger sample.
Serum and urinary TGFβ1	Humans	Qiao et al. (2017)	63 case-control studies (364 T2DM patients, 1604 T2DM and DN patients, and 2100 healthy controls)	Systematic review	TGFβ1 levels were higher in T2DM patients and were positively correlated with albuminuria	TGFβ1 could be a promising marker of DN, although future research is needed
Plasma uromodulin and cystatin C	Humans	Steubl et al. (2016)	426 individuals of whom 71 healthy subjects and 355 had CKD (stages I-V)	Cross-sectional	Multiple linear regression modeling showed significant association between uromodulin and eGFR (coefficient estimate b.0.696, 95% confidence interval [CI] 0.603–0.719, $P < 0.001$)	Uromodulin could be an earlier marker of CKD compared to creatinine and CysC
					Uromodulin was able to differentiate between patients in stage 0 and I	

					Neither cystatin C nor creatinine distinguished between stages 0 and I	
Urinary Ig G, KIM-1, NAGL, NAG	Humans	Nauta et al. (2011)	94 T1DM and T2DM, and 45 control subjects	Cross-sectional	Glomerular and tubular markers were associated with albuminuria independently of GFR (except KIM-1)	Markers of glomerular and tubular damage should be evaluated in patients with DN
Urinary RBP4,b2-microglobulin, NAGL, NAG, and IgG creatinine ratios	Dogs	Nabity et al. (2015)	20–25 dogs with X-linked hereditary nephropathy and 10–19 dogs control subjects	Retrospective	Urinary RBP4 was the most strongly correlated with serum creatinine and GFR. Although logistic regression analysis showed serum creatinine, uIgG/c, and uB2M, but not uRBP4/c, as significant independent predictors of GFR	b2-microglobulin, NAGL/c, NAG/c, IgG/ could allow early detection of CKD, and RBP4/c is a marker of CKD progression in dogs
Urinary albumin vitamin D-binding protein, RBP4, uromodulin,	Dogs	Chakar et al. (2017)	40 dogs with CKD and 9 control subjects	Cross-sectional	Increased vitamin D-binding protein and RBP4 were detected in early stages of CKD with or without albuminuria	Vitamin D binding protein and RBP4 should be studied as an early marker of CKD, whereas uromodulin could be a marker of progression of CKD in dogs
					Dogs with CKD had undetectable or lower RBP4 than controls, although among dogs in early stages of CKD there were no differences	
Plasma and urinary VEGF	Humans	Kim et al. (2004)	147 patients with T2DM and 47 healthy controls	Cross-sectional	VEGF concentration was higher in T2DM patients and was associated with albuminuria	VEGF might be a useful marker of DN in patients with T2DM
Plasma VEGF	Humans	Hovind et al. (2000)	199 patients with T1DM and DN, and 188 patients with T1DM and normoalbuminuria	Cross-sectional	Men with DN had higher concentration of VEGF than normoalbuminuric patients	Sex differences might affect VEGF concentration
Urinary cytokine (IL-8, MCP-1. TGF- β1, VEGF): urine creatinine ratios	Cats	Habenicht et al. (2013)	26 cats with CKD and 18 healthy cats	Cross-sectional	Cats with CKD had a significantly lower urinary levels of VEGF and higher urinary levels of IL-8 and	Further research is needed to evaluate the utility of measure urinary cytokines in cats with CKD, but it markers might

(continued)

Table 3 (continued)

Marker	Species	Author (reference)	N	Study design	Main results	Potential clinical application
					TGF-β1 compared to healthy cats	reflect kidney inflammaniton and fibrosis
Plasma suPar	Humans	Salim et al. (2016)	2292 patients from Emory cardiovascular biobank whose renal function was sequentially evaluated	Longitudinal	suPAR concentration increased before a decline in estimated GFR was observed	Kidney function of patients with elevated suPAR should be monitored closely
Plasma suPar	Humans	Curovic et al. (2019)	667 patients with T1DM and different levels of albuminuria	Longitudinal	suPAR predicted cardiovascular events and a decline in GFR but was not associated with albuminuria	suPAR is useful to detect early DN
Plasma suPAR	Humans	Theilade et al. (2015)	667 patients with T1DM and 51 control subjects	Cross-sectional	suPAR levels were higher in patients with cardiovascular disease and in patients with albuminuria. Multivariate logistic regression analysis showed an association between suPAR and albuminuria in patients with DM	suPAR and its relations with albuminuria should be investigated
Plasma suPAR	Humans	Guthoff et al. (2017)	258 patients at risk of T2DM	Longitudinal	Higher suPAR levels are associated with an increased risk of new-onset microalbuminuria in subjects at risk for type 2 diabetes	suPAR might be a useful marker of early DN in T2DM patients
Panel of 42 serum biomarkers	Humans	Colombo et al. (2019b)	840 patients with T2DM	Longitudinal multicenter study	Kim-1 and β2-microglobulin improve prediction of renal function decline	Until further validation Kim-1 and β2-microglobulin could be useful in clinical trials to select those patients with higher risk of DN
Panel of 297 serum biomarkers	Humans	Colombo et al. (2019a)	1174 patients with T1DM	Longitudinal multicenter study	Predictive information can be obtained using just two biomarkers (CD27 and KIM-1)	Few biomarkers are necessary to gain prediction of DN diagnosis.
Panel of 207 serum biomarkers	Humans	Looker et al. (2015)	154 cases (40% reduction in GFR) and 153 controls	Longitudinal multicenter study	14 biomarkers were associated with CKD progression (SDMA, SDMA/ADMA ratio,	These panel detected some novel biomarkers that require further investigation

					Kim-1, creatinine, β2-Microglobulin, α1 Antitrypsin, Uracil, N-terminal prohormone of brain natriuretic peptide, C16-acylcarnitine, Hydroxyproline, Fibroblast growth factor-21, Fatty acid-binding protein heart, Creatine, Adrenomedullin)	
Panel of 402 plasma biomarkers	Humans	Kammer et al. (2019)	481 T2DM patients with incident or early CKD (comparing patients with stable GFR and patients with a rapid decline of GFR)	Longitudinal multicenter study	KIM-1 was the most important predictor of GFR decline	KIM-1 might be a useful marker of DN in patients with T2DM

Abbreviations: *eGFR* estimated glomerular filtration rate, *DN* diabetic nephropathy, *T1DM* patients with type 1 diabetes, *T2DM* patients with type 2 diabetes, *SDMA* symmetric dimethylarginine, *NGAl* neutrophil gelatinase-associated lipocalin, *AER* Albumin excretion ratio, *AGEs* advanced glycosylation end-products, *AKI* acute kidney injury, *CKD* chronic kidney disease, *DN* diabetic nephropathy, *UNCR* urinary NGAL-to-creatinine ratio, *KIM-1*, kidney injury molecule −1, *IgG* immunoglobulin G, *MCP-1* urinary monocyte chemoattractant protein-1, *NAG* N-acetyl-β-D-glucosaminidase, *GST-α* alpha glutation S transferasa, *GST-μ* mu glutation S transferasa, *IL-8* interleukin 8, *TIMP-1* tissue inhibitor of metalloprotease-1, *VEGF* vascular endothelial growth factor, *RBP4* retinol binding protein 4, *suPAR* soluble urokinase type plasminogen activator receptor, *TGFβ1* transforming growth factor β-1

Kidney Disease Diagnosis and Treatment), SUMMIT (Surrogate markers for micro and macrovascular hard endpoints for innovative diabetes tools) and BEAt-DKD (Biomarker Enterprise to Attack Diabetic Kidney Disease) are providing and will provide important results in the near future.

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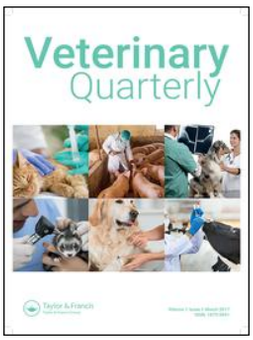
ARTICLE IV



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Kidney function and glucose metabolism in overweight and obese cats

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ABSTRACT

Background: In people, obesity and prediabetes mellitus might predispose to chronic kidney disease (CKD).

Aims: To assess the association of overweight [Body condition score (BCS) >5] and glucose metabolism alterations, with established or potential markers of CKD. In addition, fructosamine and fasted blood glucose were compared as predictors of early abnormal glucose metabolism.

Methods: 54 clinically healthy cats were included in a cross-sectional study comprising 25 neutered males and 29 (28 neutered) females aged 7.2 (5.5–9.4) years. Two potential markers of CKD, namely urinary free active transforming growth factor-β1-creatinine ratio and urinary retinol binding protein-creatinine ratio were measured along with other parameters to assess CKD. A receiver operating curve was used to identify the best sensitivity and specificity of fructosamine to identify cats with fasting glucose >6.5 mmol/L.

Results: No association was found between BCS and markers of CKD. Fructosamine was greater in cats with fasting glucose >6.5 mmol/L compared to those with fasting glucose ≤6.5 mmol/L. A fructosamine concentration ≥250 µmol/L was able to detect cats with hyperglycemia with a sensitivity of 77% and a specificity of 65%. Furthermore, fructosamine was more strongly correlated with fasting glucose than albumin-corrected fructosamine ($r = 0.43$, $p = 0.002$ vs $r = 0.32$, $p = 0.026$). Cats with higher fructosamine had lower serum symmetric dimethylarginine concentrations.

Conclusion: The present study does not suggest an effect of obesity on renal function in domestic cats.

Clinical relevance: Fructosamine might be of value for the diagnosis of prediabetes mellitus in cats.

