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INFLUENCE OF OVERWEIGHT ON ROUTINE PARAMETERS OF RENAL FUNCTION IN DOGS

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“It always seems impossible until it’s done“
by Mandela

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ABSTRACT:

INFLUENCE OF OVERWEIGHT ON ROUTINE PARAMETERS OF RENAL FUNCTION IN DOGS

Obesity is one of the most common nutritional disorders in dogs and its prevalence has grown exponentially in recent years alongside with obesity in humans. It is from an accumulation of adipose tissue in such a way that it affects the patient health. In humans, a chronic increase of body weight is a risk factor to develop renal dysfunction. Therefore, the purpose of this study was to investigate the effect of weight gain on renal function in dogs. Renal function was determined using traditional markers of renal dysfunction (blood urea nitrogen and creatinine, urine specific gravity and urine protein-creatinine ratio). A total of 16 beagles were studied, 8 non-obese, which kept a stable ideal body weight, and the remaining 8, gradually increased weight for 24 weeks. Both groups were fed a commercial adult maintenance diet high in fat and protein. The obese group was fed 1.3 times more than the maintenance energy requirements, resulting in an average of 0.328 kg increase in weight per month. Renal markers were measured at times 1, 12 and 24 weeks and the results did not showed a significant difference between the groups. These results suggest that the degree of overweight that was achieved in this study did not cause renal dysfunction based on routine kidney markers.

KEY WORDS: obesity, overweight, canine, renal insufficiency, obesity-related glomerulopathy, proteinuria

RESUMO:

A INFLUÊNCIA DO EXCESSO DE PESO EM PARÂMETROS DE AVALIAÇÃO DA FUNÇÃO RENAL DE ROTINA

A obesidade, uma das doenças nutricionais mais comuns em cães tendo a sua prevalência crescido exponencialmente nos últimos anos, a par com a obesidade nos humanos. É uma doença crónica que advém do excesso de acumulação de tecido adiposo de tal forma que afeta a saúde. No homem, uma condição corporal aumentada é um fator de risco para disfunção renal. O objetivo deste estudo foi investigar o efeito do aumento de peso sobre a função renal em cães. A função renal foi determinada através de marcadores renais convencionais de insuficiência renal (ureia e creatinina sérica, densidade urinária, rácio proteína-creatinina de urina). Um total de 16 beagles foram usados, 8 não obesos que mantiveram um peso corporal estável, e os 8 restantes, que aumentaram progressivamente o peso durante 24 semanas. Ambos os grupos foram alimentados com uma dieta comercial de manutenção, rica em gordura e proteína. Ao grupo obeso foi fornecido 1,3 vezes mais que os requisitos energéticos de manutenção, fazendo com que este grupo aumenta-se 0,328kg mais por mês. Os marcadores renais foram medidos às 1, 12 e 24 semanas em ambos os grupos, não tendo demonstrado diferenças significativas, sugerindo que o grau de excesso de peso obtido neste estudo não causa alterações na função renal.

Palavras-chave: obesidade, excesso de peso, canideo, insuficiência renal, obesidade relacionada com glumerolopatia, proteinúria

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LIST OF ABBREVIATIONS AND SYMBOLS

ACE - Angiotensin-converting enzyme
ACVIM - American College of Veterinary Internal Medicine
APOP - Association for Pet Obesity Prevention
APPA - American Pet Products Association
BAT - Brown adipose tissue
BCM - Body cell mass
BCS - Body condition score
BMI - Body mass index
BP - Blood pressure
BW - Body weight
BUN - Blood urea nitrogen
CCAAT – Cytosine-cytosine-adenosine-adenosine-thymidine
C / ebpα - CCAAT / enhancer binding protein α
CKD - Chronic kidney disease
CRP - C-reactive protein
DER - Daily energy requirement
DEXA - Dual energy x-ray absorptiometry
DM - Dry matter
ESRD - End-stage renal disease
FM - Fat mass
FCR - Flat-coat retriever
GFR - Glomerular filtration rate
GI - Gastrointestinal
IL - Interleukin
Inos - Nitric oxide synthase
IRIS - International Renal Interest Society
Kcal - Kilocalories
Kg - Kilogram
LBM - Lean body mass
LDL – Low Density Lipoprotein
LIM - Length of the lower leg
ME - Metabolic energy
MER - Maintenance energy requirement
MRI - Magnetic resonance imaging
Nefas - Non-esterified fatty acids
NO - nitric oxide

NRC - National research council

OA - Osteoarthritis

PK - Pharmacokinetic

PDSA - People's Dispensary for Sick Animals

% BF- Percentage of body fat

PPARY - Peroxisome proliferator-activated receptor y

POMC - Pro-opiomelanocortin

RAAS - Renin–angiotensin–aldosterone system

RBW - Relative body weight

RER - Resting energy requirement

SDMA - Symmetric dimethylarginine

SNS - Sympathetic nervous system

TG - Triglycerides

TNF-α - Tumor necrosis factor alfa

UK - United Kingdom

UPCR - Urine protein-creatinine ratio

US - United States

USG - Urine specific gravity

VLDL - Very low density lipoproteins

WAT- White adipose tissue

WHO - World Health Organization

PART I - INTERNSHIP REPORT

As part of the Integrated Master's Degree in Veterinary Medicine from the Faculty of Veterinary Medicine, University of Lisbon, I completed the internship with a total duration of six months. The internship took place in the Laboratory of Animal Nutrition, Department of Nutrition Genetics and Ethology, at the Faculty of Veterinary Medicine of the Ghent University, from the end of January to the end of June 2016. In this Laboratory work researchers from all over the world, who perform studies in the areas of nutrition, conduct food-related experiments with dogs, cats, chickens, ponies, pigeons and pigs for third parties, but also do nutritional analysis in the framework of services and clinic. Furthermore, the staff provides dietary advice to veterinarians, pet owners, businesses, government and other agencies. These include clinical consultations concerning dietetics. The main research themes of the lab are feed intake regulation and energy homeostasis, the impact of nutrition on intestinal microbiota and host physiology, the role of nutrition in immune competence and inflammatory diseases and the role of trace elements in the metabolism.

I had the opportunity to experience a new working environment and interact with highly dedicated scientists on a daily basis. The atmosphere was friendly and people were welcoming, which contributed to a memorable experience.

During these months, I performed tasks in the area of clinical nutrition of small animals, under the supervision of Prof. Dr. Myriam Hesta. My supervisor also gave me permission to follow her nutritional advice consultations as well as nutrition classes with other master students at the hospital. I also had the pleasure to work with Daisy Liu, who gave me the opportunity to follow and help in her PhD research about the effect of obesity on renal function in dogs. Based on this study I was able to develop and write my Master's dissertation about the "Influence of overweight and obesity on some parameters (creatinine, blood urea nitrogen (BUN), urine protein\creatinine ratio (UPCR) and specific gravity) of the renal function in dogs".

With the support from resident students Veerle Vandendriessche and Wendy Wambacq and the teaching assistant Sophie Dupont, and based on our weekly meetings and the nutrition clinical cases they gave me to resolve, I was able to gain a broad range of skills in a short period of time. I performed literature review and analyzed different clinical nutrition cases in dogs and cats in the literature to include in my project. I gained valuable knowledge on interpreting the nutritional necessities in different life stages and also in different disease stages, and how nutrition can treat specific disease conditions such as allergies or food intolerances, nephrology bladder stones. I also learnt, at an advanced level, to understand and be more critical about commercial feed, and I improved my English language skills considerably.

In summary, this internship, with its challenges and opportunities, contributed for the successful conclusion of this dissertation.

PART II - REVIEW ON OBESITY/OVERWEIGHT AND KIDNEY DYSFUNCTION

I. Introduction

In recent years, obesity has become an escalating global problem in humans. It has become a significant worldwide health epidemic, especially in developing countries, and is already considered the disease of the twenty-first century (WHO, 2016). The World Health Organization (WHO) estimates that 39% of adults (aged 18 years and over) were overweight in 2014, and 13% were obese (WHO, 2016). In 1980 the numbers were quite different, with 23.2% of the population being overweight and only 5.4% were effectively obese (WHO, 2016). This means that the prevalence of obesity more than doubled between 1980 and 2014 (WHO, 2016). The same is happening with our pets: the prevalence of pet obesity is increasing (German, 2006a; Raffan, 2013; Sandøe, Palmer, Corr, Astrup & Bjørnvad, 2014).

This health problem is not just the accumulation of large amounts of adipose tissue, but is also associated with important metabolic and hormonal changes in the body (Zoran, 2010). Obesity also leads to a reduction in longevity in dogs. A study carried out with a group of 48 Labrador retrievers showed that the group with dietary restriction remained leaner and lived longer (median lifespan of 13 years compared to 11.2 years for the moderately overweight group) (Kealy, et al., 2002; Lawler et al., 2005; Osto & Lutz 2015). Obesity in humans is widely recognized as a risk factor for cardiovascular disease and for various metabolic disorders such as type II diabetes and hypertension, but also kidney diseases (German, 2006a). Actually, increases in chronic kidney disease (CKD) prevalence have paralleled increases in the prevalence of overweight and obesity in humans (Hall et al., 2014). However, studies have indicated that even in the absence of diabetes type II or systemic arterial hipertension, which together account for more than 75% of all cases of end-stage renal disease (ESRD), obesity itself can contribute to kidney damage or has a negative impact on the progression of renal disease (Griffin, Kramer & Bidani, 2008; Hall et al., 2014).

Similar relationships may hold true for dogs. However, there isn't an evident correlation between obesity and kidney disease yet in this species. Experimentally induced obesity in dogs performed by Henegar et al. (2001) showed that blood pressure, pulse rate, glomerular filtration rate (GFR) and renal plasma flow were all significantly higher in obese versus non-obese dogs. Also, at histological analyses the kidney structure of obese dogs demonstrated enlarged Bowman's space, increased glomerular cell proliferation, increased mesangial expansion and thickened basement membranes, and increased expression of renal transforming growth factor (TGF). However, the glomerulosclerosis scores for obese

versus lean dog kidneys were not statistically different (Henegar et al., 2001). In another study that was performed with obese pet dogs in a weight-loss program showed that the increased levels of biomarkers of renal injury (homocysteine, cystatin, and clusterin) and the increased urine protein to creatinine ratios improve after weight-loss (Tvarijonaviciute et al., 2013). Based on this assumption, the present master dissertation consists primarily of a critical evaluation of the pathogenic potential of obesity, which may be responsible for the development of progressive renal disease in obese dogs, irrespective of other common obesity-related diseases. Knowledge of obesity/overweight as a risk factor for renal disease can heighten awareness and target health screening of dogs. And with more evidence from canine research studies as a tool, practitioners may be able to advocate more strongly for obesity prevention and weight reduction plans for their clients' pets.

The present dissertation is divided into 3 parts: the first is a brief description of the completed internship; the second part includes a review of the literature on the problem of overweight and obesity in dogs and the effect of overweight on renal function with focus on the possible pathophysiological mechanisms, and in the final of this part there is also a briefly discussion about the nutritional concerns when a dog has obesity and renal insufficiency; the last part aims to demonstrate the study and the results of the study in question, as well as the discussion of then.

II. The Problem of Obesity

Due to the emphasis on dogs as respected and valued members of society, the goals of canine nutrition, defined as longer life, higher quality of life and better performance, are now similar to that of human nutrition (Toll, Yamka, Schoenherr & Hand, 2010). Besides this positive factor, nowadays, it is also more and more common to see pet owners consider their pets as family members (Toll et al., 2010). This human-animal bond is characterized by an excessive anthropomorphic or anthropocentric behavior. Food and other factors play an important role in this relationship, and are responsible for the most common nutritional disorder in companion animals in industrialized countries, obesity (Kienzle, Bergler & Mandernach, 1998). This chapter will approach the problem of obesity in dogs, showing its dimension in our society, as well as the factors that can predispose dogs to this problem and the consequences that it can bring.

1 DEFINING THE PROBLEM

1.1 Definition of overweight and obesity

Obesity has been defined as excessive fat accumulation that may result in a significant impairment of health, and is the most common nutritional disorder in companion animals (National Institutes of Health. 1985; German, 2006a; Zoran, 2010; Laflamme, 2006). This state of energy imbalance is due to excessive dietary intake or inadequate energy utilization (German, 2006a). Accumulation of fat is achieved by increasing the number of fat cells (hyperplastic obesity) and/or due to increasing the size of fat cells (hypertrophic obesity) (Burkholder & Toll 2010). Body composition studies of dogs indicate that animals judged to be in ideal body condition have 15 to 25% body fat (Laflamme, 1997; European Pet Food Industry Federation, 2013). When body fat exceeds 30-40%, the animal is considered obese, while the excess of 25-35%, the animal is considered overweight (Burkholder et al, 2010; European Pet Food Industry Federation, 2013).

1.2 Prevalence of overweight and obesity

Recent years have seen a drastic increase in the rates of overweight and obesity among humans living in developed countries (German, 2006a; Zoran, 2010; Sandøe et al, 2014). The worldwide prevalence of obesity in adults aged 18 years and over more than doubled between 1980 and 2014 (WHO, 2016). The reported prevalence in dogs in several studies in United States of America (USA), Europe and Australia was between 22 percent and 44

percent, although there are differences in prevalence in the literature that may reflect differences in sampling (e.g., who has been asked (owners or vets) or local variations) (Sandøe et al., 2014; McGreevy et al., 2005; Robert & Reither, 2004). In dogs, some investigators agree that the prevalence of pet obesity is increasing in a similar fashion to human obesity (German, 2006a). In 2016, in USA, the Association for Pet Obesity Prevention (APOP) has estimated that 53.9% of USA dogs are overweight or obese, which was 1.4% more than in 2012 (Association for Pet Obesity Prevention, 2016). This means, in 2015, 41.9 million US dogs were estimated to be overweight or obese, from a total 77.8 million US dogs, according to American Pet Products Association (APPA) source data from 2016 (Association for Pet Obesity Prevention, 2016). In the United Kingdom (UK), another new report from People's Dispensary for Sick Animals (PDSA) Animal Welfare estimates that around 40% of dogs in the UK are thought to be overweight or to obese, and that the proportion of overweight pets will continue to rise (PDSA Animal Welfare, 2017).

In general, specialists agree that the nutrition-related disorder most seen in companion animals nowadays is the excess weight gain (Sandøe et al., 2014). This statistic is clinically relevant since obesity is not only a huge accumulation of adipose tissue but also involves important changes at the metabolic and hormonal level in the body (Sandøe et al., 2014). Therefore, there is clinical relevance for deciding whether the animal has an ideal, overweight or obese body condition since these two last scenarios might negatively affect an animal's health. Although, there is no ideal method to evaluate the body condition, many different methods exist.

2 DIAGNOSTIC

In the common routine most of the veterinarians diagnose obesity only through visual inspection. The subjectivity inherent in this practice makes this a method less sensitive from a clinical perspective. The assessment of body composition using objective measurements can identify and quantify excess of body weight (BW) and can set an optimal weight. Methods that estimate of the correct percentage of body fat (% BF) are the most accurate procedure to diagnose obesity (Toll et al., 2010; Laflamme, 2006; Case, Carey, Hirakawa & Daristotle, 2000).

Diagnosis of overweight and obesity in humans is relatively simple, requiring only measurement of height and weight to calculate body mass index (BMI) (BMI >25 is overweight) (WHO, 2016). In dogs, due to body conformation variations across breeds, the variation of frame size within breeds and age variation, the diagnosis of overweight and obesity is more difficult (German, 2006b). There is no ideal, definitive method for deciding whether a dog is in a thin, ideal, overweight or obese body condition (Toll et al., 2010;

German, 2006b). Many different systems have been developed for the measurement fat mass (FM) and lean body mass (BM) in companion animals, but few have been validated for use as routine tests due to some lack of accuracy, price or practicability (Toll et al., 2010; German, 2006b). These systems can also be classified into two categories: the clinically relevant techniques and the research techniques (Table 1). The next sections will discuss some of these techniques in detail. The most widely adopted procedures in the clinical routine are the quantitative procedures, namely the BW measurement combined with body condition score (BCS), which is preferred above other morphometric methods (Toll et al., 2006; German, 2006b; Elliott, 2006a).

Table 1 - Methods for body composition analysis in dogs (adapted from German, 2006b)

Clinical techniques	Advantage	Disadvantage
BW	Objective, easy and quick to use	Has little meaning when it's used alone
BCS	Easy and quick to use and doesn´t required any equipment	Subjective and needs experience
Tape measurements	Easy, quick and cheap	There are not many studies that relate the pelvic circumference to the % body fat
BMI	Easy, quick and cheap	Needs to be breed specific
Skinfold thickness measurement	Easy, quick and cheap	The elasticity of the skin can create false positives
Bioelectrical impedance analysis	Inexpensive, portable, simple, safe, quick, noninvasive	Many variables to control (hydration status, consumption of food and water, skin and air temperature, recent physical activity, conductance of the examination table, patient age, size, shape and posture in addition to electrode positioning)
Research techniques	**Advantage**	**Disadvantage**
Dilution techniques - Total body water - Extracellular fluid	Acceptable in all breeds, objective	Expensive equipment and labor for analyses, the assumption of the hydration (can change with age, sex, species, breed or disease)
Dual energy x-ray	Easy to use, low radiograph	Expensive equipment and

absorptiometry (DEXA)	radiation exposure, accurate for limb lean and fat	specialized radiology technician required and also anesthesia.

BW (body weight); BCS (body condition score); BMI (body mass index); DEXA (Dual energy x-ray absorptiometry

2.1 Common clinical methods

The combination of BW and BCS has been used to define criterion for canine obesity, because these parameters are easier to measure than body fat (Freeman, et al., 2011).

2.1.1 Body weight and Relative Body Weight

A complete physical exam should include an assessment of BW. BW is the simplest technique and it provides an approximate measure of the total energy stored in the body (Elliott, 2006a). Although BW by itself distinguish say if the patient is overweight, underweight, or in ideal body condition, when it's used complementarily with another method it´s very useful, e.g., BCS (German, 2006b; Burkholder, 2000a; Case et al., 2000). Furthermore, BW can vary from day to day, so it is important to use the same method for an individual animal each time to avoid more external variation (German, 2006b; Burkholder, 2000a; Case et al., 2000).

Relative body weight (RBW) in terms of an animal's ideal weight has been selected as a criterion for diagnosing obesity since it is easier to measure BW than body fat (Burkholder, 2000a; Case et al., 2000). RBW is the ratio between the current weight an animal and its estimated optimal weight (Burkholder, 2000a; Case et al., 2000). An overweight dog's RBW range is on average between 10 and 20% above the optimal weight and for obese dogs it is 20% or more above the ideal weight (Toll et al., 2010; Burkholder, 2000a). Although easy to apply, this method presents some critical issues. Establishing an optimum weight is a challenging task for the veterinarian. Domestic canids are the most diverse mammalian species in terms of weight and body size. Burkholder (2000a) suggest that BW is recorded when the animal reaches adulthood as a reference. Most dogs reach adult weight at 12 months, and large breeds may reach it only at 18 months (e.g. Bernese Mountain, Bullmastiff, Dogue de Bordeaux) (Hand et al., 2010). Nonetheless, if an animal does not have access to nutrition that satisfies their nutritional needs during growth, mature adult weight may not match their optimal weight. In cases of pure breeds, the American Breeder Club establishes the averages of optimal weights, although even in the same breed the optimum weight may differ 25% or more (Toll et al., 2010). Thus, determining the optimal weight requires some care.

2.1.2 Morphometric measurements

Morphometry is a technique that comprises of a set of simple, economic and non-invasive procedures to assess body composition using different body parameters (German, 2006b; Toll et al., 2010, Eliiot, 2010). According to German (2006b), there are 3 main methods, which are the BCS, the measurement of skinfold thickness and the dimensional evaluations.

2.1.2.1 Skinfold thickness measurement

This morphometric analysis is based on the relationship between the thickness of subcutaneous fat layer and total body fat (Elliott, 2006a). It is widely used in humans to calculate the %BF from measurements of the thickness of the subcutaneous fat layer that lies between the dermis and muscle (Elliott, 2006a). In dogs, this layer is deposited in significant quantities in the thoracic region, the lumbar, coccygeal areas and the intra-abdominal region, usually with a higher fat deposition in the lumbar region (Toll et al., 2010). Unfortunately, the skin in dogs is easily detached from the subcutaneous adipose tissue making skinfold measurement impractical and unreliable in this species (Elliott, 2006a).

2.1.2.2 Dimensional evaluations

Dimensional evaluations estimate mathematically and statistically the %BF by the measurements of various anatomic circumferences and lengths (Fig.1) (Elliot, 2010). Waist circumference measurement in humans, a simple and practical procedure, is an approximate index of intra-abdominal fat and total %BF (WHO, 2000). Burkholder (2000a) refer to two studies that show that the measurement of pelvic circumference in dogs is proportional to the amount of fat and that this is the dimension that alters the most with increasing weight, like in humans (Burkholder, 2000a). In these studies they measured diferents parts of the animal's body and convert this measurements into a equation (tablet 2) to estimate the percent body fat. The choosen measures to creat these equation was the height at the shoulder (HS) measured in centimeters and the pelvic circumference (PC) at the level of the flank measured in centimeters too. The pelvic circumference measurements is the one who change most with weight gain in the dogs. The reasonable correlation existed between the %BF calculations (using gender-specific or gender nonspecific equation) was determined by DEXA (Burkholder, 2000a; Mawby et al., 2004). However, to obtain reliable results of %BF through the equations, the dog cannot exceed the weight limits of the studied groups (7.3 to 34.5 kg) and the %BF limits obtained (from 1% to 33%).