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KEYWORDS
Cat; feline; obesity; metabolic syndrome; fructosamine; diabetes mellitus; nephropathy; symmetric dimethyl arginine; active transforming growth factor-β1; retinol biding protein

1. Introduction

In people, metabolic syndrome is defined as a cluster of disorders, which include abdominal obesity, abnormal glucose metabolism, dyslipidemia and hypertension (International Diabetes Federation, 2010; Matfin 2010). It is associated with a threefold risk of coronary heart disease and a fivefold risk of type 2 diabetes (T2DM) (International Diabetes Federation, 2010). Although T2DM is considered the leading cause of chronic kidney disease (CKD) in people (Pyram et al. 2012), recent studies have suggested that obesity, prediabetes and metabolic syndrome could also be independently associated with CKD (Johns et al. 2012; de Vries et al. 2014; Boronat et al. 2016; Markus et al. 2018). Feline diabetes mellitus is mostly classified as T2DM, with obesity being its main risk factor (Hoenig 2012; Nelson and Reusch 2014). Obesity can cause insulin resistance and dyslipidemia, and prediabetes has been suspected to occur in cats, too (Gilor et al. 2016), although diagnostic criteria are not well established. Fasting glucose levels may be considered impaired in cats when fasting glucose >6.5 mmol/L, whereas in the non-fasting state, it is considered that the upper normal limit is 9.2 mmol/L (Gottlieb et al. 2015; Reeve-Johnson et al. 2016; Gottlieb and Rand 2018). However, stress can increase blood glucose concentration in cats (Gottlieb and Rand 2018). In people, insulin resistance can be assessed through several simplified formulas that can be easily calculated from insulin concentration (Katz et al. 2000; Sung et al. 2010). In cats, fasting plasma insulin concentration and one of these formulas (the homeostasis model assessment, HOMA), have also been considered useful predictors of insulin sensitivity (Appleton et al. 2005), and could be potential markers of

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Table 1. Simplified estimated formulas of insulin sensitivity.

Insulin sensitivity Index	Formula
HOMA	$(I_0 \times G_0) / 22.5$
QUICKI	$1/(\log I_0 + \log G_0)$
Fasting I/G ratio	I_0 / G_0

I_0 =fasting insulin *(μU/ml)*; G_0 = fasting glucose *(mmol/L)*

dysglycemia. However, measurement of insulin is expensive and requires fasting. Therefore, methods other than fasting glucose or insulin could be useful to study disorders of glucose metabolism. On the other hand, in contrast to people, the association of obesity and diabetes mellitus with CKD has not been fully investigated in cats. Thus, the present study was conducted in a sample of healthy cats with three different objectives: I) To assess the association between overweight (BCS >5) and a set of established or potential markers of kidney damage. II) To assess the association between different indicators of abnormal glucose metabolism and these same markers of kidney damage. III) To compare fructosamine and fasted blood glucose as predictors of early abnormal glucose metabolism.

2. Material and methods

2.1. Animals

A cross-sectional study was performed at the Veterinary Teaching Hospital of the University of Las Palmas de Gran Canaria. Clinically healthy cats, aged five years or above, were consecutively included. The owners participated voluntarily after signing an informed consent. Owners fulfilled a questionnaire covering information about previous diseases (including urinary tract disorders) and medical treatments. They were also specifically asked about clinical signs of diabetes mellitus or CKD, including unintentional weight loss, polyuria and polydipsia. Cats were considered healthy based on this questionnaire, a normal physical examination, normal abdominal ultrasound and blood tests. Exclusion criteria included previous diagnosis of a severe chronic disorder, such as lower urinary tract diseases, diabetes mellitus, CKD, leukemia or other neoplasms, owner-reported clinical signs of hyperglycemia or renal disease, positive test for retrovirus infections, use of corticosteroids or non-steroidal anti-inflammatory drugs in the previous six months, or treatment with other nephrotoxic drugs such as toceranib. CKD was defined as serum creatinine ≥140 μmol/L plus urinary specific gravity (USG) <1.035 or a urinary protein-creatinine ratio (UPC) >0.4 (International Renal Interest Society 2017). Those with serum creatinine concentrations ≥140 μmol/L, but not available urine data, were excluded as a diagnosis of renal failure could not be confirmed or ruled out. Cats were classified according to their body condition score (BCS; 1-9) as normal-weight (BCS = 5), as overweight (BCS = 6-7) or obese (BCS >7). Overweight and obese cats were combined into the overweight group (BCS >5) to simplify the interpretation of the results. All cats were assessed after a minimum of 12 hours of fasting (without water deprivation), and underwent physical examination, blood and urine sampling (cystocentesis or home collection), and an abdominal ultrasound. Systolic blood pressure was measured whenever possible, using a Doppler ultrasonic (Doppler Vet BP®, Mano Médical, Taden, France) or a high definition oscillometric (VetHDO®, S + B MedVet GmbH, Babenhausen, Germany) device, following international guidelines (Taylor et al. 2017).

This study was approved by the Animal Welfare Ethics Committee, University of Las Palmas de Gran Canaria, Spain with reference number 10/2018.

2.2. Analytical procedures

Blood samples were obtained in serum separator tubes, which were centrifuged and aliquoted within 20 minutes. Some of those aliquots were frozen. Creatinine, urea, glucose, alkaline phosphatase activity, alanine aminotransferase activity, total proteins, globulins, albumin and glucose were measured in fresh or refrigerated serum samples within 24 hours in all cats, whereas serum fructosamine, cholesterol, triglycerides and symmetric dimethylarginine (SDMA) were measured in refrigerated serum samples within 24 hours in 12 cats, and in frozen serum samples in 42 cats, all by spectrophotometry.

Insulin was measured with a commercial, feline insulin ELISA kit (Mercodia, Uppsala, Sweden). Insulin sensitivity was assessed through simplified estimation formulas [HOMA, quantitative insulin check index (QUICKI) and fasting insulin to glucose ratio (I/G)] (Appleton et al. 2005) (see Table 1).

USG, urinary dipstick and urinary sediment examination were performed within 24 hours after urine collection. Those urine samples collected by the owner were preserved under refrigeration until the analysis was done; and those collected by cystocentesis were analyzed at the moment. Urine samples were aliquoted and frozen for later measurement of UPC by colorimetry (Animal Lab, Gran Canaria, Spain), and of the potential markers of renal disease: urinary free active transforming beta growth factor-creatinine ratio (uaTGFβ1:Cr) [Human Free Active TGF-β1 (BioLegend, San Diego, USA)] (Lawson et al. 2016) and retinol binding protein-creatinine ratio (uRBP:Cr) [human RBP sandwich ELISA kit (Immundiagnostik AG, Bensheim, Germany)] (van Hoek et al. 2008), were measured by

commercial ELISA methods, following the manufacturers' instructions. A standard curve was performed in each assay and all standards and samples were run in duplicate on the same plate. Absorbance was read at 450 nm within 30 minutes. The assay detection limits for Free Active TGF-β1 and RBP sandwich were 2.3 pg/ml (provided by the manufacturer) and 1.37 μg/l (provided by Hoek et al) (van Hoek et al. 2008), respectively.

2.3. Statistical analysis

Minimal sample size was calculated (Massachusetts General Hospital Biostatistics Center (MGHB) 2019) considering the standard deviation (0.41 μmol/L) and difference in means (0.50 μmol/L) in SDMA between cats with and without CKD, obtained from a previous study (Hall et al. 2014). A sample size of 16 cats in each group was required for a probability of 90 percent that the study would detect a difference of 0.50 μmol/L between groups of healthy cats and cats with CKD at a two-sided 0.05 significance level.

Distribution of quantitative variables was assessed through histograms. Data are presented as medians and interquartile ranges. Categorical variables are expressed as number of cats and percentages. Comparisons between groups were performed using the pairwise Mann–Whitney's U test, and correlations between variables with the Spearman's test. The comparisons and correlations were performed in two steps: first, to assess the associations between overweight or BCS and markers of kidney damage; and secondly, between markers of glucose metabolism and markers of kidney damage.

Albumin-corrected fructosamine was calculated according to the following formula (Reusch and Haberer 2001). fructosamine corrected for albumin (μmol/L) = observed fructosamine value (μmol/L) x median albumin concentration 31 (g/L)/observed albumin concentration (g/L).

A receiver operating curve (ROC) was used to find the fructosamine cut-off value with the best sensitivity and specificity for the detection of cats with fasting glucose >6.5 mmol/L (Gottlieb et al. 2015; Reeve-Johnson et al. 2016; Gottlieb and Rand 2018).

Statistical analysis was performed with SPSS Statistics Version 25.0 (IBM, Madrid, Spain).

3. Results

A total of 68 cats were examined for inclusion. Eight cats were excluded because CKD could neither be diagnosed nor ruled out, as their serum creatinine concentrations were ≥140 μmol/L and urine samples were not available. Two normal-weight cats were excluded because of CKD, and four additional cats (two normal-weight and two overweight) because of ultrasound findings consistent with urinary tract diseases (hydroureter (1), hydronephrosis (2), and bladder stones (1)). Thus, 54 clinically healthy cats were included, 25 neutered males and 29 (28 neutered) females, aged 7.2 (5.5-9.4) years. The breed distribution was as follows: domestic short hair (42), domestic long hair (4), mixed Persian (3), Persian (2), Siamese (2), and Angora (1). In total, 17 cats (6 male, 11 female) had normal BCS (BCS = 5), whereas 37 (19 male, 18 female) were overweight. Among these cats fructosamine could be measured in 52 cases, SDMA in 51 cats, uaTGFβ1:Cr in 29 cats, and uRBP:Cr in 28. Two cats had not fasted for 12 hours, so they were not included for the assessment of glucose, insulin or lipids. Therefore, for the assessment of glucose and lipid metabolism, 17 (6 male, 11 female) cats with BCS = 5, and 35 cats (17 males, 18 females) with BCS >5 were analyzed. Among them, fasting glucose was measured in 51 cats and fasting insulin was measured in 32 cats.