Because of the variaion in body types among breeds these measurements may not always correlate well with %BF (Burkholder, 2000a; Mawby et al., 2004). Despite this, the advantage of these evaluations is that they would be helpful to convince the owners that their animals are indeed overweight and in need of weight loss (Burkholder, 2000a; Mawby et al., 2004).

Figure 1 - Anatomical sites to measure zoometric variables in dogs (Adapted from Burkholder, 2000a).

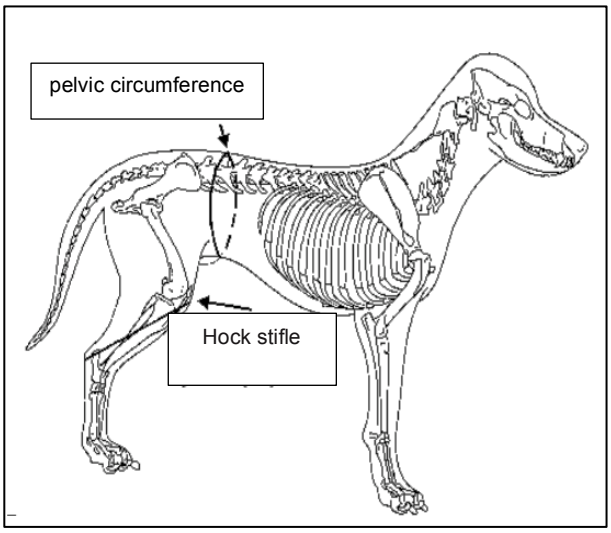


Table 2 - Equations for converting morphometric measurements into fat mass percentages (Adapted from Mawby et al., 2004).

Dogs
Gender specific formula:
% BF in males = -1.4 (HS in cm) + 0.77 (PC in cm) + 4
% BF in females = -1.7 (HS in cm) + 0.93 (PC in cm) + 5
Non-gender specific formula:
% BF in both sexes = -0.0034 $(HS \text{ in cm})^2$ + 0.0027 $(PC \text{ in cm})^2$-1.9 / BW(kg)

BF – body fat; Cm – centimeters; HS- hock stifle; PC- pelvic circumference at the level of the flank; BW – body weight; Kg – kilogram

2.1.2.3 Body condition scores

BCS is a semi-quantitative but subjective method for evaluating body composition, particularly the percentage of body fat, and for estimating the degree of over- and underweight (Freeman et al., 2013; European Pet Food Industry Federation, 2013; Michel, 2012; Elliot, 2010; Toll et al., 2010; German, 2006b; Burkholder, 2000a).

This scoring system requires prior training and observer experience to carefully follow the criteria that are defined, which are the visual assessment of the pet and palpation to assess body fat over the ribs, abdomen, lumbar area, and tail base (Freeman et al., 2013;

European Pet Food Industry Federation, 2013; Elliot, 2010; Toll et al 2010). Various systems of 3-, 5-, 6- and 9-points were proposed (Elliott, 2006a; Hand et al., 2010). The two most common in clinical practice are 5- (Edney and Smith, 1986) and 9-point systems (Laflamme, 1997), where the classification for ideal body condition is 3 and 5, respectively. The most widely accepted and validated system is the 9-point system (European Pet Food Industry Federation, 2013; Hand et al, 2010; German et al. 2006; Burkholder, 2000a; Laflamme, 1997). Laflamme (1997) demonstrated a good correlation between BCS and %BF measured with DEXA, a noninvasive technique that can be used to estimate body composition, in which for each unit increase in BCS on a nine-point scale there is approximately 5% increase in body fat (Burkholder, 2000a). Moreover, studies show good concordance between BCS measurements from diferent experienced observers (Raffan et al., 2006; Mawby et al., 2004).

Table 3 shows the BCS (9-point and 5-point system) in dogs, including a description of the palpation location and the corresponding percentages of body fat as well as the increase or decrease of BW under or above optimal BW. Using the BCS in conjunction with BW gives a clinician a more complete perspective on a patient's body condition and provides a good basis for determining energy requirements (Michel, 2012; Burkholder, 2000a). The combination of these two measures can be extremely helpful for owners that usually do not recognize if their animal is overweight or obese and thus may contribute to the success of weight-loss programmes (Toll et al., 2010).

Table 3 - Guide to 9-point and 5-point Body Condition Scores in dogs (European Pet Food Industry Federation, 2013) (page 11-13)

Score		Location Feature	Estimated body fat (%)	% BW below or above BCS 5
9 points	5 points			
1 Emaciated	1	Ribs and bony prominences are visible and easily palpable with no fat cover. Severe abdominal tuck when viewed from the side and an exaggerated hourglass shape when viewed from above. Tail base prominent, raised bone structures with no tissue between the skin and bone. Obvious loss of muscle mass and no discernible body fat.	≤4%	- ≥40%
2 Very Thin		Ribs and bony prominences are visible and easily palpable with no fat cover. Severe abdominal tuck, when viewed from the side	4-10%	- 30-40%

Continued from page 11 (11-13)				
2 Very Thin		and a marked hourglass shape when viewed from above. Tail base prominent, raised bone structures with no tissue between the skin and bone. Minimal loss of muscle mass.	4-10%	- 30-40%
3 Thin	2	Ribs and bony prominences are easily palpable with minimal fat cover. Marked abdominal tuck when viewed from the side and an obvious waist when viewed from above. Tail base raised bony structures with little tissue between skin and bone.	5-15%	- 20-30%
4 Slightly underweight		Ribs and bony prominences are easily palpable with minimal fat cover. Abdominal tuck when viewed from the side, and a well-proportioned waist when viewed from above. Tail base raised bony structures with little subcutaneous tissue.	10-20%	- 10-15%
5 Ideal	3	Ribs and bony are not visible, but easily to palpable with thin layer of fat, as well other bony prominences are palpable with a slight fat cover. Abdominal tuck is present when viewed from the side, and a well-proportioned lumbar waist when viewed from above. Tail base smooth contour or some thickening, bony structures palpable under a thin layer of subcutaneous fat.	15-25%	0%
6 Slightly overweight		Ribs and bony prominences can be felt under a moderate fat cover. Abdominal tuck and waist are less pronounced. Tail base smooth contour or some thickening, bony structures remain palpable under moderate layer of subcutaneous fat.	20-30%	+10-15%
7 Overweight	4	Ribs and bony prominences can be felt under a moderate fat cover. Little abdominal tuck when viewed from side or waist, and back slightly broadened when viewed from above. Tail base Smooth contour or some thickening,	25-35%	+20-30%

Continued from page 12 (11-13)				
7 Overweight	4	bony structures remain palpable under subcutaneous fat.	25-35%	+20-30%
8 Obese		Ribs and bony prominences are difficult to palpate, under a thick fat cover. Other bony prominences are distended with extensive fat deposit. Tail base appears thickened, difficult to palpate bony structures. General ventral bulge under abdomen, no waist, and back markedly broadened when viewed from above. Fat deposits over lumbar area and neck.	30-40%	+30-45%
9 Grossly Obese	5	Ribs and bony prominences are very difficult to feel under a thick fat cover between bone and skin. Tail base appears thickened, bony structures almost impossible to palpate. General pendulous ventral bulge under abdomen, no waist, back markedly broadened when viewed from above. Fat deposits over lumbar area, neck, face, limbs and in the groin. A dip may form on the back when lumbar and thoracic fat bulges dorsally.	>40%	+>40%

2.2 **Research techniques**

Body fat can be accurately measured by other sophisticated techniques, e.g. dilution techniques, bioelectrical impedance analysis, computerized axial tomography, magnetic resonance imaging, total organic determination of potassium and DEXA (German, 2006b). Often, these methods are expensive and require specialized equipment and/or general anesthesia and are not practical or available for clinical applications (German, 2006b; Elliot, 2010). DEXA is a noninvasive and quick technique to measure the bone mineral, whole body fat, and non-bone lean mass (Son, d'Avignon & Laflamme, 1998). Because it is easily adaptable for use in small animals, it has been extensively studied and used to determine an accurate estimation of body composition in dogs (Toll, Gross, Berryhill & Jewell, 1994; Munday, Booles, Anderson, Poore & Earle, 1994; Mawby et al., 2004;). This x-ray technique uses photons of two different energy levels (70 and 140 kVp) (Mawby et al., 2004). These two different energy levels cross differently through the bone, the muscle and

the fat, (Elliott, 2006b), which allows one to calculate the mineral density, the lean mass and the fat mass with a high degree of precision (Toll et al., 1994; Munday et al., 1994; Elliott, 2006b). However, this technique is neither practical nor economical for common use in veterinary clinics, because dogs must be sedated or anesthetized for the procedure and requires expensive initial set-up costs and experienced personnel to interpret results (Gosselin, Wren & Sunderland, 2007; Lauten, Cox, Brawner & Baker, 2001).

3 ADIPOSE TISSUE

Adipose tissue has been considered throughout the years as a diffuse and wrongly defined tissue with as main role the storage of energy in the form of triglyceride, and insulation and protection of other body organs. Nowadays, adipose tissue is considered to be a much more complex organ containing a variety of cell types (Zoran, 2010; German, Ryan, German, Wood & Trayhurn, 2010a). In this chapter, a brief overview of the characteristics of adipose tissue (histology, distribution and physiology) and new perspectives of this tissue will be given.

3.1 Histology

Adipose tissue is a special kind of conjunctive tissue which, although containing adipocytes as the most prevalent cells (about 50%), comprises of various others types of cells, including pre-adipocytes, mesenchymal stem cells, fibroblasts, endothelial cells, pericytes, and immune cells such as macrophages, dendritic cells, mast cells, granulocytes and lymphocytes, as well as nerve cells bound to the autonomic nervous system (Haugen & Drevon, 2007; Sethi & Vidal-Puing, 2007; Sharkey, 2007; Prins, 2002). The primary and popular classification of adipose tissue divides this tissue into two types: white adipose tissue (WAT) and brown adipose tissue (BAT) (Zoran, 2010; Joazeiro, 2008; Haugen et al., 2007).

WAT is characterized by cells that contain only a droplet of fat and whose colour, depending on the type of diet, can range between white or yellowish, which is due to accumulation of carotenes dissolved in fats (Joazeiro, 2008). These adipocytes can accommodate much larger lipid droplets than any other cell type (Haugen et al., 2007). These cells have a considerable capacity for expansion and when filled with lipids they become spherical (Joazeiro, 2008). In one mature white adipocyte the lipid droplet comprises approximately 85% of the cellular volume, and the nucleus and other organelles are usually compressed against the cell membrane (Haugen et al., 2007; Joazeiro, 2008). Therefore, they differ in size, having diameters of up to 100 μm, which is larger than most

other cell types (Haugen et al., 2007). Although considered unilocular lipid droplets, when viewed under the electron microscope these cells also have, in addition to the main lipid droplet, much smaller droplets, however devoid of surrounding membrane (Joazeiro, 2008).
BAT is different from WAT because of the presence of multilocular lipid droplets, whose colour is due to the abundant vascularization and numerous mitochondria present in the cytoplasm (Joazeiro, 2008; Haugen et al., 2007). This tissue is actively involved in thermogenesis and is found in higher proportions in neonates (Haugen et al., 2007; Joazeiro, 2008).
However, in obesity multiple changes occur in the adipose tissue. In obesity an excess of caloric intake causes the adipose depots to expand (Fuster, Ouchi, Gokce, Walsh, 2016). This expansion is mediated by an increase in adipocyte numbers (hyperplasia) and an enlargement of adipocyte size (hypertrophy) (Fuster et al., 2016). The hyperplasia is mediated by the formation of functional adipocytes (adipogenesis) (Fuster et al., 2016). In contrast, adipocyte hypertrophy typically leads to lipid-laden, dysfunctional adipocytes that undergo cell death and contribute to adipose tissue inflammation and dysfunction (Cildir, Akincilar, Tergaonkar, 2013). These quantitative and qualitative changes in the cellular composition of adipose tissue are mainly composed of mononuclear cells, such as macrophages, the most abundant immune cell in the adipose tissue of obese individuals (Cildir et al., 2013). Studies in animals demonstrate that adipose tissue expansion is accompanied by macrophage infiltration, and switch in macrophage activation to a more proinflammatory state, and also high expression of inflammatory cytokines (Weisberg et al., 2003; Lumeng, Bodzin, Saltiel, 2007). Also, there is recruitment of T cells, B cells, macrophages, neutrophils, and mast cells that are increased in visceral adipose tissue of obese individuals or diet-induced obese mice (Cildir et al., 2013). By contrast, specific subsets of T cells – helper T cell type 2, regulatory T cell, and invariant natural killer T cell – and eosinophil numbers are decreased in the obese adipose tissue (Cildir et al., 2013).

3.2 Adipogenesis

Stem cells and pre-adipocytes play an indispensable role in the expansion of adipose tissue that occurs in obesity (Fischer-Posovaszky et al., 2007). These cells are recruited when existing adipocytes reach a critical level of hypertrophy, and consequently in adipose tissue hyperplasia (Trujillo & Scherer, 2006). The peroxisome proliferator-activated receptor y (PPARy) and the CCAAT / enhancer binding protein α (C / EBPα) are the main regulators of adipogenesis (Sethi et al., 2007).

3.3 Distribution

WAT is mainly distributed between the subcutaneous and visceral compartments. The subcutaneous level forms the adipose panicle, a layer disposed between the dermis and the muscular layer. At the visceral level, the deposits are located at the thoracic (mediastinal) and abdominal (omental, mesenteric, perirenal, retroperitoneal, parametrial, periovaric and epididymal) levels (Sharkey, 2007; Trujillo et al.,2006). In humans, the pathologic consequence of obesity is mainly influenced by the deposition of fat into visceral deposits instead of subcutaneous deposits (Saisho et al., 2013; Kley, Caffall, Tittle, Ferguson, Hoenig 2008). This phenomenon has been called metabolic syndrome in humans, and is associated with abdominal obesity (accumulation of visceral adipose tissue), blood lipid disorders, inflammation, insulin resistance or type II diabetes, and increased risk of developing cardiovascular disease (Ader et al., 2014; Hoenig, Traas & Schaeffer, 2013). Similar symptoms have been reported in dogs too (Hoenig, Pach, Thomaseth, Devries & Ferguson, 2012; German et al.,2009). However, a true metabolic syndrome has not described, which may be due to differences in risk factors for cardiovascular disease and blood lipid abnormalities (Hoenig et al., 2012; German et al.,2009).

BAT has a more limited and residual distribution in the adult, surrounding most of the vital organs (heart, kidney, aorta, circulatory routes, among others) (Sharkey, 2007; Trujillo et al.,2006).

3.4 Physiological functions

There are various functions of adipose tissue that make it a vital organ. In addition to the classical functions of adipose tissue, including thermal insulation (fats are poor heat conductors) and mechanical protection, adipose tissue is recognized mainly because of the large energy storage in the form of triglycerides (Zoran, 2010; Sharkey, 2007; Prins, 2002). During periods of increased ingestion and/or decreased energy expenditure, the surplus energy is effectively stored in WAT in the form of lipids, mainly triglycerides (TG). These are hydrolysed in free fatty acids (FFA) and glycerol by the action of lipoprotein lipase. These FFA penetrate into the adipocytes and are transported, intracellular, through the fatty acid binding proteins. They are activated on Acyl-CoA and esterified in the endoplasmic reticulum in different classes of lipids like TG, phospholipids and cholesterol esters. Subsequently, these energy reserves are used by the body in situations of caloric restriction, physical exercise and between meals, in order to meet the energy needs of other organs (Haugen et al., 2007; Beylot, 2007).

The mobilization of TGs is made from lipolysis (hydrolysis of intracellular TG), which results in the release of glycerol and fatty acids. Glycerol is used at the gluconeogenesis level to synthesize new glucose molecules. Fatty acids are circulated in the form of non-esterified fatty acids (NEFAs), transported by albumin, which will later be used by muscles (oxidation), liver [complete CO2 oxidation, incomplete ketone bodies, synthesis of TG, secretion and storage of very low density lipoproteins, (VLDL)] and adipose tissue (re-esterification of fatty acids), for example (Haugen et al 2007; Beylot, 2007). In normal conditions the adipocyte has the capacity to integrate a significant number of hormonal and nervous signals, in order to maintain an adequate balance between synthesis (lipogenesis) and catabolism (lipolysis) of TG, responding to the physiological needs (Haugen et al., 2007; Sethi et al., 2007). In addition, the adipocyte is able to limit an abnormal plasma increase of NEFAs (Haugen et al 2007; Lafontan, 2005). The adipose tissue also stores cholesterol and fat-soluble vitamins, in particular vitamins D and E (Sethi et al., 2007).

3.5 Adipose tissue as an endocrine organ

Previously, it was believed that adipose tissue was a relatively inert tissue primarily responsible for the storage of excessive energy in the form of TG (Sethi, Antonio & Vidal-Puig, 2007). However, beginning in 1994 with the discovery of leptin, a regulator of energy homeostasis, it has become clear that adipose tissue is a metabolically active organ (Sethi et al., 2007). The white adipose tissue (WAT) is now recognised as an active endocrine organ (Fig. 2) that communicates with the brain and peripheral tissues by secreting several hormones and protein factors, termed adipokines (Trayhurn, 2005). This term is restricted only to the proteins secreted by adipocytes themselves, and this way exclude all the other proteins secreted by other cells that exist in WAT such as macrophages (Trayhurn & Wood, 2004). Over the last 10 years, there have been over 100 different adipokines have been discovered and characterised in humans and rodents (German et al., 2010). Adipokines act centrally to regulate appetite and energy expenditure, and peripherally affect insulin sensitivity, oxidative capacity, and lipid uptake (Gray & Vidal-Puig, 2007). Nevertheless, as a dynamic organ, the adipokine profile changes in response to the amount and condition of adipose tissue (Gray & Vidal-Puig, 2007).

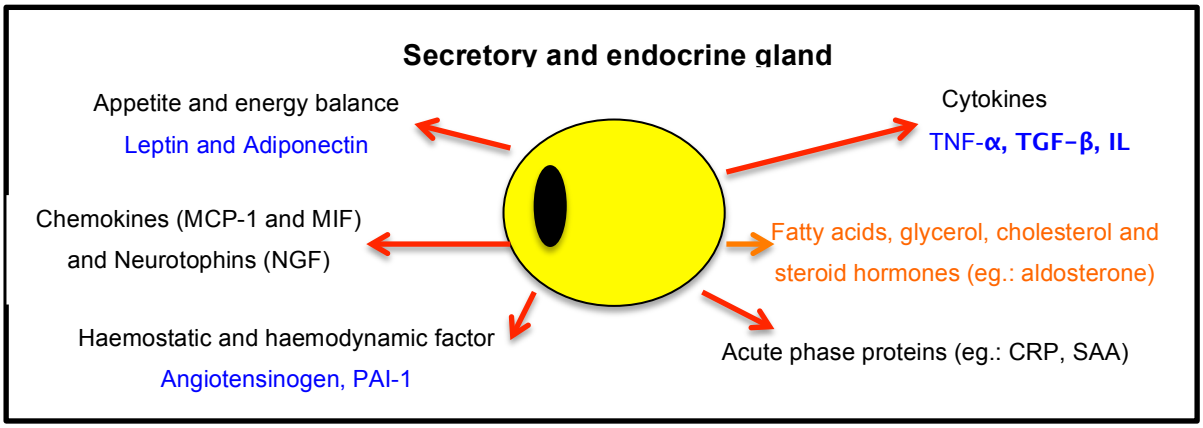
Adipokines include classic cytokines (e.g., TNFα, IL-6), antigenic factors, growth factors, and proteins involved in vascular homeostasis, regulation of the arterial pressure (eg., Angiotensinogen), lipid metabolism, homeostasis of glucose (eg. adiponectin), the complement system, as well as proteins of the acute phase and response to the stress (Trauhurn & Beattie, 2001; Trayhurn et al., 2004; Haugen et al., 2007).

One of the most well know adipokines and best characterized in dogs is leptin and it's secretion by the adipocytes is proportional to fat mass, which means in obese individuals

plasma levels are higher (regardless of race, gender and age) and decreases with weight loss (Considine et al. 1996; Ishioka et al., 2002). Experimentally induced obesity in dogs demonstrated that the increased fat mass, with increased BCS, resulted in measurable increases in serum leptin and the reduction in fat mass showed the opposite (Ishioka et al., 2007; Ishioka et al., 2002).

Leptin acts in multiple centers in the central nervous system (CNS), especially at the hypothalamic level (Kershaw & Flier, 2004). The primary action of leptin is to regulate the energy balance by decreasing appetite and stimulating energy expenditure (thermogenesis) (Rahmouni et al., 2005; Mathieu, Poirier, Pibarot, Lemieux & Després, 2009). Specifically, leptin acts on the nervous system to increase satiety (Kershaw et al., 2004). However, in obese humans and pets, the higher leptin concentrations do not seem to suppress appetite, leading to a hypothesis of leptin resistance (Nishii et al., 2006; (Vazquez-Vela, Torres, Tovar, 2008). Leptin resistance can be explained by several mechanisms, including defects in leptin transport across the blood–brain barrier by the saturation of the transport systems for leptin (Caro et al., 1996), impaired neuronal leptin signaling in target neurons (Munzberg, Flier, Bjorbaek, 2004) or altered signaling in downstream target cells and neuro-circuits (Faouzi et al., 2007). This leptin resistance is selective: peripheral leptin receptors continue to function and this may be important in the pathogenic metabolic effects of hyperleptinemia that occurs in obesity such as the chronic activation of the SNS and consequently hypertension that occurs in obesity and will be discussed in chapter III (Margetic, Gazzola, Regg, Hill 2002).