As expected, several variables reflecting abnormal glucose metabolism, including glucose, fructosamine, triglycerides, albumin and HOMA, were significantly greater in cats with BCS >5; and QUICKI was significantly lower in cats with BCS >5 (Table 2). Five out of 23 (21.7%) normal-weight cats, and 23 out of 26 (88.5%) cats with overweight had fasting serum glucose >6.5 mmol/L ($p = 0.01$). Fructosamine, but not albumin-corrected fructosamine, was significantly greater in cats with fasting glucose >6.5 mmol/L compared to those with fasting glucose ≤ 6.5 mmol/L (Table 3). In addition, fructosamine was more strongly correlated with fasting glucose than albumin-corrected fructosamine ($r = 0.43$, $p = 0.002$ vs $r = 0.32$, $p = 0.026$) and ROC analyses yielded a better diagnostic performance for fructosamine than for albumin-corrected fructosamine in the identification of cats with fasting glucose >6.5 mmol/L (area under the curve = 0.72 vs 0.61). Specifically, a fructosamine concentration ≥ 250 μmol/L was able to detect cats with a blood glucose concentration >6.5 mmol/L with a sensitivity of 77% and a specificity of 65% (Figure 1). Thus, albumin-corrected fructosamine was discarded for further analyses. Significant differences for fasting insulin and HOMA were observed when cats with a fructosamine ≥ 250 μmol/L were compared to cats with a fructosamine < 250 μmol/L (Table 4). Fructosamine was correlated with fasting insulin ($r = 0.53$; $p = 0.002$), HOMA ($r = 0.56$; $p = 0.001$), I/G ratio ($r = 0.39$; $p = 0.028$), and QUICKI ($r = -0.56$; $p = 0.001$).

3.1. Association between overweight and markers of kidney damage

No statistically significant differences were found for established markers or potential markers of renal

Table 2. Clinical parameters assessed in 54 clinically healthy cats ≥5 years old, classified according to their body composition score (BCS). Data are given as median and IQR.

	Cats BCS = 5 (n = 17)	Cats BCS >5 (n = 37)	p-value*
Age *(years)*	7.0 (5.4–9.5)	7.3 (5.5–9.3)	0.963
Weight *(kg)*	**3.8 (3.4–4.3)**	**5.4 (4.9–6.7)**	**<0.005**
BCS *(1-9)*	**5.0 (5.0–5.0)**	**7.0 (6.0–8.0)**	**<0.005**
SDMA *(0-0.69 μmol/L)*	0.44 (0.32–0.64)	0.44 (0.39–0.54)	0.607
Creatinine *(71-212 μmol/L)*	132.6 (97.2–168.0)	150.3 (114.9–176.8)	0.123
Urea *(5.7-12.2 mmol/L)*	7.5 (6.5–8.7)	7.8 (6.8–9.0)	0.292
UsG (1.035-1060)	1050 (1044–1052)	1050 (1046–1055)	0.420
UPC (<0.4)	0.19 (0.09–0.43)	0.12 (0.09–0.19)	0.149
uaTGFβ1:Cr *(pg/mg)*	5.81 (2.70–8.40)	8.17 (4.63–12.65)	0.301
ALT activity *(12-130 U/L)*	42 (34–64)	50 (38–69)	0.280
ALKP activity *(14-111 U/L)*	35 (15–40)	27 (18–43)	0.730
Albumin *(22-40 g/L)*	2.9 (2.8–3.2)	3.2 (3.0–3.4)	**0.001**
Total protein *(57-89 g/L)*	7.0 (6.9–7.5)	7.6 (7.2–7.9)	**0.015**
Globulins *(28-51 g/L)*	4.1 (3.9–4.3)	4.3 (3.9–4.6)	0.308
Fasting glucose *(4.1-8.8 mmol/L)*	**5.9 (5.1–8.7)**	**7.2 (6.3–9.3)**	**0.041**
Fructosamine *(175-400 μmol/L)*	**246 (205–258)**	**257 (233–278)**	**0.046**
Corrected fructosamine *(175-400 μmol/L)*	249 (224–282)	251 (228–270)	0.733
Triglycerides *(0.23-1.4 mmol/L)*	**0.48 (0.40–0.72)**	**0.90 (0.79–1.06)**	**<0.005**
Cholesterol *(2.6-10.6 mmol/L)*	3.78 (2.95–5.48)	4.40 (3.36–5.17)	0.321
Fasting insulin *(5.5-58.9 pmol/L)*	21.2 (15.3–29.3)	31.7 (18.4–80.6)	0.053
HOMA^{+}	**0.75 (0.62–1.64)**	**1.77 (0.89–3.56)**	**0.040**
QUICKI^{++}	**0.8 (0.6–0.8)**	**0.6 (0.5–0.8)**	**0.040**
Fasting I/G ratio^{+}	0.58 (0.42–0.85)	0.84 (0.42–1.51)	0.158

Variables that showed a significant difference were highlighted in bold
ALT = alanine aminotransferase, ALKP = alkaline phosphatase BCS = body condition score, Fasting I/G = fasting insulin to glucose ratio, HOMA = homeostasis model assessment, QUICKI = quantitative insulin check index, SDMA = symmetric dimethylarginine, uaTGFβ1:Cr = urinary active transforming growth factor β: creatinine ratio, UPC = urine protein/creatinine ratio, USG = urinary specific gravity.
+The higher the value, the lower the insulin sensitivity
++The lower the value, the lower the insulin sensitivity
*p values <0.005 reflect a significant difference between cats with BCS = 5 and cats with BCS > 5
The uaTGFβ1:Cr was measured in 8 cats with BCS = 5, and 21 cats with BCS >5
The SDMA was measured in 17 cats with BCS = 5, and 34 cats with BCS >5
Fasting insulin was measured in 12 cats with BCS = 5 and 20 cats with BCS >5
Fasting glucose was measured in 17 cats with BCS = 5 and 34 cats with BCS >5
Fructosamine was measured in 17 cats with BCS = 5 and 35 cats with BCS >5

function between cats with BCS = 5 and cats with BCS >5 (see table 2). uRBP (not shown in the table) was measured in 28 cats (seven cats with BCS = 5, and 21 cats with BCS >5) but its concentrations were above the assay sensitivity in only eight cases (three with BCS = 5, and five with BCS >5); the median uRBP:Cr ratio was 0.81×10^{-4} (0.43×10^{-4}–1.1×10^{-4}) μg/mg, and no statistically significant differences were observed between groups (p = 1.0)

When assessed as continuous variable, no correlation was observed between BCS and established or potential markers of CKD [Creatinine (r = 0.019; p = 0.892); SDMA (r=-0.001; p = 0.994); USG (r = 0.136; p = 0.373); UPC (r=-0.102; p = 0.509), uaTGFβ1:Cr (r = 0.089; p = 0.645); uRBP:Cr (r=-0.048; p = 0.809)]. Systolic blood pressure was only measured in a total of 32 cats (8 cats with BCS = 5 and in 24 cats with BCS >5), and no statistically significant differences were observed [138 (116-144) vs. 137 (131-150) mmHg; p = 0.357].

3.2. Association between markers of glucose metabolism and kidney damage

Markers of kidney injury were compared between groups based on cut-offs for fasting glucose (6.5 mmol/L) and fructosamine (250 μmol/L) (see Table 3 and Table 4). There was no significant difference in any marker of kidney injury when cats were classified according to their glucose concentrations (see Table 3), whereas cats with a fructosamine <250 μmol/L showed higher SDMA than those with fructosamine ≥250 μmol/L (p = 0.021). In addition, moderate, inverse correlation was found between fructosamine and SDMA (r = −0.36; p = 0.011).

4. Discussion

As observed in previous studies (Appleton et al. 2001; Jordan et al. 2008; Hoenig 2012), we found a strong association between excess of body weight, insulin resistance and dyslipidemia, confirming the existence of a feline form of the metabolic syndrome. It is also known that cats with obesity are at greater risk of developing diabetes mellitus (Donoghue 1998; Hoenig 2012). However, the role of diabetes mellitus on feline CKD is unclear, and whether obese or prediabetic cats have a higher risk of CKD, has not been investigated yet. Since cats are frequently obese for most of their life, and they are chronically exposed to metabolic changes induced by obesity, particularly insulin resistance and the metabolic syndrome, we hypothesized that it might predispose them to develop CKD. Moreover, CKD is a prevalent disease in elderly cats (Reynolds and

Table 3. Clinical parameters assessed in 51 clinically healthy cats ≥5 years old after 12 hours of fasting, classified according to their glucose concentrations. Data are given as median and IQR.

	Fasting glucose ≤6.5 mmol/L n = 23	Fasting glucose >6.5 mmol/L n = 28	p-value*
Age *(years)*	7.1 (5.5–9.6)	7.0 (5.5–9.4)	0.688
Weight *(kg)*	**4.2 (3.8–5.2)**	**5.5 (4.5–6.8)**	**0.004**
BCS *(1-9)*	**5.0 (5.0–6.0)**	**7.0 (6.0–8.0)**	**0.001**
SDMA *(0-0.69 μmol/L)*	0.44 (0.39–0.59)	0.39 (0.39–0.54)	0.356
Creatinine *(71-212 μmol/L)*	159.1 (88.4–176.8)	150.3 (132.6–168.0)	0.220
Urea *(5.7-12.2 mmol/L)*	7.7 (6.8–9.2)	7.5 (6.7–8.5)	0.550
UsG (1.035-1060)	1050 (1044–1053)	1050 (1047–1052)	0.645
UPC (<0.4)	0.17 (0.10–0.28)	0.13 (0.08–0.19)	0.147
uaTGFβ1:Cr *(pg/mg)*	6.45 (3.03–15.88)	7.71 (4.58–10.47)	0.983
ALT activity *(12-130 U/L)*	**42 (35–57)**	**52 (42–74)**	**0.037**
ALKP activity *(14-111 U/L)*	33 (17–40)	29 (18–47)	0.715
Albumin *(22-40 g/L)*	**2.8 (2.6–3.2)**	**3.2 (3.0–3.6)**	**0.033**
Total protein *(57-89 g/L)*	7.3 (7.0–7.8)	7.5 (6.9–7.8)	0.580
Globulins *(28-51 g/L)*	4.2 (3.9–4.6)	4.3 (3.9–4.5)	0.872
Fasting glucose *(4.1-8.8 mmol/L)*	**5.8 (5.2–6.3)**	**8.3 (7.4–10.5)**	**<0.005**
Fructosamine *(175-400 μmol/L)*	**235.2 (206.5–258.4)**	**261.4 (249.4–282.2)**	**0.007**
Corrected fructosamine *(175-400 μmol/L)*	242 (215–263)	254 (228–290)	0.207
Triglycerides *(0.23-1.4 mmol/L)*	0.7 (0.4–0.9)	0.9 (0.7–1.1)	**0.009**
Cholesterol *(2.6-10.6 mmol/L)*	4.7 (3.0–5.4)	4.1 (3.4–4.4)	0.699
Fasting insulin *(5.5-58.9 pmol/L)*	20.7 (13.7–30.8)	33.9 (21.7–64.2)	0.073
HOMA^{+}	**0.8 (0.5–1.4)**	**2.3 (1.7–4.5)**	**0.004**
QUICKI^{++}	**0.8 (0.8-0.9)**	**0.6 (0.5-0.6)**	**0.001**
Fasting I/G ratio^{+}	0.7 (0.4-0.9)	0.8 (0.4-1.4)	0.762

Variables that showed a significant difference were highlighted in bold
ALT = alanine aminotransferase, ALKP = alkaline phosphatase BCS = body condition score, Fasting I/G = fasting insulin to glucose ratio, HOMA = homeostasis model assessment, QUICKI = quantitative insulin check index, SDMA = symmetric dimethylarginine, uaTGFβ1:Cr = urinary active transforming growth factor β: creatinine ratio, UPC = urine protein/creatinine ratio, USG = urinary specific gravity.
+The higher the value, the lower the insulin sensitivity
++The lower the value, the lower the insulin sensitivity
*p values <0.05 reflect a significant difference between cats with fasting glucose ≤6.5 mmol/L and cats with fasting glucose >6.5 mmol/L
The uaTGFβ1:Cr was measured in 11 cats with fasting glucose ≤6.5 mmol/L, and 16 cats fasting glucose >6.5 mmol/L
The SDMA was measured in 22 cats with fasting glucose ≤6.5 mmol/L, and 26 cats with fasting glucose >6.5 mmol/L
Fasting insulin was measured in 17 cats with fasting glucose ≤6.5 mmol/L and 15 cats with fasting glucose >6.5 mmol/L
Fructosamine was measured in 23 cats with fasting glucose ≤6.5 mmol/L and 26 cats with fasting glucose >6.5 mmol/L.

Lefebvre 2013), and most cases of CKD are of unknown cause, which could lead to the speculation that obesity could be a hidden causal factor for kidney injury. However, according to our results, concentrations of established markers of CKD, such as creatinine or SDMA, did not differ between normal-weight and overweight cats. In regard to the potential biomarkers assessed in this study, uaTGFβ has been proposed as an important mediator of diabetic nephropathy in animal models, one of which showed that urinary aTGFβ1:Cr ratio precedes the onset of azotemia by six months (Lawson et al. 2016). In relation to RBP, its urine concentration might increase when tubular damage occurs, and higher levels of urinary RBP:Cr in cats with CKD compared to healthy cats have been reported (van Hoek et al. 2008). However, these putative early markers of kidney injury were not correlated with obesity in our study. These findings add to previous studies (Greene et al. 2014; Freeman et al. 2016) suggesting that, in contrast to dogs (Henegar et al. 2001; Tvarijonaviciute et al. 2012; 2013), obesity does not contribute to the development of CKD in cats. However, prospective studies would be required to definitely evaluate whether feline kidney function is affected by obesity.

On the other hand, our results suggest that changes in kidney function might exist related to higher blood glucose concentration. SDMA is a byproduct of arginine-methylated proteins, and is mainly excreted through the kidneys (Schwedhelm and Boger 2011). Its serum concentrations are inversely correlated with glomerular filtration rate (GFR) (Hall et al. 2014), and values persistently above 0.69 μmol/L are consistent with CKD (International Renal Interest Society 2017). One previous study reported that SDMA levels were lower in cats with diabetes mellitus than controls, but also compared to cats with CKD or cats with hypertrophic cardiomyopathy (Pyram et al. 2012)[5]. The authors suggested that it might be due to osmotic diuresis or hyperfiltration. The latter is one of the earliest changes observed in people with diabetic nephropathy (Dronavalli et al. 2008), and lower concentrations of SDMA due to hyperfiltration mechanisms have also been suggested in humans (Marcovecchio et al. 2010). In the present study, although neither cats with BCS >5 nor cats with fasting hyperglycemia showed reduced concentrations of SDMA, those with fructosamine concentration >250 μmol/L did have low SDMA concentrations. Osmotic diuresis could not explain low SDMA values in cats with higher concentrations of fructosamine, since the animals included in this study did not have glycosuria. Whether or not lower concentrations of SDMA are related to hyperfiltration requires further investigation.

Our study also assessed the possible role of serum concentrations of fructosamine for the evaluation of

early abnormalities of glucose metabolism in cats. Recently, the use of blood glucose has been proposed to screen for prediabetes (Gottlieb et al. 2015; Reeve-Johnson et al. 2016; 2017; Gottlieb and Rand 2018). The authors proposed that cats with fasting glucose persistently >6.5 mmol/L could be considered prediabetic (Marcovecchio et al. 2010; Gottlieb et al. 2015; Gilor et al. 2016; Reeve-Johnson et al. 2016). However, repeated blood sampling and prolonged fasting are not easy to perform in clinical practice. In addition, blood glucose concentration is subject to the effect of stress, which could raise glucose levels up to 10.8 mmol/L (Gottlieb and Rand 2018). In the present study, high fructosamine concentrations showed a better association with markers of insulin resistance (fasting insulin and HOMA) than fasting glucose. As HOMA and fasting insulin have been considered reliable parameters to evaluate insulin sensitivity in cats (Appleton et al. 2005), this means that fructosamine could reflect decreased insulin sensitivity better than fasting glucose, maybe due to the fact that fructosamine is not affected by stress (Crenshaw et al. 1996). Fructosamine levels depend on blood glucose concentration and the half-lives of the proteins (Crenshaw et al. 1996). It has been assumed that feline fructosamine, as in dogs, could reflect blood glucose levels of the preceding one to two weeks (Dixon et al. 1953; Crenshaw et al. 1996). Furthermore, for fructosamine to exceed the normal reference range, severe hyperglycemia, lasting for 3-5 days is necessary (Link and Rand 2008). Therefore, it is considered a useful parameter to distinguish between diabetes mellitus and stress hyperglycemia (Crenshaw et al. 1996), and we

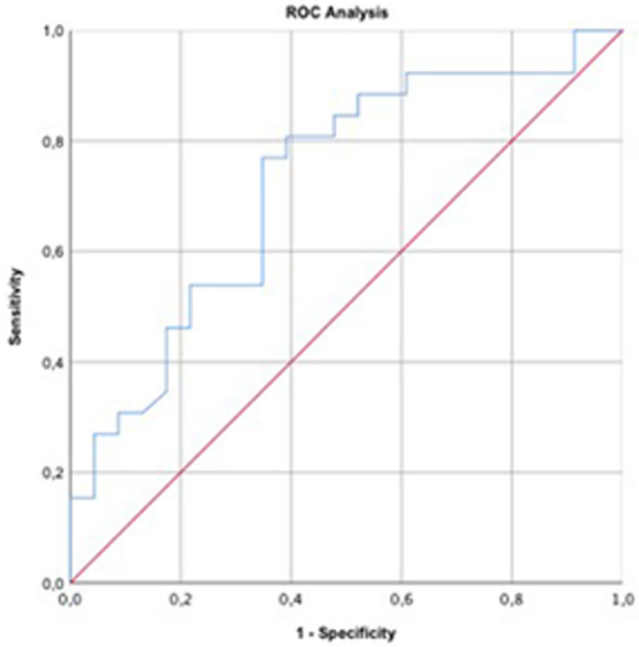

Figure 1. Sensitivity and specificity of fructosamine concentration to detect cats with a fasting glucose >6.5 mmol/L was calculated through ROC analysis (area under the curve = 0.72).