Figure 2 - View of the biological function of adipose tissue (represented schematically by a single cell), secreting hormones such as cytokines and others. (German et al., 2010a)



MCP-1 monocyte chemoattractant protein-1; MIF, macrophage migration inhibitory factor; NGF, nerve growth factor; IL - interleukin; TNF-a - tumor necrosis factor-a; TGF-b - transforming growth factor-b; PAI-1 plasminogen activator inhibitor-1; CRP- C-reactive protein; SAA - serum amyloid A

4 CLINICAL RISKS ASSOCIATED WITH OBESITY IN COMPANION ANIMALS

In humans, obesity has detrimental effects on health and increases mortality risk (WHO 2000). The diseases associated with or exacerbated by obesity arise from two mechanisms: metabolic changes associated with excess fat and the increase of fat mass itself. Obesity is not just a pathological state of overweight, adipose tissue is now recognised as an active endocrine organ having local, peripheral and central effects by secreting adipokines, as mentioned in the previous section (Trayhurn et al., 2004). On the other hand, the increase of fat mass induces physico-mechanical changes (Osto et al., 2015; German et al., 2010a; Sandøe, 2014). Like humans, dogs are also susceptible to the many and varied health consequences of obesity that we will approach now in the following section.

4.1 Clinical evaluation, physiology

In general, clinical evaluation is more difficult in an obese dog compared with a dog with ideal body condition. Physical examination of an obese patient, namely, chest auscultation, abdominal palpation and complementary diagnostic tests, especially ultrasound, are difficult to implement due to excess adipose tissue. Blood collection techniques, cystocentesis and aspiration of peripheral lymph nodes are also problematic (German, 2006b).

4.2 Anesthetic risk

As in obese humans, weight excess affects most vital organs of the obese dog as changes occur in cardiovascular, respiratory and metabolic systems (Diez & Nguyen, 2007; Love & Cline, 2015). In human medicine, anesthetic drugs are administered according to lean BW to avoid over- or under- dosing (Diez et al., 2007; Love et al., 2015). However, the risk varies with the type and dose of anaesthetic used and the duration of the surgery (Diez et al., 2007; Love et al., 2015). The major risks are an overdose of anesthetic and the prolongation of the recovery period due to the storage in body fat of soluble anesthetics in the lipids. Obesity has effects on pharmacokinetic (PK) parameters (bioavailability, volume of distribution, and clearance) because of the changes in body composition, circulating and extracellular volume, organ perfusion, and the compromise on ventilatory capacity (Love et al., 2015; Michel et al., 2012). A study performed by Boveri, Brearley, and Dugdale (2013) compared the effect of body condition on propofol requirements in dogs and demonstrated that overweight dogs required a lower IV propofol dose per kg of total body mass to allow tracheal intubation than did normal BCS animals. This suggests that IV anaesthetic doses

should be calculated according to lean body mass like humans (Boveri, Brearley & Dugdale, 2013).

Other risks are associated with concomitant disorders, which are common in these patients, such as cardio-respiratory disorders (Diez et al., 2007) that lead to an increase in mortality after surgery in obese patients (Love et al., 2015).

4.3 Longevity

Obesity leads to a reduction in longevity in dogs, as was shown in one study (Kealy et al., 2002). In the study, 48 Labrador retrievers divided in two randomly selected groups, 24 dogs for the treatment group and the other 24 for the control group, were used to evaluate the effects of 25% diet restriction on the average life expectancy (Kealy et al., 2002). The dogs in the group with dietary restriction remained leaner and lived longer; the median lifespan was 13 years while the group without restriction was 11.2 years (Kealy et al., 2002).

4.4 Endocrine and metabolic diseases

Several endocrinopathies are associated with obesity, such as insulin resistance in dog and humans or diabetes type II in humans (Lund, Armstrong, Kirk & Klausner 2006; Mattheeuws, Rottiers & Kaneko, 1984), and hyperlipidemia (Park, Lee & Oh, 2014; Jeusette, Lhoest, Istasse & Diaz, 2005).

Pancreatic β cells, in the Langerhans islets, are responsible for the secretion of insulin, which manages uptake and use of glucose in peripheral tissues (Michel et al., 2012). In humans, the constant excess of calories ingested leads to decreased tissue sensitivity to insulin (i.e. become 'insulin resistant') (Michel et al., 2012; German, 2006b). There is a positive correlation between plasma concentrations of insulin and BMI in humans (Michel et al., 2012; German, 2006b). In overweight dogs this obesity-induced insulin resistance and hyperinsulinemia has also been reported (Gayet et al., 2004). However, the degree of insulin resistance associated with diet-induced obesity appears to be dependent on age, with older dogs being more insulin-resistant than young dogs (Serisier et al., 2008a). Weight-loss leads to a recovery of insulin sensitivity and decreased insulin concentrations (Diez et al.,, 2004; Yamka et al., 2006a). Contrary to obese humans, insulin resistance in obese dogs does not spontaneously progress to type II diabetes mellitus (Verkest, Fleeman, Rand & Morton, 2011; Verkest, Fleeman, Rand & Morton, 2012).

Beside insulin resistance, obesity is one of the principal causes of hyperlipidemia in dogs. Even with moderate obesity the levels of serum triglycerides and total cholesterol are higher than lean dogs (Pena, Suarez, Bautista, Montoya & Juste, 2008; Jeusette, et al., 2005).

Both alterations are reversed by weight loss (Jeusette et al., 2005). The long-term effects of hypertriglyceridemia observed during obesity have been associated with an increased risk of acute necrotizing pancreatitis in humans (Kota & Jannula, 2012). For dogs this association has been proposed as a risk factor for pancreatitis, however, further studies have to be performed to assess cause and effect relationships (Osto et al., 2015, Weeth, 2016).

4.5 Cardiorespiratory changes and systemic hypertension

Obese dogs have an increased prevalence of cardiovascular disease in the form of congestive heart failure (Edney et al., 1986). The adverse effects of obesity on left ventricular function can be explained by three mechanisms: an increased plasma volume which increases ventricular preload, hypertension that raises left ventricular afterload, and alterations in the myocardial genome that result in systolic and diastolic dysfunction (Diez & Nguyen, 2010; German, 2006b; Diez et al., 2007). Increased blood pressure has been documented in overweight and obese dogs (Bodey & Michell, 1996; Montoya et al., 2006; Mehlman, et al., 2012). The largest study of blood pressure in dogs to date showed that the obesity effectively increases the blood pressure, however this increase was small (5 mmHg), and insufficient to lead to clinical consequences (Bodey et al., 1996). Another early study showed similar alterations (Remillard, Ross, Eddy & 1991). The American College of Veterinary Internal Medicine (ACVIM) has considered the effects of obesity on blood pressure as small and the prevalence of hypertension as very low (Brown et al.,, 2007.). However, obese dogs have left ventricular hypertrophy (Mehlman et al., 2012), which is reversible with weight loss (Neto, Brunetto, Sousa, Carciofi & Camacho, 2010.; Pelosi, Rosenstein, Abood & Olivier, 2013).

Obesity can also exacerbate, aggravate or be responsible for several respiratory diseases. The most notable examples are laryngeal paralysis and brachycephalic airway obstruction syndrome that are aggravated by obesity (German, 2006b; Diez et al., 2007; White & Williams, 1994). Obesity also exacerbates heatstroke and there is a high risk of tracheal collapse development in dogs (German, 2006b). The main symptoms in obese patients are exercise intolerance and shortness of breath (Toll et al., 2010).

4.6 Traumatic and degenerative orthopaedic disorders

Dogs with overweight have a high risk to develop traumatic and degenerative orthopedic disorders (Edney et al., 1986; Michel et al., 2012; German, 2006b). There is a greater osteoarthritis (OA) severity in dogs with BCS above ideal (Toll et al., 2010). An improvement of OA in obese dogs was demonstrated with weight loss of 6.1% onwards,

with a decrease of lameness (Marshall et al., 2010). The mechanical stress due to the weight increase appears to be the major factor associated with this relationship and to be a predisposing factor in humeral condylar fractures, cranial cruciate ligament rupture and herniated disc (Case et al., 2000; German, 2006b). Because there is also a strong association between obesity and severity of OA in others joints such as the hand joint in humans that are not a mechanically stressed, it has been questioned whether obesity has only a strictly mechanical impact (Frye, Shmalberg & Wakshlag, 2016). These findings led to further interest in the inflammatory mediators released from adipose tissue (e.g., cytokines IL-6 and TNF-a, and the adipokines leptin, visfatin, adiponectin, and resistin) (Frye et al., 2016). A recent study in humans, was performed to find the role of BW in the pathogenesis of osteoarthritis (OA) and the results have suggested that obese adipokine concentration, mainly the adiponectin, in synovial fluid may contribute to the metabolic changes associated with OA (Gross, Guillaume & Gegout-Pottie, 2014). However, which adipokines and their exact role in osteoarthritis are still unclear (Frye et al., 2016). In dogs there is paucity of information about the relationship of adipokines with osteoarthritis, and unlike in people, would be even more difficult because all major affected joints are weight bearing (Frye et al., 2016)..

Besides the fact that orthopedic problems can be cause by obesity, these orthopaedic disorders alone predisposes these individuals (humans and dogs) to be less active, which is a risk factor for obesity and thus requires an adaptation of energy intake in order to prevent overeating and excess weight and avoid the aggravation of the pathology (Michel et al., 2012; Toll et al., 2010; German, 2006b).

4.7 Effects on renal function and urinary tract disorders

In humans, obesity is known as a risk factor for CKD due to the fact that prolonged obesity can cause structural and functional changes in the kidney (e.g., greater kidney weight, higher plasma renin activity and insulin concentration, high arterial pressure and high GFR, an expanded bowman´s capsule, increased mesangial matrix, and increased thickened glomerular and tubular membranes (Chang, Ryu & Choi, 2016; Zhang & Lerman, 2015; Griffin et al., 2008). In a prospective cohort study with 62,249 metabolically healthy persons performed by Change the risk for CKD the results demonstrated that persons with BMI of overweight and obese (23 to 24.9 kg/m^2, and 25 kg/m^2 or greater, respectively) were associated with an increased incidence of CKD, with an incidence rate of 2.9 cases per 1000 person-years for overweight and 3.6 for obese compare with the 2.1 for normal weight (Chang et al., 2016).

In dogs, experimentally induced obesity resulted in increased arterial pressure (+12 +/- 3 mmHg) and high levels of plasma renin activity, which can alter the renal function and

cause histologic changes in the renal architecture like in humans (Heneger, Bigler, Heneger, Tyagi & Hall 2001). This study performed by Heneger in 2001 demonstrated that obese dogs had insulin concentrations 2.3 fold greater compared with lean dogs as well as significantly larger glomerular Bowman's space area (+41 +/- 7%), mainly because of expansion of Bowman's capsule (+22 +/- 7%) (Heneger et al., 2001). GFR was 38 +/- 6% greater and the renal plasma flow was also 61 +/- 7% higher than the lean dogs (Heneger et al., 2001). Cell proliferation in glomeruli, thickening of glomerular and tubular basement membrane, and increased mesangial matrix were also observed (Heneger et al., 2001). The concentration of biomarkers of renal injury (homocysteine, cystatin, and clusterin) also improved after weight-loss in client-owned dogs (Tvarijonaviciute et al., 2013). This issue will be deeply explained further ahead in part II, section III.

In addition to this problem, obesity in humans is also associated with a higher incidence and prevalence of nephrolithiasis (Scales, Smith, Hanley & Saigal, 2012; Taylor, Stampfer & Curhan, 2005). Increased BW is associated with lower urinary pH and increased excretion of urinary oxalate, uric acid, sodium and phosphate (Maalouf, Sakhaee, Parks, Coe, Adams-Huet, Pak, 2004; Lemann, et al., 1996). Other studies have shown that obesity may increase the risk of renal cancer, however, it is unclear (Calle & Kaaks, 2004).

In dogs, a study by Lekcharoensuk et al. (2000) reports that dogs classified as overweight had 2 times greater risk for developing nephrolithiasis (calcium oxalate), compared with dogs that were ideal in BW (Lekcharoensuk, et al., 2000). Overweight dogs also have an increased risk of developing transitional cell carcinoma of the bladder (Glickman, Schofer & McKee, 1989).

4.8 Neoplasms

In humans, there is some evidence that obesity is a risk factor for neoplasia (Lund et al., 2006). In vitro studies already showed that elevated leptin is a promoter of mammary tumors and hepatocellular carcinomas (Zou & Shao, 2008). According to Alenza, Rutteman, Pena, Beynen & Cuesta (1998), obesity in juvenile female dogs plays an important role in predisposition to mammary tumors in adulthood. Also in obese humans there are elevated levels of leptin, IL-6, resistin and TNF- α, which inhibit normal apoptotic mechanisms and promote angionesis which may promote cancer (Yin, Wang & Zhang, 2004). However, in dogs is not possible yet to associate this relationship between adipokines and tumorigenesis or associate a specific cancer to obesity because there is not enough data yet (Weeth, 2016).

5 PATHOPHYSIOLOGY OF OBESITY

The reasons for obesity are not completely evident as there are a huge number of factors that can cause this situation, but the major cause is an imbalance between energy intake and expenditure (Sandøe et al 2014; Loftus & Wakshlag, 2015; German, 2006b). According to Loftus et al. (2015), the major factors involved can be divided into two: factors that affect energy metabolism, such as resting metabolic rate, active metabolic rate, and relative activity, and those that affect energy intake and assimilation, such as the behavior of eating/feeding (hormonal and behavioral), the factors in food that affect nutrient assimilation or/ and the digestion efficiency.

Next, a small part of the pathophysiology of obesity that includes the principles of energy balance and the physiological regulation of BW will be discussed as well as the main risks factors that can contribute to obesity.

5.1 Energy balance

The fundamental principle of energy balance is as follows: changes in energy stores = energy intake - energy expenditure. According to this principle, positive energy balance occurs when energy intake exceeds expenditure, and consequently results in energy storage and weight gain (Diez & Nguyen, 2010; Toll et al., 2010; Michel et al., 2012). Contrarily, negative balance is when the expenditure exceeds intake. Though, in the long run, in a state of BW maintenance, energy intake matches energy expenditure regardless of the natural oscillations that occur everyday (Backus & Wara, 2016). A lot physiologic mechanisms have a relevant role in adapting intake to expenditure and vice versa, so as to keep a stable BW over the long term (Backus et al., 2016). There is an energy expenditure increase when energy balance is positive. This increase happens through the release of heat at the expense of ATP. On the other hand, when the energy balance is negative, there's a decrease of energy expenditure, which consequently helps to resist weight loss (Diez & Nguyen, 2010).

The total energy expenditure can be split into three components: resting energy requirement (RER), physical activity and the postprandial thermogenesis (Diez & Nguyen, 2010; Toll et al., 2010). Total energy expenditure varies according to the regularity and intensity of physical activity, the most variable component (Backus et al., 2016). In one dog, the RER usually represents between 55% and 70%, the physical activity between 30% and 40% and the postprandial thermogenesis 10% of the daily energy requirements (Toll et al., 2010).

RER is the quantity of energy that a dog requires to maintain normal physiologic functions at rest (Toll et al., 2010; Michel et al., 2012). Physiological functions are determined by lean

mass, which represent 90-95% of RER versus 5-10% of fat mass (Michel et al., 2012; Diez & Nguyen, 2006; Diez & Nguyen, 2010).

The postprandial thermogenesis represents the production of heat associated with ingestion, digestion, assimilation, and metabolism of food and is influenced by the nutritional and caloric composition of the food, the number of meals per day and the nutritional status of the animal (Michel et al., 2012; Toll et al., 2010; Case et al., 2000). Physical activity represents the movement produced by skeletal muscle contraction that results in an increase in energy expenditure (Backus et al., 2016). This last component may represent an important factor for the management of obesity.

5.2 Physiological regulation of body weight

Most dogs maintain an ideal, constant BW due to a biologic and complex set of neural, hormonal and biochemical mechanisms that keep the balance between energy intake and expenditure within fairly precise limits (Druce & Bloom, 2003). This physiologic mechanism that stabilizes BW are complex and still poorly understood (Backus et al., 2016). A small part of this mechanism is the control of food intake, which regulates BW (Losada, 2005). Food intake is, according to Backus et al. (2016) a conditioned behavior compelled by motivations to initiate or terminate a meal. These motivations are regulated by a feedback system with afferent and efferent mechanisms, which continually receive and send information to the central nervous system that give order to eat and not to eat (hunger and satiety) (Backus et al., 2016). The afferent system is divided in the sensory signals such as appearance, odor, and taste (Miján de la Torre, 2004; Losada, 2005). The others are signals from the digestive tract such as stretch and chemoreceptors that provides satiation feedback (Backus et al., 2016). Later post-ingestive signals (e.g., increased insulin, decreased glucagon) additionally serve as satiation feedback affecting meal termination (Backus et al., 2016). These signals reach the central nervous system through peripheral innervations. The stimulation of the hypothalamus regulates the food intake through two centers: hunger center and satiety center (located in the ventromedial nucleus) (Losada, 2005). The hypothalamus plays a major role in regulating energy homeostasis: it integrates peripheral, neural and hormonal afferent signals of satiety and energy reserve, directing efferent signals that affect either energy storage or energy expenditure (Backus et al., 2016).

Beside this factor adiposity signals also affect food intake (Backus et al., 2016). There is an internal mechanism that measures amount of body fat and in turn acts to adjust food intake in accord with the amount of energy needed to maintain a set-point in body fat (Backus et al., 2016). A most clearly agreed on adiposity signal is plasma concentration of a protein called leptin, one of the most known hormones in obesity, is produced by adipose tissue in

proportion to its reserves in fat mass (Miján de la Torre, 2004, Considine et al., 1996). It acts on the satiety centers of the hypothalamus and is responsible for modulating an activity of several effector neuronal systems and axonal pathways, resulting in decrease of food intake and energy expenditure increases when the level of leptin are high and vice-versa (Miján de la Torre, 2004; Backus et al., 2016).

Insulin concentration in plasma is also considered by some investigators to be an adiposity signal (Porte & Woods, 1981). Although insulin is a hormone principally secreted by pancreatic endocrine cells and not adipose, concentrations of insulin rise in plasma with increasing body fat in dogs (Porte et al., 1981). A feedback role for insulin is that it crosses the blood-brain barrier, and it bind to receptors on the neural circuit that affect the food intake, and by intracranial insulin infusions at physiologic doses reduce food intake (Porte et al., 1981; Woods & Begg, 2015). When adiposity increases, the average circulating insulin concentration rises because of a resistance of insulin (Hoenig, Thomaseth, Waldron & Ferguson, 2007). Research in dogs indicates such insulin resistance could result from diminished transport of peripheral insulin to the brain (Hoenig et al., 2007). This high plasma insulin in obesity could be a braking signal functionally preventing further undesired gain in adiposity from continued overeating (Woods et al., 2015).

5.3 Risk factors

Although the pathophysiologic mechanisms responsible for the development of obesity are complex and multifactorial, one must also consider the individual components that can impact physical activity and the energy expenditure (Backus et al., 2016). Age is one of these factors. The frequency of obesity in dogs increases with age (Robertson, 2003; Colliard, Ancel, Benet, Paragon & Blanchard, 2006). Throughout the animal's adult life the energy requirements reduces as well as the quantity of lean mass that results in a reduction of the daily energy requirements (Speakman, Van Acker, Harper, 2003). The frequency of obesity in animals with less than 2 years old is low and after that age the frequency increases; the age of diagnosis is between 5 and 8 years (more than 50% are between 7-8 years and approximately 70% are over 9 years old) (Armstrong & Lund, 1996). Another factor can be the type of breed because some breeds (e.g., Labrador retriever, Boxer, Cairn King Charles Spaniel, Cocker Spaniel, Beagle) are predisposed to obesity, and may be likely a genetic factor as is the case of the pro-opiomelanocortin (POMC) gene in Labrador retrievers. Raffan et al. showed that a frame-shift deletion mutation in POMC is strongly associated with weight, adiposity, and appetite and also in flat-coat retriever (FCR) dogs (Raffan, et al. 2016; Zoran, 2010; German, 2006b; Edney et al., 1986).

Lack of exercise can be another factor and one of the principal factors in the development of obesity due to a reduced total energy expenditure and RER, and a decrease in lean body mass (LBM) (Diez & Nguyen, 2010).

Some endocrine diseases can be associated with obesity too, such as hypothyroidism, and may also be secondary to hyperadrenocorticism (Diez & Nguyen, 2010). Hypothyroidism results in reduced energy needs at rest, which in turn may predispose to obesity. In hyperadrenocorticism there is an overproduction of corticosteroids by the adrenal cortex, which induce hyperphagia and secondarily overweight (German, 2006b). Also some drugs, such as glucocorticoids and anti-epileptic drugs, induce hyperphagia and secondarily overweight (Elliott, 2006b).

Gonadectomy is known for raising the risk of obesity in dogs (Edney et al., 1986; Robertson, 2003; McGreevy et al., 2005; Colliard et al., 2006). One study of the prevalence of obesity in dogs performed in Australia by McGreevy et al. (2005) demonstrated that 8.7% of sterilized females are obese vs. 5.3% intact obese females; 9.5% of castrated males are obese vs. 3.2% intact obese males. Neutering affects the food intake, which could be due to the withdrawal of the gonad steroid hormones, estradiol and testosterone (Backus et al., 2016). A study demonstrated that after of ovariectomy, the daily energy requirement decreased 30% in bitches (Jeusette, Detilleux, Cuvelier, Istasse & Diez, 2004). In a study conducted by Houpt, Coren, Hintz & Hilderbrant (1979), ovariohysterectomized bitches increased their weight significantly in the first 10 postoperative days, and also the food intake, compared to the control group. The lost of estrogens and androgens also seems to decrease the metabolic rate and decrease of physical activity that might occur due the decreased sexual behaviour and roaming (Toll et al., 2010; Zoran, 2010; German, 2006b).

There another factor that can contribute is the way of feeding (the quantity or how it is offered) or specific characteristic of foods such as palatability and energy density. For example, high-fat diets have lower satiating power than high protein low-fat diets and can increase the risk of obesity (Gerstein, Woodward-Lopez, Evans, Kelsey, Drewnowski, 2004; Kaiyala, Prigeon, Kahn, Woods, Schwartz, 2000). An epidemiological study indicated that dogs fed once a day are heavier (Kienzle, Bergler, Mandernach, 1998; Robertson, 2003). Also, the offering of treats and food leftovers are considered risk factors by Robertson (2003). Because humans have a tendency to humanize their pets and communicate through food, owners consequentially end up overfeeding their animals contributing to excess weight (Diez & Nguyen, 2010; Toll et al., 2010; Zoran, 2010).

III. Physiopathology Relationship Between Obesity and Renal Insufficiency

1 RENAL FUNCTION AND ASSESSMENT

The kidneys are essential for the maintenance of organism homeostasis (Joazeiro, 2008). They perform several functions, among them are the clearance of toxins of the metabolism or foreign substance, regulation of fluid balance, maintenance of acid-base, regulation of sodium, potassium and other electrolytes, regulation of blood pressure, and production of various hormones, such as erythropoietin (Verlander, 2014).

All these functions are performed by the functional unit of the kidney, the nephron, which consists of two distinct anatomical and functional structures, glomeruli and tubules (Verlander, 2014). As it crosses the glomeruli, the blood arriving through the afferent arteriole loses a filtrate which is essentially protein-free, which travels through the tubules and is modified by excretion and reabsorption performed by tubular epithelial cells (Verlander, 2014). Thus, the nephron performs three successive physiological acts, glomerular filtration, tubular reabsorption and tubular excretion, with the end product being urine (Verlander, 2014). Urine contains most of the body's metabolites, which are eliminated in varying amounts along with water (Verlander, 2014). As the amount of nefrons decreases, the renal function could be impaired and renal insufficiency develops. Renal insufficiency occurs when there are approximately 75% of functional nephrons damaged (Verlander, 2014). When renal insufficiency occurs the kidneys are not able to maintain their normal functions of regulation, excretion and also endocrine functions (DiBartola, 2010). This incapacity results in retention and accumulation of uremic toxins and an imbalance on the acid-base and on the equilibrium of chemical electrolytes (DiBartola, 2010). Clinically it is defined by the presence of azotemia, characterized by high concentrations in blood of toxic compounds such as urea and creatinine, and by decreasing the concentration of urine, resulting in hyposthenuria (DiBartola, 2010).

Evaluation of glomerular filtration rate (GFR) is considered the gold standard and most sensitive test to evaluate renal function (Heiene, Lefebvre, & Watson, 2015). GFR describes the flow rate of filtered fluid. Several methods for measuring GFR have been validated in dogs, including plasma clearance techniques using non-radioactive markers such as creatinine and iohexol (Heiene et al., 2015). A decrease in GFR beyond the normal range means that kidney function is deteriorating and also allows to evaluate the severity and the progression of renal diseases, especially chronic kidney disease (CKD) (Heiene et al., 2015). However, is considered impractical in most veterinary clinics because it requires repeated blood sampling over a period of several hours following intravenous administration of a suitable marker (Heiene et al., 2015).

In the clinical practice serum concentrations of blood urea nitrogen (BUN) and creatinine are the most commonly used tests for indirectly estimating GFR (Verlander, 2014). Urea excretion occurs by glomerular filtration in the kidney, and BUN concentrations are inversely proportional to GFR (DiBartola, 2010). However, urea can be influenced by several conditions such as dehydration, the diet, and clinical conditions characterized by increased catabolism (e.g. starvation, infection, fever) (DiBartola, 2010). Because of all these non-renal variables, BUN alone is poor as an indicator of GFR (DiBartola, 2010). Creatinine excretion is accomplished almost exclusively by glomerular filtration, and is not significantly reabsorbed or secreted by the renal tubules (Heiene et al., 2015). Its concentrations have an inverse relationship with GFR, which means, when GFR decreases, creatinine serum increases (Heiene et al., 2015). An advantage of this marker is it is less influenced by feeding than BUN concentration (DiBartola, 2010). However, it may be affected by breed and body size (Gleadhill, 1995).

The usefulness of BUN combined with serum creatinine is a very useful clue to the presence of a prerenal or postrenal component to azotemia (DiBartola & Westropp, 2014). When there is a high BUN/creatinine ratio the clinician should begin a search for extrarenal factors such as high protein intake, that increases urea but can maintaine the creatinine concentration, or gastrointestinal bleeding or also catabolic states as fever (DiBartola & Westropp, 2014). A low BUN/creatinine ratio suggests inadequate protein intake and reduced urea synthesis as in advanced liver disease (DiBartola & Westropp, 2014). BUN and creatinine, taken together, are valuable screening tests in evaluating renal disease (DiBartola & Westropp, 2014). Though they may fall short as absolute indicators of renal function, they are useful in following progression of disease (DiBartola & Westropp, 2014).

Another renal marker important for assessing renal function is evaluating the presence of proteinuria. Persistent proteinuria with inactive urine sediment is associated with CKD as well as with its progression. The greater the magnitude of proteinuria is, the greater the risk of renal disease progression and mortality (Jacob et al., 2005; Lees, Brown, Elliot, Grauer, Vaden, 2004). Proteinuria is defined as the presence of any type of protein in the urine, such as albumin, globulins, and Bence Jones proteins (Gregory, 2013). Clinical significance of proteinuria depends on its severity and persistence. In the absence of hyperproteinemia, hematuria and urinary tract inflammation, persistent proteinuria usually indicates kidney disease and severe proteinuria is associated with glomerular disease (Jacob et al., 2005). Proteinuria of renal origin results of a loss of selective glomerular filtration resulting in an increased amount of plasma protein in the filtrate or an impaired reabsorption of the filtered protein, which can happen without an initial substantial decrease in the number of functional nephrons (Gregory, 2013).

Other marker that can be an indicator of kidney dysfunction is the ability to concentrate urine. Loss of concentrating ability can be one of the earliest indicators of kidney

dysfunction, which is generally recognized when two-thirds of nephrons are non-functional (Forrester et al., 2010). In CKD, the renal interstitial osmolality gradient is decreased because of increased urine flow per nephron or because of inability to establish and maintain the medullary concentration gradient (Forrester et al., 2010).

The challenge is try to find ways to recognize kidney diseases earlier in their course, before clinical signs are evident, to permit institution of any available measures (prevention, treatment or monitoring) that might slow progression of the disease and prevent development of complications. This is why novel early biomarkers (for example serum cystatin C and SDMA concentrations) of CKD are being researched because the routine renal markers used in practice are insensitive for early diagnosis (Hall, Yerramilli, Obare, Yerramilli & Jewell, 2014).

Accompanying the incidence of obesity in recent years, several studies have been conducted investigating the repercussions of this disease on the functioning of various organ systems, including the kidney, that will be approach next (Joazeiro, 2008).

2 "HIDDEN EPIDEMIC"

In humans, obesity is a potential risk factor for the development of kidney disease because, as previously described, it increases the risk of developing major risk factors of CKD, such as diabetes type II and hypertension (WHO, 2017; Kovesdy, Furth, Zoccali, 2017). Numerous studies have shown an association between obesity and both the development and the progression of CKD (WHO, 2017; Kovesdy et al., 2017). The first reports on a possible relationship between obesity and renal dysfunction date back to 1970s, when Weisinger et al. proposed that the cause of reversible proteinuria in obese patients is renal venous hypertension (Weisinger, Kempson, Eldridge, Swenson, 1974).

In 2001, Kambham et al. published an extensive review with findings of 6618 biopsies in humans, in which they found a 10-fold increase in the incidence of nephropathy secondary to obesity, from 0.2% in the period 1986-1990 to 2% in 1996-2000 (Kambham, Markowitz, Valeri, Lin & D'Agati, 2001). This data was confirmed by Chen et al. (2004), who identified a strong association between obesity-related CKD and to microalbuminuria (Chen et al., 2004). Moreover, individuals affected by obesity have an 83% increased risk of developing CKD (Kovesdy et al., 2017). Data also revealed that there is a difference in gender as well, 24.9% of women with CKD were associated with overweight or obesity while in men it was 13.8% (Kovesdy et al., 2017). Furthermore, an association between BMI and CKD was found even independent of other pathologies associated with obesity (diabetes type II, systemic arterial hypertension). The higher the BMI was, the greater the risk of developing CKD, which led many authors to attribute obesity by itself in the role of inducing of renal

injury (Chang et al., 2013; Foster et al., 2008; Praga et al., 2000). In large human population-based studies, higher BMI is associated with low estimated GFR, with more rapid loss of estimated GFR over time and with higher incidence of ESRD (Pinto-Sietsma, et al., 2003; Ejerblad et al., 2006; Foster et al., 2008). Higher BMI was also associated with the presence and development of proteinuria in individuals even without kidney disease (Foster et al., 2008). Other studies described an association between higher abdominal girth and albuminuria, decreased GFR or incident ESRD independent of BMI level (Kramer et al., 2016; Thoenes et al., 2009). In general, the associations between obesity and renal disease persist even when the common effects associated with obesity are absent, such as high blood pressure or diabetes mellitus, which suggest obesity has a independent impact on kidney unrelated to these complication (Kovesdy, et al., 2017).

In ours pets, the few data on obesity and renal insufficiency that exist do not allow us to make an association between obesity and renal dysfunction as in humans. However, studies in experimental dogs show similar findings with regard to obesity-related renal injury (Heneger et al., 2001). The incidence of obesity has also been increasing in our pets, as was previously discussed, so one can speculate that the incidence of kidney disease may also be increasing due to obesity.

3 PATHOPHYSIOLOGY OF OBESITY-RELATED RENAL DISEASE

The first sign of renal injury secondary to obesity is the increase of proteinuria or albuminuria, caused mainly by glomerular hyperfiltration, or renal venous hypertension (Chang et al., 2013; Foster et al., 2008; Praga et al., 2000). According to Chagnac et al. (2003), weight loss improves glomerular hemodynamics (Chagnac et al., 2003). Morales et al. (2003) evaluated two groups of human patients (randomly assigned in a 2:1 ratio) of 30 overweight with diabetic and nondiabetic proteinuric nephropathies, to either follow a low-calorie normoproteinic diet or maintain their usual dietary intake for 5 months. (Morales, Valero, León, Hernández & Praga, 2003). At the end of five months, they found that the group with the low-calorie normoproteinic diet loss 4.1%, which result on a reduction of 31% on proteinuria by reducing hyperfiltration secondary to obesity, reinforcing the effects of overweight on glomerular function, even with a moderate weight loss (Morales et al., 2003). Furthermore, the evaluation the renal structure by biopsy identified an association of obesity-related glomerulopathy (ORG) with the occurrence of sclerosis and hypertrophy in the glomerular region, which are an important causes of and contributors to CKD (Kambham et al., 2001).

The studies in obese dogs seem to show a similar relation as in humans. Experimentally induced obesity in dogs fed a high-fat diet showed higher mean arterial pressure and

plasma renin activity, an increase of glomerular filtration rate and also histological changes (expansion of Bowman's capsule, increased mesangial matrix, thickening of glomerular and tubular basement membranes, and increased cell proliferation in the glomerulus) when compared to lean control group (Henegar et al., 2001). An inverse study performed with obese pet dogs that lost between 10-40% of their BW had evidence of improved renal function, with increased urine specific gravity, decreased UPCR and decreased serum creatinine (Tvarijonaviciute et al., 2013).

Although there is currently a consensus among researchers that obesity contributes to renal impairment, the mechanisms by which these disorders limit organ function have not yet been fully elucidated. Two possible pathways may mediate these deleterious renal consequences of obesity. The first pathway, the indirect effects on the kidney, include effects from coexisting conditions such as diabetes, or hypertension, both of which are the most common cause of CKD and of which it is recommended for CKD screening (Kovesdy et al., 2017; Hall et al., 2014; Griffin et al., 2008). The second pathway, the direct effects on the kidney, is the adiposity effect (Kovesdy et al., 2017; Hall et al., 2014; Griffin et al., 2008). Some hypotheses that have been explored are the compressive effect of visceral adipose tissue on the renal parenchyma; the activation of the sympathetic nervous system (SNS) and the activation of the renin angiotensin aldosterone system (RAAS) (Kovesdy et al., 2017; Hall et al., 2014; Griffin et al., 2008). In next sections, how adipose tissue can interacts with normal renal function, as well as the possible mechanisms by which obesity may contribute to the development of hypertension, renal dysfunction and eventually CKD will be discussed.

3.1.1 Physical compression of the kidneys

In humans and dogs, excess visceral fat is associated with an increase of intra-abdominal pressure (Kopple & Feroze, 2011; Kopple, 2010; Bloomfield, Sugarman, Blocker, Gehr & Sica, 2000) reaching levels as high as 35 to 50 mm Hg in some dogs with central obesity (Sugarman et al., 2000). In dogs an increase of intra-abdominal pressure by 25 mm Hg for 4 weeks produced a 28 mm Hg rise in arterial pressure (Bloomfield et al., 2000). The kidney is a retroperitoneal organ surrounded by the perirenal space and pararenal anterior and posterior space, which are occupied by fat that extends into the renal sinus and penetrates to the medulla (Verlander, 2014). Due to the low complacency of the renal structures, increased amount of fat in this space act as compressive forces on the capsule, causing an increase of hydrostatic pressure and renal interstitial fluid (perinephric hypertension) (Bloomfield et al., 2000; Sugarman, Windsor, Bessos & Wolfe 1997). The increased intrarenal pressure, in turn, compresses the loop of Henle and the peritubular capillaries (vasa reta), which reduces the flow through the renal tubules, leading to an increased

tubular reabsorption of sodium (Bloomfield, et al., 2000; Kopple, et al., 2010; Hall et al., 2004). This increase of sodium reabsorption is accompanied by a compensatory renal vasodilation and an increase in GFR to maintain the sodium balance in obese individuals (Chagnac et al., 2000; Xu et al., 2017). However, other mechanisms responsible for the increase in GFR in obese individuals are not fully elucidated. Some evidence suggests that it may be mediated primarily by the macula densa feedback mechanism. Macula densa cells sense changes in sodium chloride level, and will trigger an autoregulatory response (Verlander, 2014). When there is a drop in salt concentration, macula densa cells responds through two mechanisms: First, it triggers dilation of the renal afferent arteriole, decreasing afferent arteriole resistance and, thus, offsetting the decrease in glomerular hydrostatic pressure caused by the drop in blood pressure; second, macula densa cells release prostaglandins, which trigger granular juxtaglomerular cells lining the afferent arterioles to release renin into the bloodstream (Verlander, 2014). This could explain why obesity is associated with a reduction on afferent arteriolar resistance and increases in renal blood flow, GFR, and stimulation of renin release (Hall et al., 2003). In chronic obesity this compensatory mechanism (renal vasodilation, glomerular hyperfiltration plus the increases in arterial pressure with increase glomerular wall tension) may cause renal injury, glomerulosclerosis, and ultimately nephron loss with a decline of GFR (Xu et al., 2017; Kovesdy et al., 2017; Hall et al., 2014; Hall, et al., 2004).

3.1.2 Lipotoxicity

Despite the physical effect that obesity exerts on the renal parenchyma, ectopic deposition of lipids into non-adipose tissues, such as the kidney, often occurs in obesity (Hall et al., 2014). This is associated with the disorders caused by the exacerbated metabolism of fatty acids in non-fatty tissues, such as skeletal muscle, pancreatic islets, myocardium and kidneys and results in lipotoxicity (Kopple, et al., 2010; Jeanne & Sturley, 2009). Lipotoxicity is an accumulation of lipid intermediates in non-adipose tissue, leading to cellular dysfunction and induce apoptosis (Kopple, et al., 2010; Garbarino et al., 2009). Studies have previously documented that the deposition of adipose tissue at the abdominal level leads to an increase in the concentration of circulating non-esterified fatty acids (NEFAs) (Bjorntorp, 1990). At the liver level, NEFAs stimulate VLDL production, which will transport large amounts of triglycerides to various peripheral tissues, including the kidney (Bjorntorp, 1990). At the intracellular level the triglycerides are stored as lipid droplets (de Vries et al., 2014; Weidemann & Krebs 1969). Inside of the cell, lipids are not neutral or inert and the accumulation of toxic metabolites derived from their metabolism, such as diacylglycerols or ceramides, can lead to important cellular alterations such as mitochondrial dysfunction and endoplasmic reticulum stress (Weidemann et al., 1969; Unger & Orci, 2000; de Vries et al.,

2014). These changes lead to a decrease in the ability to oxidize the NEFAs and consequently to their accumulation (Unger et al., 2000). On the other hand, the NEFAs participate in all phases related with glucose metabolism, from use by tissues to the production and storage in liver (Savage, Petersen, Shulman, 2007). These deregulations (accumulation of toxic metabolites, decrease ability to oxidize NEFAs and their accumulation on tissues) inhibit the entry of glucose in the tissues, and is inversely correlated with the level of insulin sensitivity (Savage et al., 2007).

This ectopic deposition of lipids into non-adipose tissues has been associated with CKD. Renal biopsies of humans with obesity-related glomerulopathy demonstrated extensive accumulation of lipids in mesangial cells, podocytes and tubular cells, which was associated with clinically significant metabolic changes, such as insulin resistance (Straub et al., 2013). There is evidence in animal models that explain how excess renal lipids are related with renal injury and consequently ORG. Studies focus mainly on the effect of intracellular lipid accumulation on the different constituents of the renal parenchyma, such as the mesangial cells that lose their contractility capacity, or the podocytes that seems to be a stimulus for apoptosis, or a poorly adaptive response in the remaining podocytes (de Vries et al., 2014; Chen, Liu, Zeng, Li, Wang, 2006; Bobulescu, 2010). In dogs, we are not aware of a similar study. However, there are studies that showed lipotoxicity in other organs, such as the investigation performed by Adolphe et al in 2014 that used computed tomography to show that the increments of visceral adipose tissue during weight gain is more strongly correlated with metabolic and cardiovascular (increased of systolic left ventricular free wall thickness) alterations than with subcutaneous fat, that occur within only 12 weeks of obesity in an obese dog model (Adolphe, Silver, Childs, Drew & Weber 2014). They also showed that fasting serum glucose concentrations increased significantly with obesity (Adolphe et al., 2014). Weight-loss resulted in improved pancreatic b-cell function and insulin sensitivity (Adolphe et al., 2014).

3.1.3 Systemic arterial hypertension

Further more, the mechanisms of obesity that lead to renal injury are not restricted to the metabolism change of esterified fatty acids or to the physical compression of the kidneys. In humans one of the main consequences of overweight is the increase of blood pressure (WHO, 2000; Hall, Brands & Henegar, 1999). Between 65% and 78% of cases of hypertension are attributed to obesity (Mathieu, Poirier, Pibarot, Lemieux & Després, 2009). Clinical trials have demonstrated that weight loss in obese individuals, with or without hypertension, leads to a significant decrease in blood pressure (Hall, Crook, Jones, Wofford, Dubbert, 2002; Neter, Stam, Kok, Grobbee & Geleijnse, 2003). The increase in blood pressure (BP) is also associated with obesity in dogs, both in the domestic population

and in experimental models (Rocchini, Moorehead, DeRemer & Bondie, 1989; Bodey & Michell 1996; Montoya et al., 2006).

The mechanisms by which obesity induces hypertension are not fully understood. There are several different factors that contribute to its development. Changes in renal function leading to changes in pressure natriuresis and sodium retention appear to play an important role in all forms of hypertension, associated or not with obesity (Hall, Kuo & Silva, 2003; Hall, et al., 1993). Several mechanisms seem to contribute to this phenomenon, such as physical compression of the kidneys (already mentioned in 3.1.1 Physical compression of the kidneys), the increase of the activity of the SNS, increase of leptin and the increase of the RAAS activity. The latter three mechanisms will be discussed next.

3.1.3.1 Sympathetic nervous system:

Some clinical trials in humans suggest that obese and hypertensive individuals have higher activity of the SNS compared to lean individuals (Jones, Davy, Alexander & Seals, 1997; Grassi et al., 1995).

The normal renal effect of the increased of sympathetic activity are: 1) the increased tubular reabsorption of sodium that results in sodium retention; 2) increased glomerular filtration rate and renal blood flow due to vasoconstriction and increased renal vascular resistance; 3) increased renin secretion that leads to increases production of AngII (Rahmouni, Correia, Haynes & Mark, 2005). In humans pharmacologic blockade of adrenergic activity results in a reduction of sodium retention and a higher reduction of blood pressure in obese compared to lean individuals (Wofford, Anderson, Brown, 2001). Furthermore, studies with a high-fat diet in experimental dogs revealed that the denervation of the kidney resulted in a marked reduction of sodium retention and the development of hypertension (Kassab et al., 1995). These observations suggest that the chronic sympathetic activation induced by obesity, especially the renal sympathetic nerves, is the main contributor to the development and maintenance of obesity hypertension (Hall et al., 2014; Silva, Bentes, Daher & Matos, 2017). The proposed mechanisms that may be behind the increased activation of the SNS in obesity are hyperleptinemia, hyperinsulinemia, the increase in angiotensin II, and the physical compression of the kidneys due to visceral fat (already mentioned in 3.1.1 Physical compression of the kidneys) (Hall et al., 2014; Silva et al., 2017).