Table 4. Clinical parameters assessed in 52 clinically healthy cats ≥5 years old after 12 hours of fasting, classified according to their fructosamine concentrations. Data are given as median and IQR.

	Cats Fructosamine <250 µmol/L N = 22	Cats Fructosamine ≥250 µmol/L N = 30	p-value
Age *(years)*	7.8 (6.0–9.7)	6.0 (5.4–9.3)	0.173
Weight *(kg)*	**4.6 (3.8–5.3)**	**5.4 (4.3–6.7)**	**0.021**
BCS *(1-9)*	**6.0 (5.0–7.0)**	**7.0 (5.5–8.0)**	**0.028**
SDMA *(0-0.69 µmol/L)*	**0.54 (0.39–0.69)**	**0.39 (0.39–0.54)**	**0.021**
Creatinine *(71-212 µmol/L)*	159.1 (123.8–176.8)	150.3 (132.6–168.0)	0.707
Urea *(5.7-12.2 mmol/L)*	8.0 (7.2–9.2)	7.5 (6.5–9.0)	0.219
UsG (1.035-1060)	**1045 (1043–1051)**	**1050 (1048–1053)**	**0.032**
UPC (<0.4)	0.18 (0.10–0.26)	0.14 (0.09–0.25)	0.134
uaTGFβ1:Cr *(pg/mg)*	8.17 (5.21–11.35)	7.18 (3.56–13.41)	0.821
ALT activity *(12-130 U/L)*	46 (37–55)	56 (38–70)	0.210
ALKP activity *(14-111 U/L)*	33 (18–36)	33 (18–47)	0.470
Albumin *(22-40 g/L)*	**3.0 (2.8–3.2)**	**3.2 (3.0–3.4)**	**0.029**
Total protein *(57-89g/L)*	7.2 (6.9–7.7)	7.5 (7.1–7.8)	0.219
Globulins *(28-51 g/L)*	4.2 (3.9–4.5)	4.3 (3.9–4.5)	0.930
Fasting glucose *(4.1-8.8 mmol/L)*	**5.0 (5.9–6.7)**	**7.4 (6.5–9.2)**	**0.002**
Fructosamine *(175-400 µmol/L)*	**219.9 (202.3–228.5)**	**285.1 (267.1–315.1)**	**<0.005**
Triglycerides *(0.23-1.4 mmol/L)*	0.6 (0.5–1.0)	0.8 (0.6–1.0)	0.117
Cholesterol *(2.6-10.6 mmol/L)*	4.0 (3.0–5.1)	4.4 (3.5–5.0)	0.298
Fasting insulin *(5.5-58.9 pmol/L)*	**22.9 (13.0–31.5)**	**32.2 (20.9–63.4)**	**0.033**
HOMA^{+}	**0.8 (0.6–1.7)**	**2.0 (1.1–4.0)**	**0.011**
QUICKI^{++}	**0.8 (0.6–0.9)**	**0.6 (0.5–0.7)**	**0.017**
Fasting I/G ratio^{+}	0.6 (0.4–0.9)	0.8 (0.5–1.7)	0.191

Variables that showed a significant difference were highlighted in bold.
ALT = alanine aminotransferase, ALKP = alkaline phosphatase BCS = body condition score, Fasting I/G = fasting insulin to glucose ratio, HOMA = homeostasis model assessment, QUICKI = quantitative insulin check index, SDMA = symmetric dimethylarginine, uaTGFβ1:Cr = urinary active transforming growth factor β: creatinine ratio, UPC = urine protein/creatinine ratio, USG = urinary specific gravity.
+The higher the value, the lower the insulin sensitivity
++The lower the value, the lower the insulin sensitivity
*p values <0.05 reflect a significant difference between cats with fructosamine concentration <250 µmol/L and cats with fructosamine concentration ≥250 µmol/L
The uaTGFβ1:Cr was measured in 13 cats with <250 µmol/L, and 15 cats with ≥250 µmol/L
The SDMA was measured in 21 cats with fructosamine <250 µmol/L, and 29 cats with fructosamine ≥250 µmol/L
Fasting insulin was measured in 16 cats with fructosamine <250 µmol/L and 16 cats with fructosamine ≥250 µmol/L
Fasting glucose was measured in 21 cats with fructosamine <250 µmol/L and 28 cats with fructosamine ≥250 µmol/L.

hypothesized that it might be useful for the detection of feline prediabetes. In addition, fructosamine has some advantages compared to other methods: it is not time-consuming or expensive, nor does it require fasting. Prospective studies would be needed to evaluate this marker as a tool to diagnose prediabetes. In addition, it should also be highlighted that there is a lack of standardization in the methodology of fructosamine measurement among laboratories. Therefore, for a correct interpretation of fructosamine values, it should always be measured under the same methodology and laboratory (Sparkes et al. 2015; Idexx Reference Laboratories Support 2019).

Some limitations are acknowledged in this study. First, its cross-sectional character does not allow to establish causal inferences. Another important limitation is that the sample size was small and calculated to detect inter-groups defined differences in SDMA concentrations. Therefore, it might be underpowered to detect differences in other markers of kidney damage and its results should not be overestimated. Another limitation might be related to fasting. Cats were fasted for at least 12 hours; however, the postprandial period in cats could last longer, and it could affect concentrations of fasting blood glucose and fasting insulin (Appleton et al. 2001; Farrow et al. 2012).

In conclusion, the present study does not suggest an effect of obesity on renal function in domestic cats, whereas some changes in kidney function, reflected by SDMA concentrations, might be associated to mild chronic hyperglycemia. Finally, we propose fructosamine for the diagnosis of prediabetes mellitus, though optimal cut-offs should be investigated.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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ARTICLE V

Overweight and obesity are not associated with loss of kidney function in healthy domestic cats

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Abbreviations**:**

ALT: alanine aminotransferase

ALKP: alkaline phosphatase activity

BCS: body condition score

CKD: chronic kidney disease

GFR: glomerular filtration rate

IQR: interquartile range

SDMA: symmetric dimethylarginine

uaTGFβ1:Cr :urinary free active transforming beta growth factor-creatinine ratio

UPC: urinary protein-creatinine ratio

USG: urinary specific gravity

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Abstract

Background: Obesity has been proposed as an independently risk factor for chronic kidney disease (CKD) in people. Whether or not it has a role in feline kidney function is unknown.

Objective: To prospectively evaluate the effect of overweight on the concentration of symmetric dimethylarginine (SDMA) and creatinine in a cohort of healthy cats.

Methods: Forty healthy cats ≥5 years old were included, 14 with a body condition score (BCS)=5 (5 males, 9 females) and 26 with a BCS>5 (13 males, 13 females). Cats were reexamined every 6 months, whenever possible, for up to 18 months. SDMA and creatinine were measured at baseline and follow-up visits. Urinary free active transforming growth factor beta-creatinine ratio (uaTGFβ1:Cr) was measured at baseline. Intra and Inter-subject variability in regard to SDMA and creatinine repeated measurements were assessed through a linear mixed model. Correlation of baseline uaTGFβ1:Cr with consecutive measurements of SDMA and creatinine were assessed through a linear mixed model.

Results: No effect was found for time (p=0.629) of follow-up, overweight (p=0.935) or their interaction (p= 0.874) on SDMA, though a significant effect was found for age (p=0.004) [older cats showing higher SDMA]. Regarding creatinine, the linear mixed model did not show an effect of time (p=0.93), age (p=0.50), overweight (p=0.44) or the latter's interaction with time (p=0.11). Baseline uaTGFβ1:Cr ratio was not correlated with SDMA (p=0.367) or creatinine (p=0.157) at follow-up.

Conclusions and clinical importance: This study suggests that, in short term, obesity does not contribute to CKD in healthy cats.

Introduction

Obesity has risen to epidemic proportions in people and companion animals. Approximately 39% of the adult population is overweight and 13% is obese.[1] In a similar manner, 35-50% of domestic cats are estimated to be overweight or obese.[2] Furthermore, obese cats show similar complications to people with obesity, such as dyslipidemia and insulin resistance. Obesity and physical inactivity are the main risk factors for the development of type 2 diabetes in both humans and cats.[2.3] In addition, in humans, diabetes is the first cause of chronic kidney disease (CKD); and recent studies have suggested that obesity and metabolic syndrome could also be independently associated with CKD.[4] Glomerular hyperfiltration and albuminuria, which are common findings in early diabetic nephropathy, have been detected in non-diabetic obese patients[5,6]; and people with higher body mass index could be at higher risk of CKD.[7] In addition, structural kidney changes have been observed in obese patients in association to ectopic lipid accumulation or fatty kidney.[8]

In cats, disorders associated with CKD include diseases of the lower urinary tract, renal lymphoma, infections, hyperthyroidism, nephrotoxic drugs, and genetic kidney diseases, although often the cause of CKD is unknown[9], while the role of obesity on the development of CKD has been considered to be minor. Recently, one study showed that concentrations of SDMA and other renal markers were not different between cats with normal body condition score (BCS) and cats with a BCS >5 (1-9), suggesting that obesity may not have an effect on CKD.[10] Similarly, one retrospective epidemiological study, reported that, after ruling out dehydration, cats with greater body weight were less likely to develop CKD.[11] In addition, body weight was significantly associated with survival time in cats with CKD in other study. However, a U-shaped relationship was

also described, both the lowest and highest body weights were associated with shorter survival times.[12] Since association between obesity and CKD has hardly been studied in cats, further studies may shed light on this topic.

The aim of this study was to prospectively assess the effect of overweight on SDMA and creatinine in a cohort of healthy cats.