3.1.3.2 Leptin

In humans, studies suggest that leptin is an essential element to regulate the activity of SNS and may have a role in hypertension in obesity. An experimental study with mice lacking the ability to synthesize leptin due to a nonsense mutation in the leptin gene (ob/ob

mice) and the db / db mice with nonfunctional leptin receptors, both develop severe obesity (Ziyadeh et al., 2000). However, only the db / db mice had histological renal lesions, suggesting the possible involvement of leptin in ORG secondary to obesity, even though, they did not become hypertensive (Ziyadeh et al., 2000). A similar finding was reported in humans with deficiency of leptin by gene mutations. Although these individuals have a high risk to develop morbid obesity and many of the elements that make up the metabolic syndrome, the SNS activity remained controlled and also the blood pressure was normal (Ozata, Ozdemir, Licinio, 1999). Other experimental studies have shown that intra-cerebroventricular or intravenous infusions of leptin to equalize the blood concentrations to ones in obesity had resulted in high renal sympathetic nervous activity and consequently raised the arterial pressure (Hall et al., 2002; Hall, Silva & Carmo, 2010).

This increased arterial pressure seems to be mediated by SNS because the blocking of alpha- and beta-adrenergic receptors in a stage of high chronic leptin levels in obesity can abolish the rise in blood pressure (Hall et al., 2002; Hall et al., 2010). The mechanism by which leptin leads to increased SNS activity seems to involve the activation of the hypothalamic pathway of the pro-opiomelanocortin pathway (POMC) (Hall et al., 2014). The POMC is a precursor polypeptide that is inside of hypothalamus in the arcuate nucleus and they express in the paraventricular nucleus and lateral hypothalamus, releasing melanocyte-stimulating hormone, which then acts as an agonist for melanocortin 3 (MC3R) and 4 receptors (MC4R) (Coll, A.P., 2007). These neurons, in turn, send information to the nucleus of the solitary tract in the brainstem to alter appetite, SNS activity, and arterial pressure (Coll, A.P., (2007). The pharmacological blockade of the MC3R receptors in the SNS and especially MC4R, appears to abolish the acute effects of this leptin effect on the activation of renal SNS in mice, as well as the effects on the chronic increase of blood pressure (Haynes, Morgan, Djalali, Sivitz & Mark, 1999; da Silva, Kuo & Hall, 2004). Also in mice lacking leptin receptors in POMC neurons similar effects were observed (do Carmo, da Silva, Cai, Dubinion, Hall, 2011). Humans with POMC or MC4R mutations show early-onset morbid obesity, as well as many other obesity comorbidities such as insulin resistence, hyperlipidemia or hyperinsulinemia but do not have increase of SNS activity or hypertension (Tallam, da Silva & Hall, 2006; Greenfield, 2011). Thus, it is possible that the POMC-MC4R system constitutes one of the main pathways between leptin, the activation of the SNS and the development of hypertension. Beside this mechanism leptin has the ability to increase natriuresis in response to a decrease of Na transport in the tubules. Several studies have demonstrated that acute leptin infusion stimulates the release of nitric oxide (NO) in endothelial cells and blood vessels, with NO being shown to decrease Na reabsorption in the tubules by decreasing Na, K, -ATPase (Briffa, McAinch, Poronnik, Hryciw, 2013). Therefore, acute leptin exposure in the kidney may increase NO expression in an attempt to regulate obesity-associated hypertension. However, chronic leptin

exposure further compounds hypertension in the obese by increasing renal sympathetic activity (Briffa et al., 2013). Chronic hyperleptinemia has been shown to increase the activity of Na, K, -ATPase, causing Na retention by either increased renal SNS activity and/or decreased NO production (Beltowski, Jamroz-Wisniewska, Borkowska, Wójcicka, 2004). Bełtowski et al. identified that hyperleptinemia causes NO deficiency primarily due to increased renal oxidative stress, which inhibits the protective role of NO in increasing Na excretion (Beltowski, Wójcicka, Marciniak, Jamroz, 2004).

3.1.3.3 Renin–angiotensin–aldosterone system

The RAAS is a neuro-endocrine axis closely related to both blood pressure levels and normal kidney function (Verlander, 2014). Numerous studies to date have demonstrated that the RAAS is abnormally activated in obesity and may contribute to the increase of blood pressure in this context (Hall et al., 2002; Hall et al., 1997; Tallam et al., 2006). Experimental studies in obese dogs have demonstrated that the use of inhibitors of the enzyme conversion of angiotensin, as well as angiotensin receptor blockers, could prevent the appearance of arterial hypertension (Robles et al., 1993; Hall, Shek & Brand, 1997). In addition, arterial hypertension in obese individuals appears to be particularly sensitive to agents that block RAAS activity compared to non-obese hypertensives, suggesting that this system may have an increased importance in the hemodynamic effects associated with obesity (Reisin et al., 1997; Alonso-Galicia, Brands, Zappe & Hall, 1996).

Renin is a proteolytic enzyme, which has as main function to cleave angiotensinogen, transforming it into Angiotensin I (Verlander, 2014). Renin, is synthesized, stored and secreted by renal juxtaglomerular cells and its plasma concentration is the factor limiting the rate of angiotensin II formation (Verlander, 2014). Renin secretion is stimulated by 3 mechanisms: by the baroreceptors of the juxtaglomerular cells present in the wall of the afferent arteriole in response to a drop in blood pressure; by dense macula cells in response to a drop in ion concentration that reaches this specialized part of the nephron; by the β 1 adrenoreceptors of the juxtaglomerular cells when there is sympathetic stimulation (Verlander, 2014). Thus, the increase sodium reabsorption that occurs in the loop of Henle in obese individuals and the consequent decrease in the amount of this ion that reaches to macula densa may be one of the explanations for the increase of the plasma renin activity associated with obesity (Kopple, et al., 2010; Hall et al., 2004). Increase in SNS activity observed in obese individuals, as previously mentioned, may also contribute to an increase in plasma levels of renin.

Angiotensin I is processed by the angiotensin-converting enzyme (ACE) into angiotensin II, which is the most active element of RAAS (Verlander, 2014). Angiotensin II stimulates sodium retention and aldoesterone secretion directly, factors that contribute to an increase in blood pressure (Hall et al.,1999). In addition, angiotensin II leads to contraction of efferent glomerular arterioles, which consequently leads to increased peritubular capillary sodium reabsorption as well as increased glomerular hydrostatic pressure (Hall, Brands & Henegar, 1999). The activation of RAAS, especially through angiotensin II, may contribute to the appearance of glomerular lesions in obese individuals through the elevation of the glomerular pressure caused by the increased blood pressure (Hall et al., 2014; Hall et al., 1999). On the other hand, the increase in angiotensin II appears to be related to renal ischemic and secondary damage due to the vasoconstriction and the decreased renal blood flow (Long, Price, Herrera-Acosta & Johnson, 2004). Finally, angiotensin II stimulates the proliferation and hypertrophy of vascular and mesangial cells; activates renal fibroblasts, leading to their differentiation in myofibroblasts; stimulates the production of TGF-b; induces oxidative stress and stimulates the production of chemokines that may cause local inflammation and result in a significant impairment of renal function (Xu et al., 2017; Aroor et al., 2013).

3.1.4 Low chronic inflammation state

The exaggerated expansion of adipose tissue results in an aberrant production of pro-inflammatory adipokines such as tumor necrosis factor-α (TNF-α), monocyte chemotactic protein-1 (MCP-1), interleukin-6 (IL-6), that leads to a state of low-grade inflammation (Trajcevski et al., 2013). In addition to the expansion of adipose tissue, there are increases in macrophages migration to the adipocyte region, which increase the expression of these inflammatory substances (Armstrong & Lusby, 2011).

Studies have shown the correlation between elevated expressions of these pro-inflammatory cytokines or chemokines (TNF-α, IL-6, MCP-1) in adipose tissue with renal inflammation (increased TNF-α, MCP-1, IL-6 and infiltrated macrophages) in rodent models of obesity (Stemmer, et al., 2012; Mori et al., 2014). In obese dogs increases in serum concentrations of inflammatory markers (increased C-reactive protein and IL-6, but not TNF-α) have also been found (Frank et al., 2015), with increased macrophage infiltration in adipocyte tissue (Weisberg et al., 2003; Lumeng et al., 2007), and some studies, but not all, have been found decreases in such markers and other cytokines (MCP-1, TNF-α) with weight loss (Tvarijonaviciute et al., 2012; German et al., 2009; Wakshlag et al., 2011).

Among the large number of pro-inflammatory adipokines, TNF-α, that can be synthesized by adipocytes, macrophages, mast cells, fibroblasts and neuronal cells (Armstrong et al., 2011), is one of the main critical mediators of adipose tissue inflammation. The infusion of

TNF-α in rat induced rapid changes in adipocyte gene expression, favouring pro-inflammatory cytokine production with a reduction of adiponectin, an anti-inflammatory adipokine (Ruan et al., 2002).

Beside the increased of TNF-α level, the production of MCP-1, a chemokine produced by adipocytes and macrophages, is usually increased too with excessive fat storage (Bremer & Jialal, 2013). This chemokine is recognized as one of the mediator of adipose tissue inflammation because of its effects on macrophage recruitment into the adipose tissue (Kanda et al., 2006). The deficiency of MCP-1 or its receptor was shown to induce a reduction of macrophage infiltration in the adipose tissue in obese experimental models (Kanda et al., 2006).

In obesity these two pro-inflammatory adipokines are upregulated while anti-inflammatory adipokines such as adiponectin are down-regulated (Declèves & Sharma, 2015). This anti-inflammatory adipokines, adiponectin, is one of the most abundant adipokines produced by the adipocytes and it is decreased in obesity (Kadowaki et al., 2006). The anti-inflammatory effect of adiponectin, acts by stimulating enzyme AMP-activated protein kinase (AMPK) that have protective effects on podocytes (Rutkowski et al., 2013). The AMPK activation is a key regulator of lipid storage in kidney (Declèves et al., 2015). A significant lipid accumulation in proximal tubular cells along with impaired brush border and podocytes, as associated with increased of indicators of cell damage and inflammation by the present of high nitrotyrosine levels suggesting tubular dysfunction. However, this effect can be blocked by adiponectin or AMPK activation (Sanchez et al., 2011)

In obese individuals, as well as in dogs, the leptin is in high levels, as mention previously, which is responsible for upregulating TGF-β and TGF-β receptor II (Young, Morgan, Butler, Mark & Davisson, 2013; Nasrallah & Ziyadeh, 2013). These induce type I and type IV collagen fiber formation in the mesangium, promoting fibrosis, and overactivates SNS, which induces renal hemodynamic changes and renal damage (Young, Morgan, Butler, Mark & Davisson, 2013; Nasrallah & Ziyadeh, 2013).

The chronic, low state of inflammation that accompanies the accumulation of WAT and the increases of inflammatory marker concentration in serum provides evidence that obesity-induced inflammation may have an important pathogenic role in the development and progression of kidney injury.

3.1.5 Structural and functional changes of kidneys due to obesity

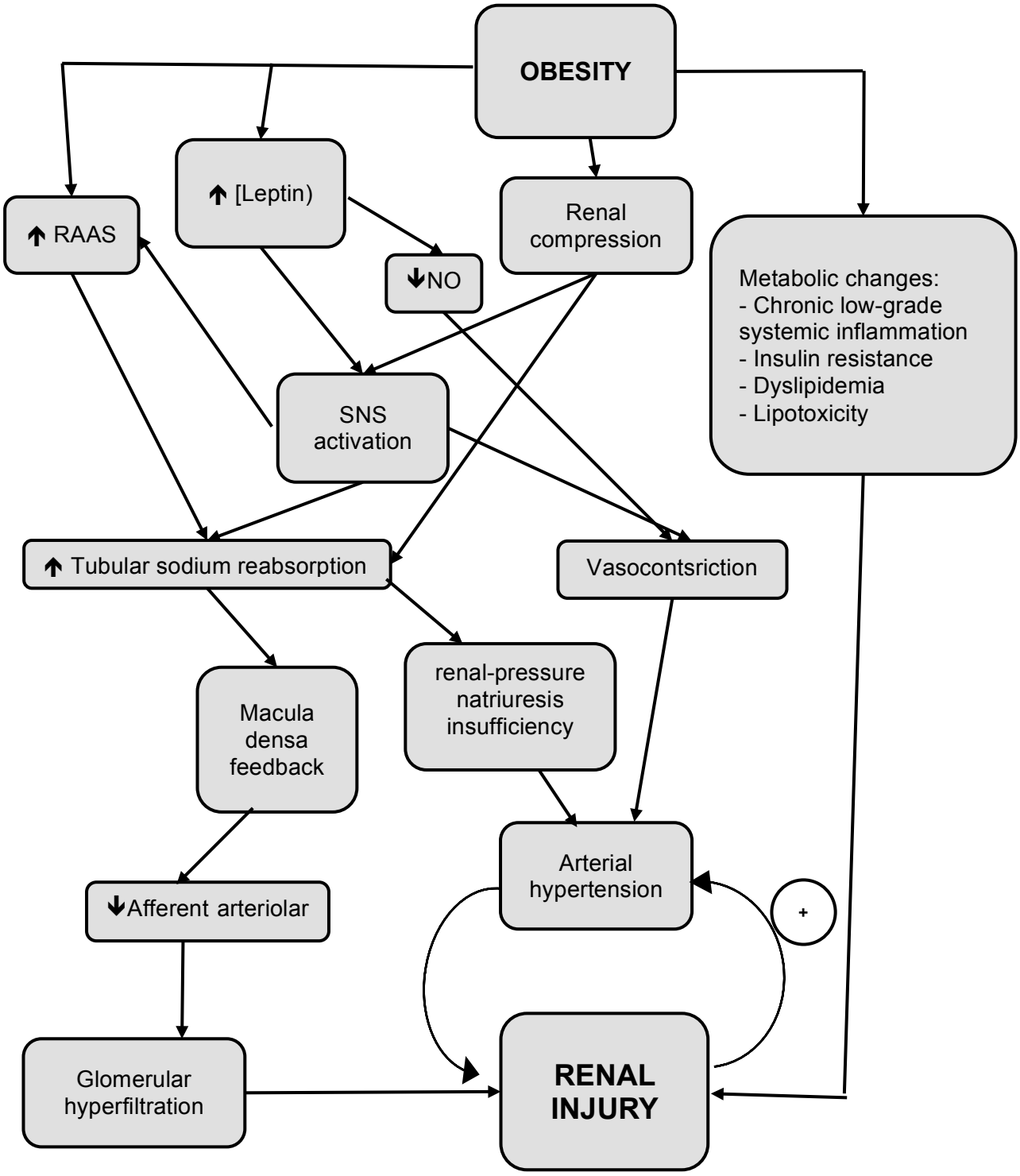
In the study performed by Heneger et al. (2001) structural alterations of the renal parenchyma (enlargement of Bowman's space, increased glomerular cell proliferation, increased mesangial matrix, thicker basement membranes, and increased expression of glomerular transforming growth factor) were observed a few weeks after a rapid increase in

weight in experimental dogs. These early renal changes occurred with only modest hypertension, no evidence of diabetes, and only mild metabolic abnormalities (Heneger et al., 2001). According Hall et al., deposition of mesangial matrix, glomerular basement membrane thickening, and fibrotic changes in obesity, at an early stage may protect the kidney of glomerular capillary overstretching despite increased glomerular hydrostatic pressure due to renal vasodilation and elevated arterial pressures (Hall et al., 2014). However, long-term arterial hypertension leads to renal damage and reduced filtration surface area thus contributing to a positive feedback mechanism that further increases glomerular pressure and leads to additional renal injury and eventually progressing to CKD (Hall et al., 2014). This slowly developing vicious cycle may be accelerated if other metabolic derangements induced by obesity are present such as hyperglycemia, inflammation, oxidative stress, and dyslipidemia (Hall et al., 2014)..

In Humans, glomerulosclerosis (segmental and focal) is the histological type of glomerular disease most often associated with obesity (Ahmed & Khalil 2007; Kasiske & Napier, 1985). Segmental and focal glomerulosclerosis associated with obesity is distinguished from idiopathic segmental and focal glomerulosclerosis because the former has lower incidence of nephrotic syndrome, is more benign, and has slower progression to renal failure (Ahmed et al., 2007).

Clinically, in humans, obesity-associated nephropathy manifests itself with nephrotic proteinuria prior to the onset of azotemia, and it is independent of other renal disease-associated conditions (Kambham, Markowitz, Valeri, Lin & D'Agati, 2001). The magnitude of proteinuria correlates with the severity of obesity and there is a significant correlation between weight loss and proteinuria reduction (Kambham et al., 2001; Griffin et al., 2008). In dogs, it is not clear yet. In the study with dogs with experimentally induced obesity, as mentioned, urine protein excretion was not determined prior to or following forced increases in BW (Heneger et al., 2001). A recent prospective study that evaluated the effect of weight loss in dogs with naturally occurring obesity showed a significant reduction in UPCR occurred after weight loss (Tvarijonaviciute et al., 2013). However, another study that evaluated the association between obesity and proteinuria did not revealed a significant difference in urine protein and albumin excretion between the overweight/obese dogs and dogs in ideal body condition (Tefft, Shaw, Ihle, Burton & Pack, 2014).

Figure 3 - Pathophysiology of the association between obesity and kidney disease. Adapted from Silva et al., 2017



NO – nitric oxide; RAAS – renin–angiotensin–aldosterone system; SNS – Sympathetic nervous system

IV. Nutritional Treatment

In this section, the treatment of obesity will be briefly discussed as well as the combination of obesity with CKD, how we should face it and what are the advantages and disadvantages of carrying out a weight loss plan according to the stage of CKD.

As already mentioned, obesity, without other concomitant diseases, is one of the simplest pathological conditions to diagnose but on the other hand, difficult to treat because besides choosing the right diet and designing the dietary plan, it´s necessary for the veterinarian to have dedication, patience and time as well as for the owners to recognize and understand the problem, and committing themselves to follow the plan (Churchill & Ward, 2016; Murphy, 2016). The basis of the treatment for weight-loss in overweight pets is calorie-restriction and avoiding regain of the lost weight (Michel et al., 2012; Toll et al., 2010). In order to develop a weight-loss program the first step is to perform a full physical exam. The results from the exam will help to find the conditions that may have influenced the weight gain (concomitant pathologies as hypothyroidism or hyperadrenocorticism), or that should be considered when defining the weight-loss plan (e.g., CKD), as well as the conditions that may influence the ability of the animal to exercise (Churchill et al., 2016; Murphy, 2016). Another imperative step for the success of a weight-loss plan is to establish a complete dietary history to understand who feeds the animal, how and when it is fed, which diet, and the amount of food the dog eats (Michel et al 2012; Toll et al 2010). After that, the three main keys components to be to take into account are alteration of behavior, the activity level and the diet (Debraekeleer, 2005; Case et al., 2000). To understand these three main keys better, first we have to approach the energy and how to restrict it as the basis of a weight loss plan is to increase energy expenditure in relation to energy intake (Toll et al., 2010).

1 ENERGY RESTRICTION

The level of energy restriction depends mainly on the degree of weight excess, the actual energy intake, and the projected duration of weight loss (Diez & Nguyen, 2010, Fascetti et al 2012). The underlying rationale in a weight loss plan is that weight loss should be gradual to minimize lean tissue loss and to avoid a regain of BW at the end of the programme (Laflamme & Kuhlman, 1995). Determining caloric restriction in dogs can be obtained by several methods. One of them is by calculation based on current food intake , which requires an accurate diet history to allow an estimation of patient's daily caloric intake. This calculation will correspond to the maintenance energy requirement (MER) for the current body weight. The MER is the amount of energy required to maintain an animal in a state of

energy balance, or in other words, the amount of energy needed to maintain an animal at its current weight (and body composition) (Toll et al., 2010). A reasonable starting point for daily calorie intake in a weight loss plan is to feed only 60-70% of that MER calculated (Toll et al., 2010). Unfortunately, an accurate diet history is not always easy to get because the owner could give an inaccurate report due to concerns of having been "feeding too much" or do not know the right amount they are giving. This makes it difficult to calculate the correct MER, and consequently cause a risk of restricting too much the energy or the opposite (Toll et al., 2010). Therefore, an alternative and more frequently used option is RER. RER correspond to the energy that the animal expends when it is lying down. It is often measured in animals that are not fasting, and therefore it may contain some energy associated with the digestion of food (Toll et al., 2010). This term of resting energy is more often used in pet because is difficult to have an animal totally cooperate under this conditions: postabsorptive state under thermoneutral conditions (i.e., no additional energy expenditure is required specifically to maintain body temperature) with the subject lying but awake and in complete muscular repose) (Toll et al., 2010). The patient's RER should be calculated using an estimate of the optimal BW based on BCS (RER in Kcal = 70 x body weight [BW]kg^0.75), because this reflects the energy needs of metabolically active tissues (e.g., cardiac muscle, the central nervous system) (Toll et al., 2010; Michel et al., 2012). Using RER as a basis for weight loss has been shown to be effective and well tolerated (Wakshlag et al., 2012; Michel et al., 2012; German et al., 2011). Regardless of which way is used, this is just a starting point because some overweight pets may already be eating close to those calculated amounts, and further calorie restriction will be required to achieve weight loss.. This will need adjustment over time to achieve a right rate of weight loss until the patient reaches the correct weight, which depends on each pet and their circumstances (Brooks et al., 2014). Laflamme showed in 1995 that the greater the caloric restriction, the greater is the weight loss and more quickly. However, the same dogs with higher rate of weight loss were the ones with greater predisposition to regain the weight when they came off of the diet (Laflamme & Kuhlman, 1995). Moreover, Laflamme & Hannah in 2005 has shown that different rates of weight loss may provide additional benefits. In that study they showed that a rate of 1% of body weight loss per week could limit risk of nutrient deficiency, loss of lean body mass, and rebound weight gain (Laflamme & Hannah, 2005). Therefore AAHA Weight Management Guidelines recommend that rates of successful weight loss range from 0.5% to 2% of weekly body weight loss for dogs (Brooks et al., 2014)

Finally, a diet and dosage must be chosen for the animal and the animal should be rechecked every two to four weeks for weight and to adjust the feeding management to reach the target (table 5). (Michel et al., 2012; Toll et al., 2010).

Table 4 – Summary of a weight loss programme for healthy overweight dogs (Michel et al., 2012)

1º physical examination including BCS;
2º Obtain a diet history;
3º Weigh the patient and set the weight loss goal;
4º Estimate the patient's optimal BW;
5º Estimate the patient's current MER from the diet history or RER from the optimal BW;
6º Set the level of caloric restriction;
7º Choose the diet and calculate the food dosage;
8º Recheck the patient every two to four weeks for weight. Adjust feeding management to target weight loss at 1% to 2% of BW/ week and address any problems or owners concerns.

BCS – body consition score; BW – body weight; MER – maintenance energy requirement; RER – rest energy requeriment; BW – body weight

2 DIETARY CONSIDERATIONS

One of the first considerations of a weight lost diet is to ensure that it is well balanced. All nutrient requirements, such protein, essential fatty acids, vitamins and minerals must be present in amounts sufficient to support normal physiologic processes and retention of lean body tissue, even when calorie intake is insufficient to maintain body weight, otherwise the animal will have deficiencies (Toll et al., 2010). The goal of this diet should be to restrict only energy (Toll et al., 2010). To achieve this, pet food manufacturers decrease the energy density of foods by reducing fat and simultaneously increasing the fiber, air or moisture content of the food, which will be discussed below (Toll et al., 2010).

In addition to caloric intake, it is important to consider the sources of those calories. Energy (calories) is derived from three macronutrients: protein, fat, and carbohydrates. A selection of an optimal proportion of these macronutrients should be considered when selecting a diet for each pet. Pets with comorbidities such as CKD, additional nutritional considerations are necessary. However, this consideration depends on the stage of CKD. In humans, the obesity paradox has been observed where renal patients with advanced CKD or end stage renal disease have lower mortality rates (Kovesdy, Anderson, Kalantar-Zadeh, 2007; Beddhu, Pappas, Ramkumar, Samore, 2003). However, these protective effects of higher BMI showed that is of short-term and is in contrast with the deleterious effects of longer term of obesity (Snyder, Foley, Gilbertson, Vonesh, Collins, 2003). Also, in dogs, higher BCS at the time of diagnosis was associated with improved survival (Parker, Freeman, 2011). In this study, underweight CKD dogs with a body condition score (BCS) of 1-3/9 had a significantly shorter median survival time compared with moderate (BCS 4-6/9) and overweight dogs (BCS 7-9/9), with no difference between moderate and overweight (Parker, Freeman, 2011). There are several putative short-term benefits such as better

nutritional status typically seen in obese individuals, which provides better protein and energy reserves in the face of acute illness, and a higher muscle mass with enhanced antioxidant capacity (Beddhu, Pappas, Ramkumar, Samore, 2003). Other hypothetically beneficial characteristics of obesity include a more stable hemodynamic status with attenuation of stress responses and increase of sympathetic and renin-angiotensin activity by adipose tissue neutralizing the adverse effects of tumor necrosis factor alpha and sequestration of uremic toxins by adipose tissue (Horwich et al., 2001; Jandacek et al., 2005). Therefore, the implementation of a weight loss program in patients with later-stages of CKD (International Renal Interest Society (IRIS) stage 3-4) may be not reasonable and a therapeutic kidney diet would be more appropriate to control the symptoms and to minimize them (e.g., azotemia, uremia, proteinuria). However, prescribing a weight loss diet for a obese dog in earlier stages (IRIS stage 1 and 2) with mild renal azotemia (lower end of the range lies within reference ranges) and clinical signs usually mild or absent (e.g., inadequate urinary concentrating, abnormal renal palpation or renal imaging findings, proteinuria of renal origin, abnormal renal biopsy results) would be beneficial. Nonetheless the diet should meet some requirements beside the low energy density and fat restriction such as moderate high quality protein and, low in phosphorus, moderate to high fiber. This requirement will be discussed below.

Protein and phosphorus:

High quality protein diets with reduced energy density help to maintain lean body mass during weight loss in dogs (Hannah & Laflamme 1998; Laflamme et al., 2005). Another benefit of these diets is the thermic effect, as protein ingestion results in increased energy expenditure in contrast with equal energy consumed from fat or carbohydrate (Toll et al., 2010, Loftus et al., 2015). Furthermore, the satiety effect of a high-protein diet is higher than with high-fat or carbohydrate diets (Diez et al., 2007). The quantity of protein recommended by Toll et al. (2010) for weight-loss is at least 25% dry matter (DM) crude protein to help prevent loss of lean body mass (Toll et al., 2010).

However, when kidney disease is present, the primary concern is the kidney and a general consensus of opinions supports the fact that reducing protein intake improves clinical signs in animals with kidney disease, especially in stages 3 and 4 (Ross, 2017). The rationale behind the limiting of protein intake in CKD patients is that the reduction of nonessential proteins will result in decreased production of nitrogenous waste with consequent improvement of clinical signs of uremia (Polzin, Osborne, Adams, O'Brien, 1989; Forrester, Adams, Allen 2010). A study of Polzin et al. compared the efficiency of three diets (maintenance diet with 44% protein in DM and two protein restriction diets with 8.2% and 17.2% DM) provided for 40 weeks for dogs with induced CKD (Polzin, Osborne, Stevens, Hayden, 1983). The animals that received the restricted diets showed a reduction in

mortality, reduction in BUN concentration, as well as clinical manifestations secondary to uremia (Polzin et al. 1983). Also dietary protein restriction (35%vs 14% DM) showed a decreased proteinuria in dogs with spontaneous glomerular disease (Burkholder et al., 2004). A decreased protein intake may reduce tubular hyperfunction by reducing acid load and renal ammoniogenesis. In general, protein metabolism is the largest generator of H^{+} ions, and when protein intake is reduced, diet helps to maintain the acid-base balance (Relman, Lennon, Lemann, 1961; Forrester et al., 2010). The type of protein can also have an influence. Animal protein (eg, methionine and cysteine) has higher sulphur content than vegetal protein, which contributes to the increase of renal acid load (Forrester et al., 2010). The reduced of protein intake can lead to catabolism of body energy reservoirs, if there is insufficient energy provided (carbohydrates and fats), including protein catabolism for the use of amino acids in gluconeogenesis. Therefore, it is necessary to avoid protein excess, but without imposing deficiency (Burkholder, 2000; Forrester et al., 2010). The study by Brenner, Meyer and Hostetter (1982) showed that excessive consumption of protein was associated with glomerular hypertension and hyperfiltration in rats (Brenner, Meyer, Hostetter, 1982). With the reduction of protein intake, these hemodynamic changes were reduced and preservation of the normal glomerular structure was observed (Brenner et al., 1982). As mentioned above, in a weight loss diet the high quantity of protein helps to prevent loss of lean body mass and helps in the satiety. However, for a dog with a combination of these two diseases reducing in protein would be more beneficial because it can reduce proteinuria and signs of uremia. In humans proteinuria or albuminuria is one of the principal signs of renal injury secondary to obesity (Chang et al., 2013; Foster et al., 2008; Praga et al., 2000). Also in dogs UPCR is higher in obese dogs, although they did not have proteinuria (Tefft et al., 2014).

The minimum recommended by NRC allowances for DM dietary protein in foods for healthy adult dogs 10% and the recommended allowances are 18% (Forrester et al., 2010; FEDIAF, 2013). The recommendation for dogs with obesity is at least 25% by Michel, in Fascetti and for the kidney disease is 14%-20% according to Forrester et al., 2010 (Michel, 2012; Forrester et al., 2010). Therefore, the recommendation for the combination of this two diseases is between 13 to 20% DM crude protein (Bartges and Rakitic, 2010; Michel, 2012). However it's important to still monitor for signs of protein deficiency.

Fat:

The fat in a weight-loss diet should be reduced due to its high energy density. The recommended upper limit for dietary fat for weight loss in dogs is 6-11% DM (Toll et al., 2010, Bartges et al., 2010). However, at the same time it should cover the essential fatty acids and fat-soluble vitamin requirements. In particular, the omega-3 fatty acids that have anti-inflammatory effects in primary renal failure are of interest (Michel et al., 2012, Toll et

al., 2010, Loftus et al., 2015, Bartges et al., 2010). Studies in laboratory-induced renal disease in dogs have reported that supplementation with menhaden fish oil (rich in omega-3 polyunsaturated fatty acids) was considered to be renoprotective compared with safflower oil (rich in omega- 6 polyunsaturated fatty acids) and beef tallow (rich in saturated fatty acids) (Brown et al., 1998). It was also described that dogs fed menhaden fish oil had lowered glomerular capillary pressure, reduced proteinuria, and slowed progressive decline in the glomerular filtration rate (Brown et al., 1998).

Carbohydrates:

In a weight-loss diet, carbohydrates can be used to lower glycemic index, to shift the metabolism of energy storage to energy usage and to increase satiety (Toll et al., 2010). There are three categories of carbohydrates based on composition: 1) simple sugars such as monosaccharides (e.g., glucose) and disaccharides (e.g. sucrose), 2) oligosaccharides (three to nine sugar units; e.g., raffinose, stachyose) and 3) polysaccharides [more than nine sugar units, e.g. starches (amylose, amylopectin or glycogen) hemicellulose, cellulose, pectins, gums]. In nutritional sense the polysaccharides are commonly known as complex carbohydrates. These ones are divided in starches, if they are digested by the animal endogenous digestive enzymes or “labeled fibers” if they are fermented by intestinal microbes (Gross, et al., 2010). After digestion and absorption of starches or simple sugars, the glycemic index increases immediately, in parallel with the increase in insulin levels (Diez & Nguyen, 2007). Carbohydrates that result in a low postprandial blood glucose response have a lower glycemic index and vice versa. Simple sugars are rich in glucose and have high glycemic index and consequently high insulin response (Diez & Nguyen, 2007).

The glucose in excess, can be used in lipogenesis, and can be transformed into fatty acids, which are stored as TG (Haugen & Drevon, 2007). After a meal, lipogenesis is stimulated by insulin (Haugen & Drevon, 2007). The importance of a low glycemic index and, consequently, a decreased response of insulin in a weight loss diet with reduced simple carbohydrate content is to decrease the lipogenesis and favor mobilization of fats (Diez & Nguyen, 2007).

The dietary fiber is one of the most important ingredients in weight loss diets, mainly the insoluble fiber, which besides diluting calories also increases satiation. Insoluble fiber increases the bulk in gastrointestinal (GI) tract and consequently increases the gastric distension; it decreases the absorption capacity of energy by interfering with the digestion and absorption of fat, protein and digestible carbohydrate (Michel et al., 2012; Toll et al., 2010; Loftus et al., 2015). Moreover, the utilization of fermentable or soluble fiber can improves GI health in the case of renal patients, (Toll et al 2010; Bartges et al., 2010). Fermentable or soluble fiber is a source of energy for GI bacteria. In order to grow, bacteria

can use BUN as a source of nitrogen, which subsequently increases the fecal nitrogen excretion in the form of the bacterial cell mass and decreases the urinary nitrogen excretion (nitrogen-trap effect) (Younes et al, 1995; Younes et al, 1996). Studies in partially nephrectomized rats with supplementation of fermentable fiber (gum arabic) in wheat starch-based diet showed a decrease in BUN concentration; however, there were no differences in total nitrogen excretion, but a shift from urinary to fecal nitrogen excretion (Younes, Garleb Behr, Demignéa, Rémésya, 1998). There is one study published to validate this hypothesis in dogs. Eight healthy dogs were randomly assigned to one of two treatment groups. One was fed with low-protein diet with insoluble fibre (cellulose) and the other was fed with fermentable fibres containing sugar beet pulp and guar gum mix (Wambacq, et al., 2016). The group fed with the fermentable fiber showed increased fermentation-derived propionic acid and its metabolites can be used as alternative substrates for gluconeogenesis which spares amino acids and reduces the kidneys'/liver's burden of N-waste removal through the nitrogen-trap effect (Wambacq, et al., 2016).

Other nutrients:

Phosphor:

Another important nutrient that is very important to take into account when kidney disease is present is the quantity of phosphor, which should be reduced to avoid the occurrence of hyperphosphatemia. When kidneys lose the functional capacity, an imbalance in calcium and phosphorus metabolism occurs, which leads to a stimulation of the parathyroid to produce parathyroid hormone in an attempt to maintain calcium homeostasis, because in general, hypocalcemia occurs in CKD (Diez & Nguyen, 2010). This phenomenon is called secondary renal hyperparathyroidism manifested through demineralization of bone and calcium deposition in soft tissues including the renal parenchyma (Diez & Nguyen, 2010). Slatopolsky et al. (1971) showed that phosphorus restriction reduced GFR by up to 1/4 in the dogs fed a phosphorus-restricted diet (intake less than 100 mg per day / animal). However, the serum PTH concentrations remained virtually unchanged. Nonetheless the dogs that received the unrestricted diet (intake of 1200 mg of phosphorus per day / animal), presented an increase of serum PTH by up to 20 times (Slatopolsky et al. 1971). Also, Brown et al. 1991, showed that animals fed with lower amounts of phosphorus in the diet had lower plasma concentrations of phosphate and creatinine (Brown, Crowell, Barsanti, White, Finco, 1991). The veterinary therapeutic renal foods are formulated with limited dietary phosphorus besides the protein restriction and have showed beneficial effects on dogs with naturally occurring CKD. One study was performed by Jacob et al., 2002, that showed the used of a kidney diet (14%DM protein, 0.28% DM phosphorus and 1.6% DM omega-3 fatty acids) in dogs with spontaneous IRIS stage 2 or greater CKD had a delay on the median time to uremic crisis (615 days) compared to dog on a maintenance diet (25%

DM protein, 1% DM phosphorus and 0.22% DM omega-3 fatty acids) that had higher median times to uremic crisis (252 days) (Jacob et al., 2002). At the end of this study, only 33% of dogs in the therapeutic kidney diet group died of a renal-related cause compared with 65% of dogs in the maintenance group (Jacob et al., 2002).

In a weight loss diet the concentration of phosphorus is not taken into account. However, when a weight loss program is prescribed to an obese patient with kidney dysfunction, a diet with low phosphorus content should be chosen. The recommended phosphorus levels for foods used to manage CKD are 0.2 to 0.5% DM for dogs to achieve beneficial effects according to Forrester et al., 2010 (Forrester et al., 2010).

Sodium:

Sodium is also one mineral that should be in low to moderate quantities in patients with CKD to help in the reduction of blood pressure, although there is no evidence yet in studies with dogs (Bartges et al., 2010). The rationale is based on the reduced ability of the remaining nephrons to excrete sodium, and the concern that whole body sodium accumulation would contribute to the development of hypertension (Toll et al., 2010).

Antioxidants:

Because oxidative stress in overweight patients is increased, which contributes to diseases associated with obesity, supplemental antioxidants are recommended for weight-loss foods as well as in CKD, due the benefits that they bring (Toll et al., 2010). Humans with chronic kidney disease have been shown to have oxidative stress with lower concentrations of vitamin E and vitamin C (Cochrane & Ricardo 2003; Locatelli et al. 2003). Exogenous antioxidants such as vitamin E, vitamin C, carotenoids and flavanols promote a more favorable redox state in the body in order to minimize oxidative damage (Brown, 2008). Together, antioxidant systems work collectively to eliminate and neutralize free radicals and minimize oxidative stress (Brown, 2008). Yu and Paetau-Robinson (2006) showed that supplementation with vitamin E, β-carotene, and vitamin C in cats with naturally occurring stage II chronic kidney disease reduced markers of DNA damage (Yu & Paetau-Robinson, 2006). In dogs with surgically induced renal mass reduction, Brown (2008) reported that supplementation with vitamin E, carotenoids, and lutein, slowed the rate of reduction of the glomerular filtration rate, compared to dogs that did not receive antioxidant supplementation (Brown, 2008).

Supplements:

Beside the nutrients that are important for the weight loss diet and CKD, there are supplements that can be add to the diet to help in weight-loss. Carnitine, for example, promotes shuttling of cytosolic long-chain fatty acids across the mitochondrial membrane to

promote their β-oxidation within the mitochondrial matrix, which may increase the rate of weight loss while promoting retention of lean body mass in companion animals during caloric restriction; nonetheless, the studies show inconsistent effects (Loftus et al., 2015; Toll et al., 2010). Another supplement is green tea extract. In dogs with obesity-induced insulin resistance, green tea extract (80 mg/kg) resulted in enhanced insulin sensitivity and decreased triglyceride levels after 12 weeks of supplementation (Serisier et al., 2008b).

Water:

Other important factor in renal patients is water since they are often dehydrated (Michel et al., 2012; Toll et al., 2010; Bartges et al., 2010). Canned diets have more water, and consequently they can increase the water consumed and improve the fluid balance in renal patients; Further the energy is more diluted per volume, which can help to increase the satiety (Bartges et al., 2010).

Feeding habits:

For the weight loss programme to be successful, it is very important to have total compliance and dedication of the owner and adjust the programme to the lifestyle of the patient's household as much as possible. It is suggested that: the owner starts to quantify and monitor the food intake with the right amount of specific diet calculated; all kinds of human food have to be eliminated; if the owner want to give treats, the amount per day is fixed and the calories from the treats have to be subtracted from the established daily diet; the animal should not be present in the kitchen during the cooking and at the time of the owners' meals (Fascetti et al 2012). To take advantage from the obligatory energy cost of digesting and absorptions food, the animal should be fed multiple small meals during the day, at least two portions, fed 8-12 hours apart, than a single large meal per day (Fascetti et al 2012). Also when CKD is present multiple small meals can help to avoid high post-prandial BUN concentrations, which decrease the probability of nausea and the signs of uremia (Forrester et al., 2010). Obese patients with later stage of CKD and presence of uremia and other signs of systemic disease may be partially or completely anorectic and require alternate feeding methods such feeding tube (Forrester et al., 2010). However, encouraging acceptance of the diet should be taken into account such as the palatability like warming, adding water or flavoring agents to dry food, such as tuna juice, clam juice, chicken broth, low-sodium soups, garlic, brewer's yeast or sweeteners such as honey or syrup (Forrester et al., 2010).

Exercise:

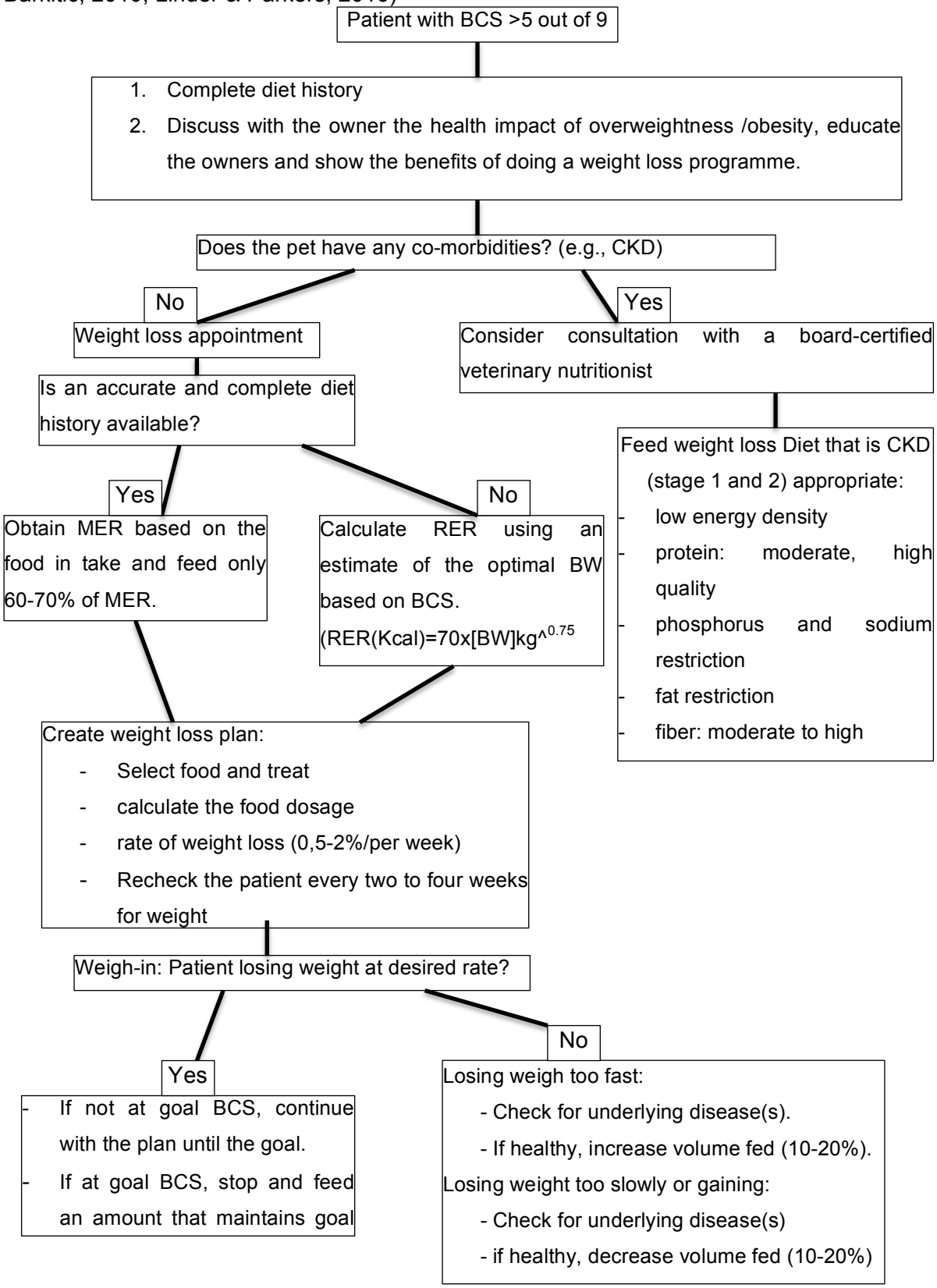
While energy restriction is the most important part of a weight reduction plan for an obese dog, increasing energy expenditure is another way to achieve a negative energy balance.