Material and Methods

A longitudinal study was performed at the Veterinary Teaching Hospital of the XX. Clinically healthy cats, aged five years or more, were consecutively included. The owners participated voluntarily after signing an informed consent form. Owners completed a questionnaire covering information about previous diseases (including urinary tract disorders) and medical treatments. They were also specifically asked about clinical signs of diabetes or CKD, including unintentional weight loss, polyuria and polydipsia. Cats were considered healthy based on this questionnaire, a normal physical examination, normal abdominal ultrasound, blood and urine tests. Exclusion criteria included previous diagnosis of severe chronic disorders, such as lower urinary tract diseases, diabetes, CKD, leukemia or other neoplasms, owner-reported clinical signs of hyperglycemia or renal disease, positive test for retrovirus infections, use of corticosteroids or non-steroidal anti-inflammatory drugs in the previous six months, or treatment with other nephrotoxic drugs such as toceranib. CKD was defined as serum creatinine ≥1.6 mg/dL plus urinary specific gravity (USG) <1.035 or a urinary protein-creatinine ratio (UPC) >0.4.[13] Those with a serum creatinine ≥1.6 mg/dL, but no available urinary sample, were excluded, as a diagnosis of CKD could not be confirmed

nor excluded. Cats were classified according to their BCS (1-9) as normal-weight (BCS = 5), overweight (BCS = 6-7) or obese (BCS >7). Overweight and obese cats were combined into the overweight group (BCS >5), to simplify the interpretation of the results.

The study lasted 18 months; during this period, cats were examined every six months. In all visits cats were assessed after a minimum of 12 hours of fasting (without water deprivation), and underwent physical examination, blood and urine sampling (cystocentesis or home collection), and an abdominal ultrasound.

This study was approved by the Animal Welfare Ethics Committee (Órgano de Ética y Bienestar Animal, OEBA-ULPGC); Reference number 10/2018

Analytical procedures:

Blood samples were obtained in serum separator tubes, which were centrifuged and aliquoted within 20 minutes. Some of those aliquots were frozen. Creatinine, urea, glucose, alkaline phosphatase activity, alanine aminotransferase activity, total proteins, globulins, albumin and glucose were measured in fresh or refrigerated serum samples within 24 hours. Cholesterol, triglycerides and SDMA were measured in refrigerated serum samples within 24 hours in 12 cats at baseline, and in frozen serum samples in the rest of cats and consecutives visits. All analytes were measured by spectrophotometry.

USG, urinary dipstick, and urinary sediment examination were performed within 24 hours after urine collection. Urine samples were aliquoted and frozen for later measurement of urinary protein-creatinine ratio (UPC) by colorimetry (Animal Lab, XX1), and for urinary free active transforming beta growth factor-creatinine ratio

(uaTGFβ1:Cr) [Human Free Active TGF-β1 (BioLegend, San Diego, USA)], by ELISA.[14] To measure the latter, a standard curve was performed in the assay and all standards and samples were run in duplicate on the same plate. Absorbance was read at 450 nm within 30 minutes. The assay detection limit for Free Active TGF-β1 was 2.3 pg/ml (provided by the manufacturer).

Statistical analysis

This study is the continuation of a previous cross-sectional study. For the latter, a minimal sample size of 16 cats per group was estimated to be needed to detect a difference of 10 µg/dL in SDMA between groups, with a 90% probability and a two-sided significance level of 0.05, after assuming a standard deviation of 8.3 µg/dL.[15,16] The mentioned study included 17 cats with BCS=5 and 37 with BCS>5. After that, two more cats were included adding up to 18 cats with BCS=5 and 38 cats with BCS>5. All these cats were attempted to be followed every 6 months for a maximum period of 18 months. Only cats with a minimum follow-up of 6 months were included in the statistical analysis.

Distribution of quantitative variables was assessed using the Shapiro-Wilk Test. Normally distributed quantitative variables are shown as mean and standard deviation. Non-parametric quantitative variables are presented as medians and interquartile ranges (IQR). Categorical variables are expressed as number of cats and percentages. Intra and Inter-subject variability in regard to consecutive measurements of markers of renal disease (SDMA and creatinine) was assessed through a linear mixed model. Additionally, correlations of consecutive measurements of SDMA and creatinine with

urinary free active transforming beta growth factor-creatinine ratio uaTGFβ1:Cr measured at baseline, were also assessed through a linear mixed model.

T-Test or Mann Whitney's U Test were used for other comparisons of normally or non-normally distributed variables, respectively, measured at a single point in time.

Differences of SDMA, creatinine and other variables at different visits (0, 6, 12 and 18 months) were evaluated through ANOVA of repeated measures of a factor for normally distributed variables, or through Friedman test for non-normally distributed variables

Statistical analysis was performed with SPSS Statistics Version 25.0 (IBM, Madrid, Spain) and the R package (R Development Core Team).

Results

A total of 56 cats were included. However, 16 of them did not return for follow-up, despite several attempts to reschedule their appointments. In total 40 cats had some follow-up; among them, 18 were neutered males and 22 (21 neutered) females. Median age was 6.8 (5.3-10) years. Breed distribution was as follows: Domestic short hair (31), , Siamese (3), Domestic long hair (1), Mixed Persian (3), Persian (1), and Angora (1). In total, 14 cats had a BCS=5 (5 males, 9 females) and 26 cats a BCS>5 (13 males, 13 females). Among cats with BCS>5, 19 had a BCS between 6 and 7 and 7 cats had a BCS>7. Their main features at baseline are displayed in table 1, and the main features of the total 40 cats along the study are displayed in table 2.

SDMA

After a median follow-up of 12 (12-12) months, SDMA was measured in 33 cats (13 cats with BCS=5 and 20 cats with BCS>5). The linear mixed model showed no effect of time (0.629), overweight (0.935) or their interaction (p= 0.874) on SDMA. In contrast, a significant effect was found for age (p=0.004) (Figure 1),

Creatinine

This variable was measured in each visit in all of the 40 cats with follow-up. The linear mixed model did not show an effect of time (p=0.93), age (p=0.50), overweight (p=0.44) or the latter's interaction with time (p=0.11) on creatinine. (Figure 2).

uaTGFβ1:Cr ratio

The uaTGFβ1:Cr was measured in 22 cats during their first visit. The median uaTGFβ1:Cr was 6.8 (4.5-11.6) pg/mg. As previously reported, no significant differences were found between cats with BCS =5 and cats with BCS >5.[11] Among these 22 cats, SDMA and creatinine were measured in 11 and 22 cats respectively at their last visit; and no differences between cats with a uaTGFβ1:Cr >6.8 pg/mg, and cats with a uaTGFβ1:Cr ≤6.8 pg/mg were found for SDMA (11.5 (10.0-12-8) vs 9.5 (7.3-16.0 µg/dL; p=0.645) or creatinine (1.8 (1.6-1.9) vs 1.8 (1.4-2.2) mg/dL; p=0.949) at their last visit. Nor did the linear mixed model show an effect of baseline uaTGFβ1:Cr on SDMA (p=0.367) or creatinine (p=0.157) at follow-up.

Discussion

In this small sample of clinically healthy cats, SDMA and creatinine, the two main markers of renal function, did not show a significant change after a median follow-up of 12 months. Neither did these markers differ between cats with BCS=5 and cats with BCS>5. Therefore, the results of this prospective study add to previous reports that suggest that obesity does does not have a relevant influence on kidney function in healthy cats.[10,11] A retrospective, epidemiological study reported that, after ruling out dehydration, cats with greater body weight were less likely to develop CKD.[11] A cross-sectional study showed similar concentrations of SDMA and other renal markers in cats with normal and high BCS.[10]

These findings are opposed to those observed in people, since higher body mass index has found to be an independent risk factor for glomerular filtration rate (GFR) decline,[17] and structural changes of the kidney have been observed in obese patients due to ectopic lipid accumulation.[8] The latter can cause renal compression, which may lead to activation of the renin angiotensin aldosterone system and glomerular hyperfiltration.[18] The fatty kidney has been linked to the development of CKD and hypertension in people.[19] Furthermore, perirenal or kidney tissue fat has also been detected in some animals with obesity. Obese rabbits have accumulation of sinus fat in their kidneys, as well as higher blood pressure, compared to controls.[20,21] In addition, pigs fed a high-fat diet develop some of the characteristics of the human metabolic syndrome, and structural kidney changes associated with kidney tissue fat deposits have been observed.[22] Similarly, in dogs, obesity can lead to increased blood pressure, hyperinsulinemia, activation of the renin-angiotensin system, glomerular hyperfiltration and structural kidney changes, such as increased mesangial matrix and

thickening of the glomerular and tubular basement membranes.[23] To our knowledge, no histological studies have been specifically performed in obese cats. The apparently differential effect of obesity on kidney function in this species will need further research.

In agreement with a previous study, we found no effect of age on creatinine concentrations in healthy cats,[24] but an effect of age on SDMA was observed. This might be related to a physiological decrement of glomerular filtration rate associated to age, as has been previously suggested in one study.[25] However, discrepancies exist about the correlation between GFR and age. One study reported differences between young (6-12 months) and aged adult cats (9-12 years) in GFR estimated with creatinine clearance, but not with iohexol clearance,[26] whereas another study including cats of a wide age range (1-17 years), did not find a correlation between age and GFR by any of these methods.[27]

In regard to uaTGFB:CR, it was considered a potentially interesting marker for the detection of early kidney damage in cats with obesity, since a previous study had shown that an increase in uaTGFB:CR preceded the onset of feline azotemia by six months.[14] However, in the present study, no variations in SDMA or creatinine that could be predicted occurred.