Therefore, to increase energy expenditure it is imperative to increase physical activity. However, this is recommended in dog without any other comorbidity. Nonetheless, in humans studies showed that regular exercise in patients with CKD increases muscle strength, prevents muscle wasting and also improves the cardiovascular health and the nutritional parameters, as well as reduces chronic inflammation (Heiwe, Jacobson, 2011).

In a weight loss programme, the introduction of physical activity should be gradual and at tolerable intensities, due to the intolerance to exertion and respiratory distress experienced by obese animals (Fascetti et al 2012; Hand et al 2010). No optimal levels of activity have been established for dogs that eat low-energy foods. However, the recommendation from AAHA is a 5 min walk three times/day for an obese dog with no orthopedic restrictions, and increase gradually until either the client's or pet's limit is reached or once a total of 30–45 min of walking/day has been achieved (Brooks et al., 2014).

Beside the increase of energy expenditure, exercise helps to maintain lean mass. In humans, the practice of exercise during a weight loss attenuates the muscle protein catabolism, shows higher levels of retention of lean mass, improves in insulin sensitivity, and increases strength and function (Votruba, Horvitz, Schoeller, 2000; Curione, Lourenco, 2005). These positive effects of exercise during weight loss in dogs have been studied too. The dual energy x-ray absorptiometry (DEXA) demonstrated that lean mass in exercised dogs (exercised 3 times/week at the university hospital, and increase daily activity level at home) during a dietary weight loss plan was maintained when compared with a control group that maintained the normal daily exercise routines during the study period (Vitger, Stallknecht, Nielsen, Bjornvad, 2016). Another study showed that exercised dogs have higher expression of genes involved in glucose utilization in muscles and also has higher preservation of lean mass, which may reflect a positive influence on insulin signaling during the weight loss protocol (Uribe, Vitger & Ritz, 2015).

Figure 4 - Algorithm – Nutritional management of concurrent canine obesity (Bartoes & Barkitic, 2010; Linder & Parkers, 2016)



PART III – STUDY OF THE INFLUENCE OF OVERWEIGHT ON ROUTINE PARAMETERS OF RENAL FUNCTION IN DOGS

1 MATERIALS AND METHODS

This dissertation is based on part of a PhD study performed by the Animal Nutrition Lab in the Department of Nutrition, Genetics and Ethology, Ghent University. The study behind this dissertation consists of a research with the duration of one and a half years for the purpose of investigating the effects of obesity and overweight on renal function. The study involves a weight gain phase and a weight-loss phase in which routine as well as specialized or novel markers of kidney function were evaluated. Examples are serum creatinine, urea, UPCR, GFR assessed using iohexol, blood pressure, and novel urinary biomarkers retinol-binding protein, immunoglobulin G, etc. Furthermore, ultrasound was also performed, using contrast with microbubbles to provide information on renal perfusion of each kidney. The body composition was also analysed every 3 months through the deuterium oxide dilution method to accurately determine the amount of fat in each animal.
The statistical analysis of this dissertation will focus only on the first 6 months of the study, and will only analyse whether weight gain in healthy adult dogs has an effect on blood urea, creatinine, urine specific weight and UPCR related to the renal function of dogs.

1.1 Study design

The study was designed to determine the relationship between obesity and kidney disease in dogs during a period of 6 months in 16 beagles.
This study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium.

1.2 Animals and diet

Sixteen healthy adult Beagle dogs averaging 5,5 years (range 8.3 to 2.5) were included in the study. Six dogs were intact females and two others were neutered females; four were intact males and four neutered males. At the start of the study, all dogs had 4 weeks of adaptation to the diet and general blood parameters (blood count and biochemistry to analyze kidney and liver function, total proteins and thyroid hormone function) were performed to verify the health condition of animals. Urinalysis and analysis of urinary sediment and urine culture were also performed to exclude infectious or inflammatory processes and to understand the origin of proteinuria. All the dogs had an initial median of

BCS 4 out of 9 (13 dogs with BCS of 4 and 3 dogs with BCS of 5) and they received the same high energetic diet for 6 months. The diet was a commercial maintenance diet, high in fat and protein and met adequate nutrient requirements of the National research council (NRC) recommendations (National Research Council, 2006). The analysed nutrient composition of the diet is shown in Table 6. The dogs were fed at an estimated maintenance energy requirement for laboratory beagles, which was adapted to the BW evolution of each individual dog (Table 5).

Table 5 – Calculation of the daily energy requirement's in the two groups

Treatment group	Control group
(kJ) MER = RER x 1.3 x Activity level	(kJ) MER = RER x Activity level
(kJ**)** RER= 293 x BW^0.75	(kJ) RER= 293 x BW^0.75
Activity level = manually assigned to each dog	Activity level = manually assigned to each dog

DER (daily energy requirement); RER (rest energy requirement); BW (body weight); MER (maintenance energy requirement); Kj (kilojoule)

Table 6 – Analysed nutrient composition (%) of the experimental diet

Diet Composition	
Dry matter	91
Crude protein	35
Crude fat	17
Crude fiber	5
Nitrogen free extract	25
Metabolizable energy	1498 KJ

Kj (kilojoule)

BCS and BW were measured weekly and the renal function was measured every 12 weeks. The body condition was determined with a 9-point Body condition scoring system (Laflamme, 1997). The 16 dogs were carefull divided into two groups in order to have equal amount of male and females and similar ages. One of the groups was define as the Control group (C) (n = 8) and the other was the group of weight gain, or treatment group (T) (n = 8. The C group had an average initial weight of 11.6 kg. The T group had an average initial weight of 11.2 kg. During the test period, the C group received MER while the T group received 1.3 times more of the RER to gradually increase their weight approximately 0.7% of BW per week to reach a state of obesity or overweight. The amount of feed given was

adapted to maintain the animals' ideal BW for the control group. They were fed once a day in the morning.

All the dogs were kept in compatible pairs in cages, 8 of them in 4.30 x 0.87 m indoor/outdoor cages, and the other 8 in 3 x 0.87 m indoor/outdoor cages. The kennels were heated during the winter and controlled daily. Light was provided between 7am and 7pm, and during the night the dogs stayed inside. All dogs had free access to water. After all the food was finished, the bowl was removed. In case of leftovers the amount was measure and recorded. All dogs were allowed to walk freely once a week for the same amount of time on the 80m^2 or 130 m^2 outdoor free areas, except on sample collection weeks, when the free walk was in the 144m2 indoor playing area.

1.3 Measurements

On week 1 of the experiment, and on weeks 12 and 24 after the start, blood and urine samples were taken. For these purposes, all the dogs were deprived of food for at least 12 hours

1.3.1 Sampling procedure

1.3.1.1 Blood sample

On weeks 1, 12 and 24, an overnight fasting pre-prandial blood sample (8 mL) was taken from the jugular vein into a Blood Collection Tube with Serum Clot Activator for urea and creatinine. Serum was obtained by centrifugation at 2000 x g, 5 min, and 21ºC. Serum urea and creatinine analysis were performed on the automated clinical chemistry analyser.

1.3.1.2 Cystocentesis

On weeks 1, 13, 25, a sterile urine sample was taken by ultrasound-guided cystocentesis. The urine was collected into a falcon tube for the kidney markers (UPCR and specify gravity). Urine specific gravity was measured by a hand refractometer and the UPCR was calculated using the formula: UPCR = protein (mg/dl)/creatinine (mg/dl), after processing the analysis.

1.4 Statistical Analysis

The data was obtained over time, so the type of statistical analysis chosen was a time analysis or a longitudinal analysis of the data, to search for differences in the evolution of the parameters over time. Data was assumed to be normally distributed and was not checked for the assumptions that parametric tests require. All statistical analyses were conducted using the statistical software programme R studio (version 3.2.5, 14/4/2016), namely packages gplots, lattice and ggplot2, to build up the graphs and package name, using the gls function for the statistical model with treatment as a fixed factor and the interaction of time and treatment to look for differences between treatments over time with dog as a random effect.

2 RESULTS

2.1 Body weight during the experiment

At the start, all dogs had an ideal BCS of 4-5 out of 9 points. Throughout the study the control group remained stable (BCS of 4-5 out of 9 points), while the treatment group increased to a BCS of 6-7 (half of the group had BCS of 7). One dog in the treatment group, however, maintained a BCS of 5 (table 7). The body weight of the control group remained stable during the six months, having just physiologic oscillations, while the weight of the treatment group show a significant increase ($p = 0{,}0001$), which corresponded to a gain of 0.328 ± 0.055 kg per month than the control. The total percentage that the treatment group increased in weight at the end of the trial was 21.5% compared with the initial weight with a BCS of 5. This increase of 21.5% fits on a BCS of 7 according to tablet on FEDIAF (Table 3) means the dog become overweight (BCS 7=+20-30%). (Table 7 and Figure 5).

Table 7 - Median and range of the body condition scores and average **±** standard deviation of weight during the experimental period (week 1, 12, 24)

	week 1		**week 12**		**week 24**	
	Control	Treatment	Control	Treatment	Control	Treatment
BCS	4 (4-4)	5 (4- 5)	4 (4-4)	5 (4-6)	4 (4-4)	6.5 (5-7)
BW*	11.58 (±1.64)	11.20 (± 1.97)	11.71 (± 1.88)	12.52 (±2.62)	11.91 (±1.65)	13.61 (±2.23)

Body condition score median (BCS); Average body weight;

* time x treatment interaction (p=0.0001) **

Below figure 5 shows that data variation across groups was very similar at each time point when live weight was measured.

Figure 5 - Overview of the distribution dog weight (kg) results throughout the experimental period (measured at 1, 12 and 24 weeks), for all dietary treatments (each point represent a dog)

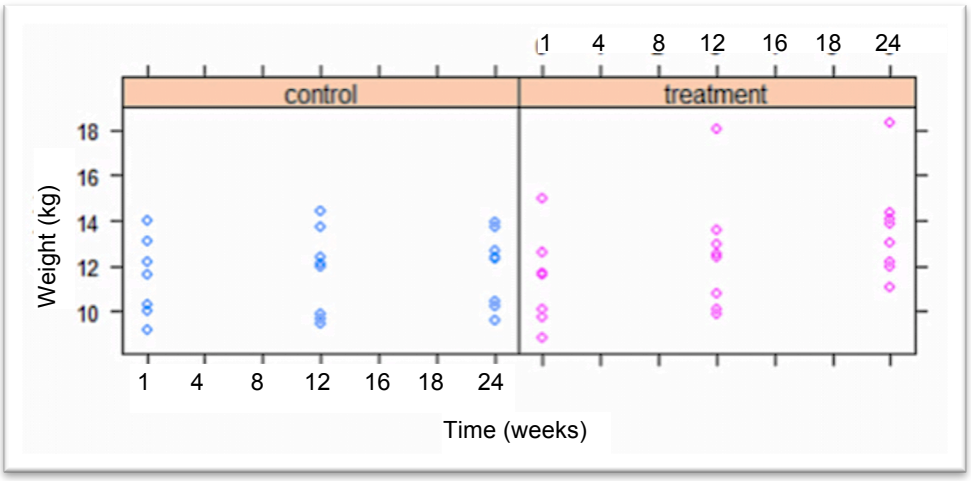
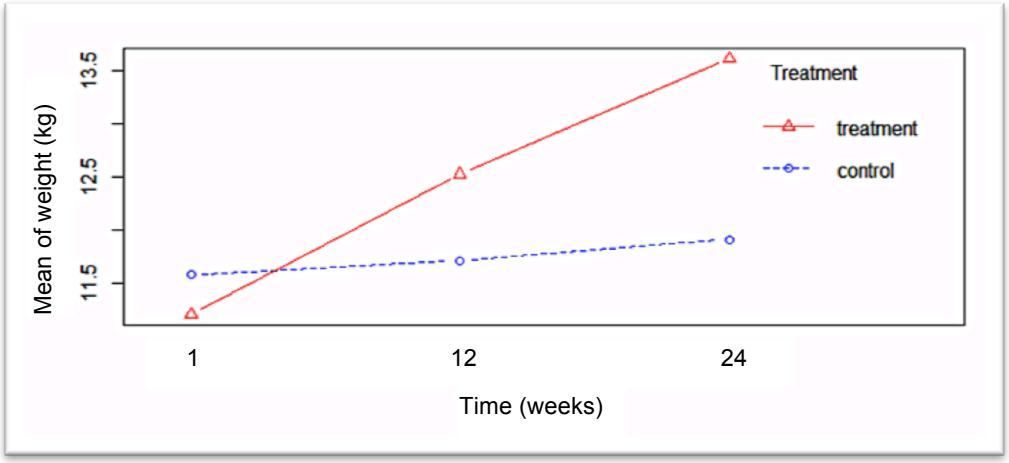


Figure 6 - Average weight (kg) for each dietary group, throughout the experimental period (measured at 1, 12 and 24 weeks)



Although figure 6 shows an increase in BW in the treatment group over time, however these results did not show a significant effect of time (p=0.136) on weight. The overall live weight at the beginning, meaning the average weight, which the two groups were equal in the trial, was 11.6 kg. It is also observed that there was no overall effect of treatment (p=0.770). The treatment reference is the control group.

2.2 Laboratory evaluation of renal function

To analyze the renal function of the animals, serum urea and creatinine, the UPCR and urine density were examined at week 1, 12 and 24. The below table 8 shows the results obtained in the renal markers chosen.

Table 8 – Average ± standard deviation results of renal markers weight during the experimental period (week 1, 12, 24)

	week 1		week 12		week 24		p-value time x treatment interaction	Reference range
	C	T	C	T	C	T		
* Blood urea (mg/dl)	30.63 ± 10.84	27.38 ± 4.27	28.25 ±5.12	30.13 ±3.09	26.63 ±5.13	27.25 ±4.10	0.707	6-57
Blood creatinine (mg/dl)	0.63 ± 0.05	0.62 ±0.06	0.58 ±0.06	0.59 ±0.10	0.61 ±0.05	0.59 ±0.07	0.364	0.3-1.3
UPCR	0.17 ±0.15	0.13 ±0.06	0.22 ± 0.23	0.24 ±0.30	0.24 ±0.19	0.21 ±0.18	0.978	<0.2
Specific gravity	1.039 ±0.01	1.037 ±0.t01	1.040 ±0.01	1.043 ±0.004	1.038 ±0.01	1.038 ±0.008	0.852	1.015-1.045

Urine protein/creatinine ratio (UPCR); Time effect Blood urea (p=0.062); Control (C); Treatment (T)

* trend time effect (0.05 < p < 0.1)

2.3 Blood urea

Figure 7 shows that blood urea, which were within the normal reference range (6-57mg/dl), did not differ between control dogs and treatment dogs. However, when plotting the average dog blood urea (mg/dl) over time for each group (figure 8) a linear decrease in blood urea over time for control dogs can be seen, whereas obese dogs show a higher blood urea concentration at 12 week compared to both 1 and 24 week. Statistical analysis shows a trend (p=0.062) for an overall effect of time on blood urea concentrations for all dogs (treatment and control dogs) (i.e., blood urea concentrations showed changes over time). On the other hand, the overall effect of treatment was not significant (p=0., 675). Furthermore, there were no significant differences between the groups over time (p=0.707).

Figure 7 - Overview of the distribution of dog blood urea (mg/dl) results throughout the experimental period (measured at 1, 12 and 24 weeks), for all dietary treatments (each point represents a dog.

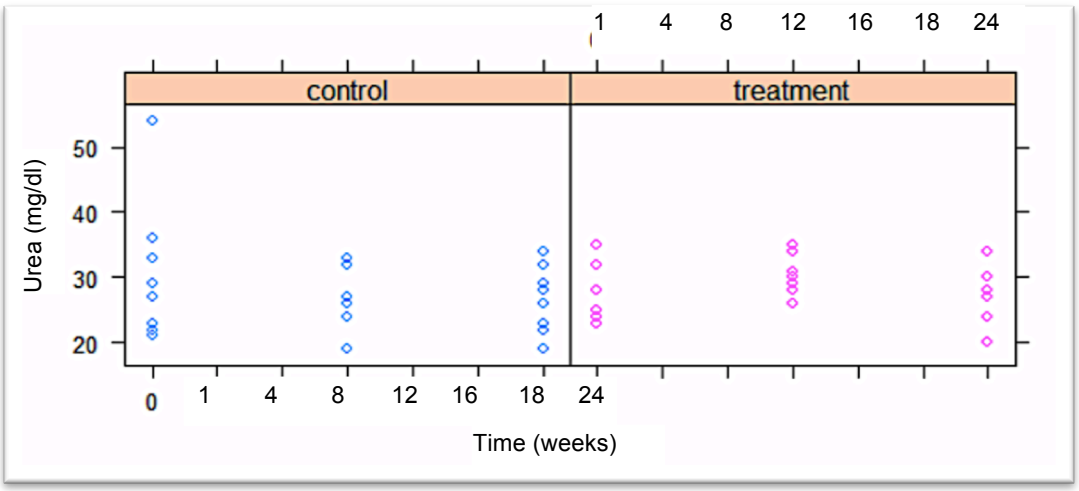
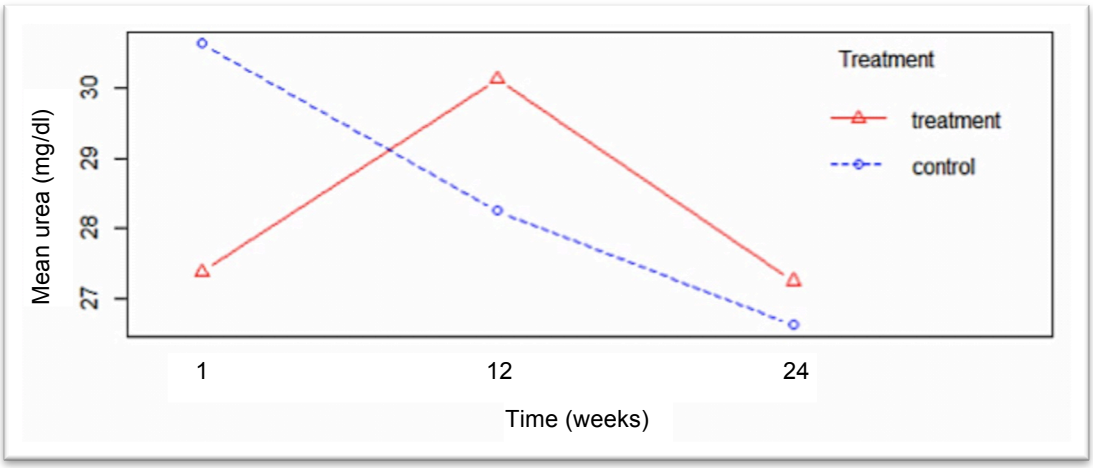


Figure 8 - Average dog blood urea (mg/dl) for each dietary treatment, throughout the experimental period (measured at 1, 12 and 24 weeks)



2.4 Blood creatinine

Figure 9 and 10 show that for the first 12 weeks, no differences in blood creatinine are to be noticed whereas at week 24, the obese dogs seem to have lower blood creatinine concentrations than control dogs. However, similar to blood urea, serum creatinine concentrations did not differ significantly between control dogs and obese dogs. The longitudinal model shows no overall effect of time (p=0.529) or of treatment (p=0.927) in serum creatinine concentrations. Further, over time, the changes in the concentrations of serum creatinine for each dietary group are not significantly different (p=0.364). However

every dog had results below of 1.4 and according to IRIS is in the normal physiologic range (0.3-1.3 mg/dl).