We acknowledge that this study has some limitations. Time of exposure to obesity may be crucial to observe changes in kidney function and may not have been long enough in this study, though we tried to minimize this effect by including cats that were at least 5 years old, knowing the incidence of obesity peaks in middle-aged (5-11 years) cats.[28] On the other hand, the number of cats included is small and, consequently, the study might be underpowered.

In summary, this study suggests that obesity does not contribute to CKD in cats. Further analysis with greater sample size and longer follow-up, or histological studies could be of interest.

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Table 1. Main baseline features of the 40 cats included in the study.

Variable	BCS=5 N=14	BCS>5 N=26	
Age (years)	5.92 (5.27-9.19)	7.17 (5.33-10.08)	0.585
Albumin (g/dL)	2.97 (0.32)	3.23 (0.82)	0.177
ALKP U/L	43.00 (38.25-69.00)	54.50 (42.50-71.25)	0.266
ALT U/L	34.00 (12.25-40.50)	26.50 (17.75-37.00)	0.900
Cholesterol (mg/dL)	146.00(113.00-211.50)	168.00 (127.00-199.00)	0.397
Creatinine (mg/dL)	1.61 (0.33)	1.76 (0.30)	0.163
Globulin (g/dL)	4.15 (3.90-4.35)	4.30 (3.80-4.50)	0.944
Glucose (mg/dL	113.00 (98.00-189.50)	130.50 (107.25-168.25)	0.601
SDMA (µg/dL)	9.00 (6.50-13.00)	11.00 (9.50-13.00)	0.499
Total proteins (g/dL)	7.26 (0.62)	7.21 (0.54)	0.382
Triglycerides (mg/dL)	46.00 (38.50-73.00)	85.50 (65.25-99.00)	0.002
UPC	0.14 (0.09-0.24)	0.10 (0.09-0.19)	0.457
Urea (mg/dL)	45.00 (38.25-50.25)	45.00 (41.00-52.50)	0.726
USG	1.050 (1044-1052)	1050 (1045-1054)	0.334
Weight (kg)	3.90 (0.87)	5.84 (1.23)	<0.005
uaTGFb1:Cr	4.52 (2.37-8.29)	7.68 (4.84-13.03)	0.134

*Two cats had not fasted for 12 hours and they were excluded for glucose measurement, cholesterol and triglycerides.

Abbreviations: ALT, alanine aminotransferase; ALKP, alkaline phosphatase activity; SDMA,symmetric dimethylarginine; UPC, urine protein/creatinine ratio; USG,urinary specific gravity.

Age, Albumin, ALKP, ALT, Creatinine, Globulin, Total proteins, Urea and Weight were measured in the total of cats

Glucose was measured in 14 cats with BCS=5 and 24 cats with BCS>5

Cholesterol was measured in 13 cats with BCS=5 and 23 cats with BCS>5

SDMA was measured in 13 cats with BCS=5 and 24 cats with BCS>5

Triglycerides were measured in 13 cats with BCS=5 and 22 cats with BCS>5

UPC was measured in 8 cats with BCS =5 and 21 cats with BCS>5

USG was measured in 9 cats with BCS=5 and 22 cats with BCS>5

uaTGFb1:Cr was measured in 6 cats with BCS=5 and 16 cats with BCS >5

Table 2. Variables measured at baseline and follow up visits (6, 12 and 18 months). In total, 40 cats had some follow-up and only 27 cats completed more than two visits.

Variable	time=0	time=6	time=12	time=18	p
Albumin (g/dL)	3.15 (0.32)	3.11 (0.24)	3.15 (0.29)	3.10 (0.14)	0.4687
ALKP U/L	28.50 (17.75-39.25)	25.00 (16.00-37.50)	31.00 (22.00-42.50)	36.00 (28.00-52.00)	0.7493
ALT U/L	50.00 (41.00-68.25)	52.00 (41.50-65.00)	51.00 (40.50-70.00)	51.00 (36.00-59.00)	0.8285
Cholesterol (mg/dL)	162.50 (125.50-195.50)	177.50 (123.50-201.75)	166.00 (148.25-191.50)	178.50(168.00-235.50)	0.2111
Creatinine (mg/dL)	1.71 (0.32)	1.73 (0.41)	1.70 (0.35)	1.64 (0.38)	0.8750
Globulin (g/dL)	4.20 (3.90-4.43)	4.00 (3.90-4.20)	4.00 (3.80-4.30)	4.10 (4.00-4.40)	0.3114
Glucose (mg/dL	121.00 (103.25-156.75)	115.50 (102.00-154.50)	104.00 (90.00-171.75)	140.00 (101.75-182.00)	0.0498
SDMA (µg/dL)	9.00 (8.00-11.00)	9.00 (7.50-12.00)	10.00 (9.00-12.00)	9.00 (8.00-10.00)	0.2977
Total proteins (g/dL)	7.35 (6.90-7.73)	7.20 (6.80-7.45)	7.10 (6.95-7.80)	7.40 (7.10-7.70)	0.0609
Triglycerides (mg/dL)	72.00 (51.00-93.00)	74.00 (55.50-107.25)	74.00 (58.75-92.25)	142.50 (86.50-220.25)	0.4437
UPC	0.10 (0.09-0.19)	0.14 (0.11-0.17)	0.12 (0.10-0.18)	0.14 (0.14-0.17)	0.0847
Urea (mg/dL)	45.00 (40.50-51.25)	43.00 (39.00-47.00)	43.00 (39.00-47.00)	47.00 (41.00-49.00)	0.0862
USG	1050.00 (1045.00-1052.00)	1047.00 (1041.25-1051.00)	1047.00 (1042.00-1048.75)	1052.00 (1046.25-1056.25)	0.4548
Weight (kg)	5.16 (1.45)	5.30 (1.59)	5.11 (1.67)	6.03 (1.58)	0.4418

*Normally distributed data are given as mean and standard deviation, whereas non-normally distributed data are given as median and IQR (25th, 75 percentile).

**Abbreviations: ALT, alanine aminotransferase; ALKP, alkaline phosphatase activity; SDMA, symmetric dimethylarginine; UPC, urine protein/creatinine ratio; USG, urinary specific gravity.

Figure 1. SDMA concentration according to age in cats with BCS=5 and cats with BCS>5 after a median follow-up of 12 months.

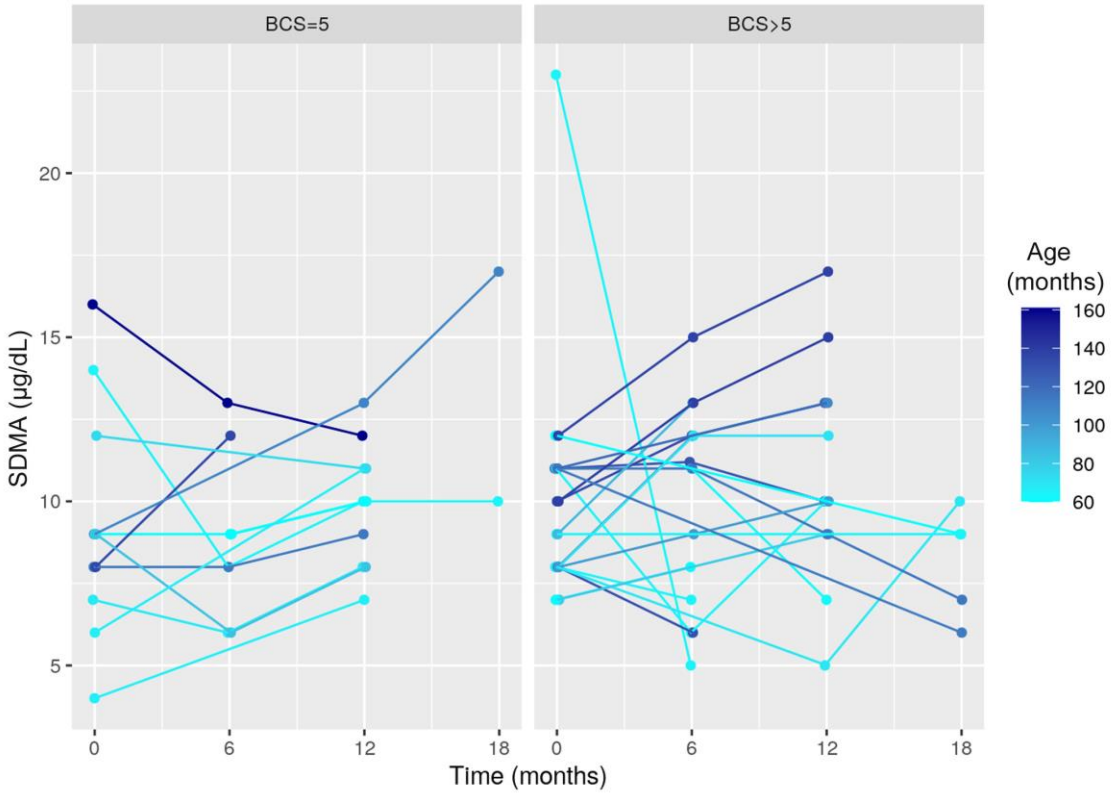
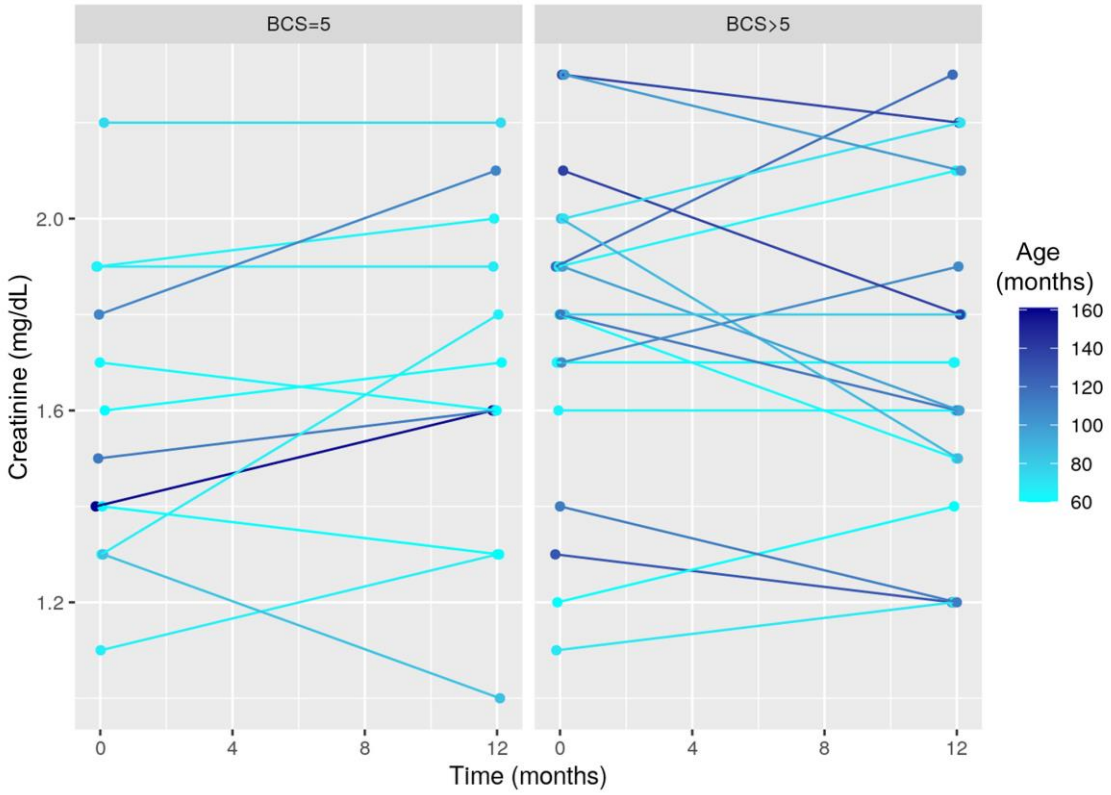