Figure 9 - Overview of the distribution dog blood creatinine (mg/dl) results throughout the experimental period (measured at 1, 12 and 24 weeks), for all dietary treatments (each point represents a dog)

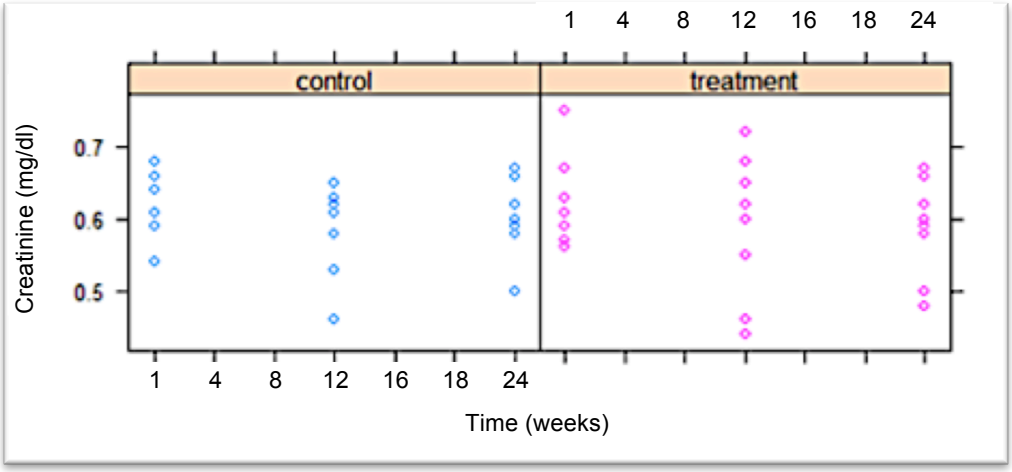
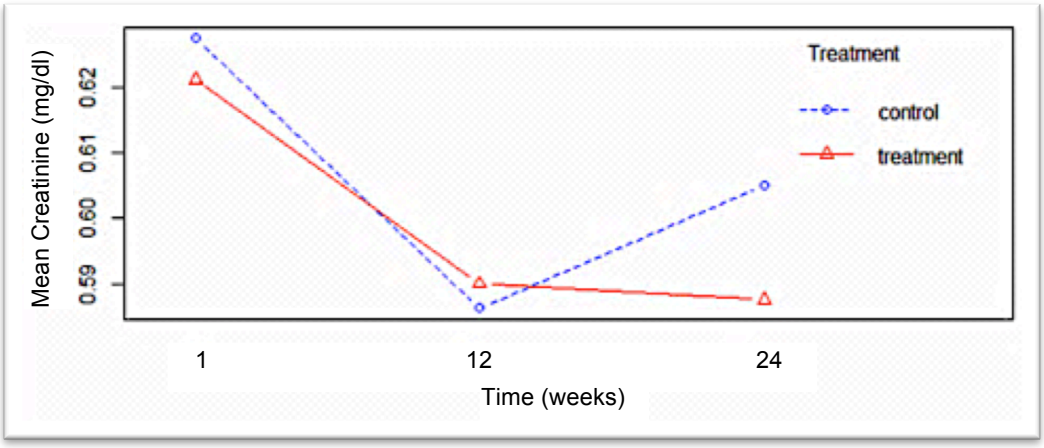


Figure 10 - Average dog blood creatinine (mg/dl) for each dietary treatment, throughout the experimental period (measured at 1, 12 and 24 weeks)



2.5 UPCR

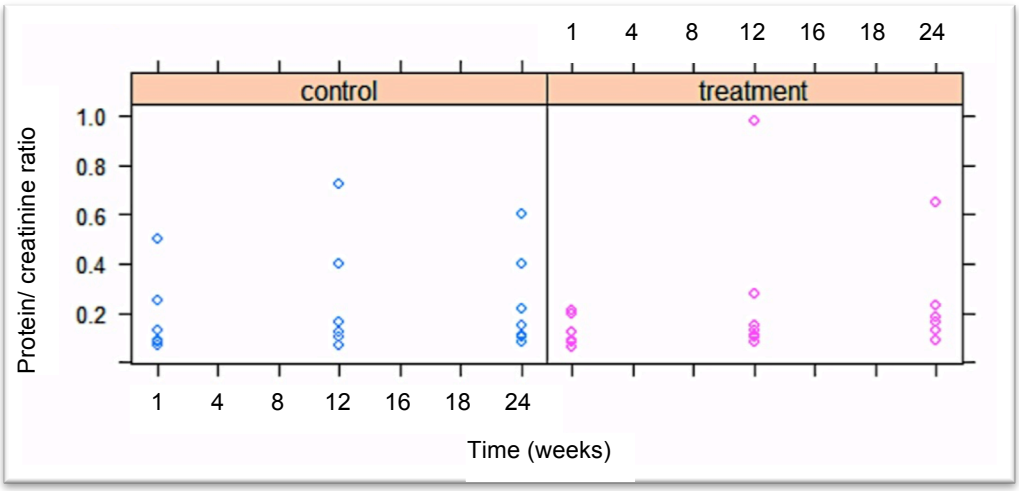
For this parameter, some data were missing during the trial, which it was impossible to build the figure by R studio. On the first urine collection at week 1 for two dogs of the treatment groups, there was a problem with the results at the lab. On the day of the last urine collection, at week 24, one dog of the control group was suffering from hyposphagma, which prevented the collection of urine.

Figure 11 shows that during the 24 weeks, 14 out of 16 dogs in both groups, had a UPCR above of 0.2, which means that they were non-proteinuric according to IRIS (UPCR <0.5). Nonetheless in the control group there were 2 dogs that had higher values. One of them

had a stable value that could be categorized as borderline proteinuric (UPCR 0.2-0.5) during the 24 weeks, while the other increased to values higher than 0.5, which corresponds with proteinuria. In the treatment group also one dog had results outside of the normal range of <0.2, having increased its values through the experimental period and became proteinuric (>0.5).

From the statistical output, there was no overall effect of time (p=0.223) or of treatment (p=0.891) in urine protein/creatinine ratio. Further, over time, the changes in the concentrations of blood creatinine for each group were not significantly different (p=0.978).

Figure 11 - Overview of the distribution dog urine UPCR throughout the experimental period (measured at 1, 12 and 24 weeks), for all dietary treatments (each point represents a dog)



2.6 Urine specific gravity

Similarly to what happened with the UPCR, there was one data point missing and R studio did not build the figure. Treatment dogs seem to show an increased urine density at 12 weeks compared to at 1 and 24 weeks. However, in figure 12 urine density did not seem to differ between control and treatment dogs over time, like with the other parameters. The results were in the physiologic range (1.015 to 1.045) according IRIS except for two dogs from the control and two from the treament that showed a high concentrated urine with values between 1.045-1.050. However from the statistical output, there was no overall effect of time (p=0.721) or of treatment (p=0.806) in UPCR. Further, over time, the changes in the concentrations of blood creatinine for each dietary group are not significantly different (p=0.852).

Figure 12 - Overview of dog urine specific weight throughout the experimental period (measured at 1, 12 and 24 weeks), for all dietary treatments (each point represents a dog)

3 DISCUSSION AND CONCLUSION

Previous studies in humans have provided compelling evidence for close associations among obesity and renal dysfunction, with presence of proteinuria or albuminuria, caused mainly by glomerular hyperfiltration, or renal venous hypertension (Chang et al., 2013; Foster et al., 2008; Praga et al., 2000). These symptoms are exacerbated over time and with the increase of abdominal fat, leads to a loss of GFR (Foster et al., 2008) and renal injuries in the glomerular region (Kambham et al., 2001) even when the common effects associated with obesity are absent. The weight loss tends to improve the renal function (Morales et al., 2003). In dogs, as study has already showed an increase the GFR and higher renal plasma flow as well renal injury related with the increase of weight (Henegar et al, 2001). Studies of weight loss in obese dogs showed that there is an improvement of renal function with a reduction in proteinuria (UPCR), increased urine specific gravity and decreased serum creatinine (Tvarijonaviciute et al., 2013).

The current study has investigated the possible association of weight gain on the renal function using common blood and urine tests used in clinical practice. Healthy kidneys remove wastes and excess fluid from the blood. Therefore, analyzing blood and urine allows the clinician to assess the health of the kidney and whether the kidneys are leaking abnormal amounts of protein. The traditional markers of renal function in current clinical use are serum urea, serum creatinine, UPCR and specific gravity. These were assessed in this study during the 24 weeks that the treatment dogs gained weight. Although the dogs in the treatment group gained 21.5% of weigh, the renal markers did not differ significantly between the two groups over the time This suggests that the degree of overweight achieved in the study might not have been enough to cause renal dysfunction based on routine kidney markers. In contrast, the dogs in study from Henegar et al. (2001) that showed altered renal function had a 50% increase in weight over their initial weight in 24 weeks.

In this study as mention the dogs increased 21.5% weight over their initial weight which means a total average increase of 2.41 kg at the final of the study. The BW increased progressively over the 24 weeks in the treatment group, as expected. However one of the dogs in the treatment group did not gain weight, since it refused to eat the daily portion provided, thereby maintaining constant BW and BCS (5 out of 9) all over the 24 weeks. Therefore the average of 0.328 kg (+/- 0.050 kg) gained per month in the treatment group could be underestimate because it takes into account the animal that did not gain weight. This 21.5% of weight gained corresponds to a BCS of 7 out of 9, which means the dogs only achieved only an overweight status. Therefore, the obese stage was not achieved in

the treatment group, which means that the weight gained may have been insufficient to cause changes the renal markers. It should also be taken into account that this 21.5% of weight gain may not correspond only to gain in fat mass. Therefore, it would have been useful to have deuterium oxide dilution data to distinguish between fat and lean body mass and give a more objective value of the weight gained.

Studies in humans revealed that obesity has a negative impact on the renal system, regardless of the presence or absence of diabetes, one of the major causes of CKD (WHO, 2017; Kovesdy et al., 2017). The first sign of renal injury secondary to obesity is the increase of proteinuria or albuminuria, caused mainly by glomerular hyperfiltration, renal venous hypertension or glomerular hypertrophy which occurs in response to increased metabolic demand (Griffin et al., 2008). Long term or severity of obesity can lead to the development of glomerulosclerosis and later in CKD (Griffin et al., 2008).

In companion dogs, it is less clear as to whether there is an association between adiposity and renal functional. However, one study with experimentally-induced obesity in dogs showed altered renal function (glomerular hyperfiltration with an associated increase of GFR) and histologic changes such as expansion of Bowman's capsule, cell proliferation in the glomeruli, thickening of glomerular and tubular basement membranes, and increased mesangial matrix (Henegar et al., 2001). In that study the method used to assess renal function was GFR measured using iothalamate. GFR is the gold standard according to IRIS to evaluate the renal function. However, is a not practical method in the clinical routine because it requires several blood collections (Heiene et al., 2015). There is currently no published study that assesses the renal function during weight gain in dogs and that why is important to evaluate renal function with routine markers in dogs that gain weight. Nonetheless, there is a study in obese dogs where renal function was evaluated during a weight loss program with routine renal markers, which showed that urea and urine specific gravity (USG) were both greater after weight loss than before loss, whilst UPCR and creatinine were less after weight loss (Tvarijonaviciute et al., 2013). Another study in healthy dogs showed numerically higher UPCR with higher BCS (median UPCR 0.13 and 0.09 for the BCS of 8 and 9, respectively compared with UPCR 0.03 for BCS of 6 and 7 and UPCR 0.02 for BCS of 5) (Tefft et al., 2014). Nonetheless all values were within laboratory reference intervals (Tefft et al., 2014).

In the current study, serum urea and creatinine was not statistically significant between the two groups over time ($p>0.05$). Serum urea increased numerically whilst serum creatinine decreased in the treatment group after 12 weeks of weight gain, while the control group had a slight decrease in the two parameters. Yamka et al. performed a study to determine which biomarkers differ between lean and overweight dogs and to show the effects of weight loss on these biomarkers (Yamka, Friesen Frantz, 2006). Their results show that overweight dogs had lower level of creatinine and urea than the lean dogs, and after a weight loss diet

with hight protein for 90 days, urea had a statistically significant increase (Yamka, et al., 2006). However, not in all previous studies found the same effect (Diez et al., 2004). Diez performed a study of weight lost with two different diets: a high protein diet and a high fiber diet (Diez et al., 2004). The results showed that after the weight loss the urea decreased in the two groups with no effect of diet (Diez et al., 2004). Also, this study showed that the group on the high protein diet had higher values of urea during the whole study than the other group with high fiber diet and this was associated with the amount of protein (Diez et al., 2004). This could be the reason for the observed results in our study. The amount of given food to the two groups was different. The treatment group received a higher amount, which corresponded to a higher amount of protein. However, is not possible to state this change was due to the switch to a high protein diet because serum urea concentrations were not collected from the previous diet. Furthermore, the study did not include a control diet with low protein content in order assess diet effect. Nonetheless the treatment group received 1.3 times RER, which means the treatment group received 1.3 times more of protein during the experimental period.

Also, other clinical conditions can increase the blood urea such as starvation, infection or fever or even GI bleeding, but none of these were present in the dogs in this study (Diez & Nguyen, 2010). Furthermore, the concentrations of urea and creatinine were within laboratory reference intervals in this study.

Notwithstanding, after 12 weeks the urea concentrations decreased again in the treatment group, showing results similar to the control group and to week 1. The serum urea concentration is also dependent on the reabsorption in the kidneys, which is influenced by the filtrate flow in the renal tubules or GFR. Thus, an increased renal perfusion decreases its reabsorption, increasing its excretion in the urine, and the opposite, a reduction of renal perfusion results in an increase in renal tubule reabsorption of urea, increasing its concentration in the blood (Diez & Nguyen, 2010; Fettman & Rebar, 2004). However, as mentioned previously high amounts of dietary protein increase the serum urea as well as clinical conditions that increase the catabolism of endogenous protein, while low-protein diets, anabolic drugs, hepatic insufficiency or portosystemic shunting can decrease urea. Nonetheless, obese individuals have a tendency to have increased GFR (Chagnac et al., 2000; Wofford et al., 2001; Wuerzner et al., 2010). As mentions in the Yamka et al. study, obese dogs had lower values of urea than lean dogs (Yamka, et al., 2006). Consequently, the measurement of GFR during the study cloud help to differentiate from where was the decrease of urea. This measure was taken during the study but was not used for this study because the objective was to evaluate only the routine parameters. However, is necessary to take into account also the normal physiological variation of urea that can be the only reason in this study, because all the values was in the normal range (6-57 mg/dl, reference

from medvet) during the study. Furthermore, it is necessary to take into account that urea is not a reliable marker for GFR (Dibartola, 2010).

The numerical decrease of serum creatinine concentration in the treatment group could be expected. As mentioned in the study of Yamka et al., obese dogs have lower values of creatinine than the lean dogs, which increase after a weight loss (Yamka, et al., 2006). Creatinine is inversely related to the GFR, which means an increase of GFR translates into a decrease in blood creatinine concentration (Lefebvre, Watson, Heiene, 2015), Thus, this result could be result of an increase in GFR, which is described in obese dogs (Henegar et al., 2001). Also in humans and experimental animal models increase of GFR is associated with obesity, which is due an increase of metabolic demands that result in glomerular hyperfiltation (Griffin et al., 2008). However, the control group had a similar trend with a decrease until week 12 and a slight increase following that point, showing no statistical significance between the two groups. Nonetheless the values of the two group were in the normal range (<1.4) mg/dl) and this result could be from normal physiologic variation that can occurs due to exercise, feeding, age, body size (Lefebvre, Watson, Heiene, 2015).

The influence of body size on serum creatinine is related with quantity of muscle mass, which means an increase of muscle mass increases serum creatinine and vice versa (Lefebvre et al., 2015). Thus, other reason for the decrease of serum creatinine concentration could be due loss of muscle mass. Both groups were restricted to a limited space, which can contribute to less exercise and consequently slight reduction of muscle mass. A study performed by Bjornvad et al. of indoor-confined, adult neutered cats showed that the BCS underestimate the levels of body fat and although they seem skinny they have high level of body fat due to a lack of exercise (Bjornvad et al., 2011). As observed in humans, physical inactivity may result in a decrease in lean body mass, causing a relatively higher percentage of body fat, despite what appears to be a healthy bodyweight (Heber et al., 1996). However, all the values obtained are in the normal ranges and that from this study it is not possible to determine whether these absolute decreases or increases are of clinical significance.

The results observed of USG (1.015 to 1.045) and UPCR (normal: <0.2 or borderline: 0.2 to 0.5) were within laboratory reference intervals during the 24 weeks except for two dogs (one from the control group and another one from the treatment group).

An increase on UPCR would be expected in the treatment group because obesity in humans can lead to proteinuria or albuminuria, and ORG (Chang et al., 2013; Praga et al., 2000). In humans, the magnitude of proteinuria also correlates with the severity of obesity (Kambham et al., 2001). Also in dog a study performed by Tefft et al., UPCR is numerically higher in obese dog mainly in BCS of 8 or 9. However none of them developed proteinuria, and compared with the lean dogs these results did not show a statistical significant

difference (Tefft et al., 2014). A study of weight loss in obese dogs showed a decrease of UPCR after a weight loss (Tvarijonaviciute et al., 2013). From Table 8 it is possible to observe that UPCR increased lightly in the two groups. The increase in the two groups could be due to a higher GFR that can occur with the increase of protein in the diet or due to the glomerular lesions already mentioned. The latter could be also the reason for two dogs, one in the control group (UPCR 0.6 at week 24) and one in the treatment group (UPCR 0.65 at week 24) that had higher levels (which can also be seen by the higher level of standart desviation 0.19 and 0.18 for control group and for the treatment group respectively on week 24). One of them was one of the oldest (9 year old) in the experiment which may mean that this dog could have some renal damage due to the effect of the age that was not detected in the initial examination and makes them more sensitive to the excess of protein in the diet (Dibartola, 2010). The other dog the had a persistent proteinuria with results of UPCR: 0.25 at week 1 which means that is in borderline (0.2-0.5); 0.98 at week 12 and 0.65 at week 24. In IRIS a patient is considered proteinuric by UPCR when has a values higher than 0.5, however the persistence of proteirunia have to be demonstrated by re-evaluating 2 to 4 weeks, that in this case was 12 week (Gregory, 2016). Proteinuria is associated with progression of CKD and the greater the magnitude of proteinuria, the greater the risk of renal disease progression and mortality (Jacob et al., 2005). In humans, proteinuria is appears in ORG patients before the onset of azotemia (Kambham et al., 2001). Also in obese dog structural changes on the glomerulus may be precursors of more severe glomerular injury associated with prolonged obesity (Henegar et al., 2001). The realization of biopsies in this study could help to demonstrate if the proteinuria found in this dog was result of ORG.

The results of USG did not show a significant difference through the time and the slight difference between the weeks can be just due physiologic variations. In dogs, urine osmolality varies widely during the day, which mean each dog, have a individual variation of USG that can be influenced by the effect of feeding or drinking behavior (Vonderen, Kooistra, Rijnberk, 1997). The hydration status of the animal is another factor that can change the USG, with deprivation of water the USG increase and a concentrate urine is present, while the opposite such as in polydipsia, a decrease of USG occurs (dilute urine) (Watson, Lefebvre, Elliott, 2015).

However in this study would expect an increase a decrease on USG in the treatment group as a result of the increase of weight and consequently renal dysfunction. In the weight loss program of Tvarijonaviciute, weight loss resulted in an increase of USG, that could be representative of improved renal funtion through an increase in tubular concentrating ability (increased USG) and a decrease in protein filtered by the glomerulus (decreased UPCR) (Tvarijonaviciute et al., 2013).

To monitor renal disease, veterinary clinicians rely primarily on serum creatinine and urea concentrations, USG, and UPCR, which quick to measure and easy to performed because only one sample of blood or urine is necessary (Murgier, Jakins, Bexfield, Archer, 2009). However, all of these tests are insensitive in detecting renal damage and dysfunction at an early stage (will be discussed further), since azotemia and impaired urine concentrating ability are only seen when at least 75% of the nephrons are damaged (Watson, 1998; Dibartola, 2010; Von Hendy-Willson & Pressler, 2011). Other diagnostic modalities such as the evaluation of the GFR that is the gold standard could have helped further explain the results in this study. However, is not practical for the routine use. Thus, it is necessary to find practical renal markers to measure renal function. Symmetric dimethylarginine (SDMA), a new renal maker being developed as indirect measures of GFR, has shown to have higher sensitivity than current renal markers used in the clinical practice, and with the possibility of detecting patients with mildly reduced renal function (Hall et al., 2014). Until now, the available data suggest that plasma (or serum) concentration of symmetric SDMA reflects GFR in dogs (Nabity et al., 2013) and may be more sensitive than blood creatinine for detecting early stages of CKD (Hall et al., 2016), and also less affected by loss of lean body mass (Hall et al., 2015).

Other main possibility for lack of significant changes in routine renal marker values as a result of weight gain are due to the fact that the dogs were not obese long enough to allow the detection of obese-related glomerulopathy, meaning that the study period might have been insufficient. Nevertheless, in one study of dogs with induced obesity, significant histologic, biochemical, and functional renal changes were noted with only a 7- to 9-week duration of obesity. In that study, however, the dogs experienced in just 9 weeks a 50% weight gain over their initial weight (Henegar et al., 2001) as already mention previously. Another possible cause for the results is that the number of animal groups was low, and in addition to that fact one of the dogs of the treatment group did not gain much weight, as already mentioned.

In conclusion, the overweight dogs did not show the presence of glomerular disease, which can be evidenced by proteinuria, increase of creatinine or decrease of specific gravity, evaluated by routine markers of renal function in otherwise healthy overweight and obese dogs. Evaluation of a larger number and more markedly obese dogs may determine if ORG develops in obese dogs, or perhaps other renal markers should be used. Routine markers used in this study were not able to detect any changes in renal function at the beginning of obesity development. Therefore, it necessary to discover more specific and sensitive tests to evaluate renal function in the context of obesity-related kidney disease, given that there is a high risk of progression towards irreversible renal damage. Future studies will need to address the need of developing markers that enable early detection of renal dysfunction, as

well as to identify causal factors that might predispose to such dysfunction. New serum biomarkers for early renal injury have started to gain attention in veterinary medicine, namely the SDMA, which is beginning to become widespread throughout the veterinary community.

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