Figure 2. Creatinine concentration according to age in cats with BCS=5 and cats with BCS>5 with at least 12 months' follow-up.



CONCLUSIONS

1. Diabetes is associated with chronic kidney disease in adult cats. After adjustment for age, cats with diabetes are four times as likely to have chronic kidney disease compared to cats without diabetes.

2. Established and potential markers of renal disease are not associated with body condition score in cats. Therefore, obesity does not seem to be linked to chronic kidney disease in adult cats.

3. Cats with increased fructosamine have lower SDMA than those with normal fructosamine. Abnormal glucose metabolism may be associated with glomerular hyperfiltration in cats.

4. Increased fructosamine is more strongly associated with measures of insulin sensitivity than increased fasting blood glucose.

5. Feline overweight and obesity do not have an impact on renal function during a median follow-up of 12 months.

ANNEXE I

Additional published or submitted work during the predoctoral fellowship.

1. Book Chapters:

- Melián C, Pérez-López L. Other Adrenal Cortical Tumours and Pheocromocytoma. In: Fracassi F, Peterson ME, Feldman E Handbook of Feline Endocrinology. 1st ed. Milán: EDRA; 2019. p: 392-401.

2. Journal articles:

- Melián C, Pérez-López L, Saavedra P, Ravelo A, Santos Y, Jaber JR. Ultrasonographic evaluation of adrenal gland size in healthy dogs and in dogs with hyperadrenocorticism. Veterinary Record. 2020 (Submitted).

  Journal Impact Factor: 2.101

  Rank: 16/141 Q1, Veterinary Sciences

- Arenas C, Pérez-Alenza MD, García-San José P, Llauet L, Pérez-López L, Melián C, Feldman E. Serial cortisol concentrations, urine and ACTH stimulation test results to monitor trilostane treatment in dogs with pituitary dependent hyperadrenocorticism. J Vet Intern Med. 2020. DOI: 10.1111/jvim.15830.

  Journal Impact Factor 2.286

  Rank: 12/141 Q1, Veterinary Sciences

- Melián C, Pérez-López L. Tratamiento y control de la diabetes en gatos. [Monitoring and treatment of diabetes in cats]. Revista Clínica de Medicina Felina. Multimédica Ediciones Veterinarias. 2020;5.
  ISSN:2604-6687

- Pérez-López L, Melián C. Algoritmo para el diagnóstico de la enfermedad Addison. [Algorithm for the diagnosis of Addison's disease]. Medicina interna práctica de pequeños animales. Endocrinología, Nefrología urinario 2020. Vol. April: 6-7.
  ISSN: 2462-7356

- Melián C, Pérez-López L. La alimentación en perros y gatos con diabetes [Feeding in dogs and cats with diabetes]. Ateuves 2019;83:13-15.
  ISSN: 1885-8481

- Pérez-López L, Brito-Casillas Y, Wägner AM, Melián C. Diabetes Mellitus en un perro con carcinoma follicular de tiroides. [Diabetes mellitus in a dog with follicular thyroid carcinoma]. Clin Vet Peq Anim 2018; 38(1):23-27.
  ISSN: 1130-7064

3. Conference papers:

- García-San José P, Pérez-Alenza MD, Arenas C, Llauet L, Pérez-López L, Melián C, Feldman E. Assessing various parameters to assess trilostane response, including cortisol concentrations, in dogs treated for naturally occurring pituitary dependent hyperadrenocorticism. XIII Southern European Veterinary Conference. Seville (Spain), 7-9th November 2019.

- Pérez-López L, Jaber JR, Ravelo A, Santos Y, Melián C. Ultrasonographic evaluation of adrenal gland thickness in healthy dogs and in dogs with hyperadrenocorticism. Congress of the European Collegue of Veterinary Internal Medicine – Companion Animals. Milán (Italy) 19-21th September 2019.

- Pérez-López L, Brito-Casillas Y, Melián C, Wägner AM, Boronat M. Correlación de la fructosamina y la glucosa basal con el sobrepeso u obesidad en gatos sin diabetes. [Correlation of fructosamine and fasting glucose with overweight and obesity in cats without diabetes]. XII Southern European Veterinary Conference. Madrid (Spain), 10-20th October 2018.

- Pérez-López L; Melián C. Diabetes Mellitus en un perro con carcinoma follicular de tiroides. [Diabetes mellitus in a dog with follicular thyroid carcinoma]. IV Congreso de Animales de Compañía de Canarias. Las Palmas de Gran Canaria (Spain). 20th -22nd October 2016.

4. Press:

   - L. Pérez-López, AM. Wägner. La obesidad en nuestras mascotas. La Provincia; 12 Mayo de 2019.

ANNEXE II

1. Grants

   - Pre-doctoral fellowship by University of Las Palmas de Gran Canaria (2016-2020).
   - Research Grant of "El Consejo Social of the ULPGC" in 2018: 2.000 euros for expenses of the doctoral thesis.
   - Erasmus Traineeship Grant to perform a three month externship at the Veterinary Teaching Hospital of Ghent University.

2. Awards

   Best poster Award of the European Society Endocrinology at the 29th Annual ECVIM-CA Congress (Milán, Italy, 19-21th, 2019).

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To whom it may concern

DATE	PAGE	OUR REFERENCE
02 March 2020	1/1	Externship Certificate

To whom it may concern,

This is to certify that Laura Pérez López was engaged with our organization over a period of 3 months from October 2019 to January 2020. During her externship Laura rotated in nutrition, internal medicine, and medical imaging services at the University Veterinary Teaching Hospital of Ghent University. She attended weekly seminars, she reinforced clinical knowledge, and she gained experience in diagnostic imaging techniques. Furthermore, as a result of her externship, a collaboration arose between departments of medical imaging, nutrition and the research group were Laura belongs: Diabetes and applied endocrinology from University of Las Palmas de Gran Canaria. A project entitled: " Utility of shear wave elastography to detect early chronic kidney disease in cats" was sent to the ethical committee. The approval has not been received yet, but we all look forward to perform this project soon.

Sincerely yours,

Professor Myriam Hesta
DVM, PhD Vet Sci, Dip ECVCN

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UNIVERSITY

Agradecimientos

A Ana Wägner, gracias por haberme dado esta oportunidad y por tu confianza en mí. Así como por el tiempo y trabajo que has dedicado a esta tesis, ya que sin tu ayuda y dirección no hubiera sido posible. Además tu energía y tu actitud siempre tan positiva fueron las mejores influencias con las que pude tropezar.

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A Pedro Saavedra, gracias por tu valiosa aportación a esta tesis y por buscar esos ratitos para atenderme con esa amabilidad que te caracteriza.

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A las personas que depositaron su confianza en este estudio, y que velando por lo mejor para sus animales, decidieron participar. Ellos son los verdaderos responsables de que este proyecto se haya realizado.

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A Alicia y Alberto, por su apoyo.

A mi familia, en especial a mi padre, mi madre y mis abuelos por su infinito cariño y apoyo incondicional. Y por supuesto a Alberto, gracias por aportarme tanto cada día.