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Effect of Weight Loss in Obese Dogs on Indicators of Renal Function or Disease

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Background: Obesity is a common medical disorder in dogs, and can predispose to a number of diseases. Human obesity is a risk factor for the development and progression of chronic kidney disease.

Objectives: To investigate the possible association of weight loss on plasma and renal biomarkers of kidney health.

Animals: Thirty-seven obese dogs that lost weight were included in the study.

Methods: Prospective observational study. Three novel biomarkers of renal functional impairment, disease, or both (homocysteine, cystatin C, and clusterin), in addition to traditional markers of chronic renal failure (serum urea and creatinine, urine specific gravity [USG], urine protein-creatinine ratio [UPCR], and urine albumin corrected by creatinine [UAC]) before and after weight loss in dogs with naturally occurring obesity were investigated.

Results: Urea ($P = .043$) and USG ($P = .012$) were both greater after weight loss than before loss, whilst UPCR, UAC, and creatinine were less after weight loss ($P = .032$, $P = .006$, and $P = .026$, respectively). Homocysteine ($P < .001$), cystatin C ($P < .001$) and clusterin ($P < .001$) all decreased upon weight loss. Multiple linear regression analysis revealed associations between percentage weight loss (greater weight loss, more lean tissue loss; $r = -0.67$, $r^2 = 0.45$, $P < .001$) and before-loss plasma clusterin concentration (greater clusterin, more lean tissue loss; $r = 0.48$, $r^2 = 0.23$, $P = .003$).

Conclusion and Clinical Importance: These results suggest possible subclinical alterations in renal function in canine obesity, which improve with weight loss. Further work is required to determine the nature of these alterations and, most notably, the reason for the association between before loss plasma clusterin and subsequent lean tissue loss during weight management.

Key words: Canine; Clusterin; Cystatin C; Homocysteine.

Chronic kidney disease (CKD) is a major cause of morbidity and death in dogs and, given that it typically diagnosed relatively late in the course of the disease, dogs with advanced changes represent only a fraction of all dogs with CKD.[1,2] Human obesity is a risk factor for the development and progression of CKD,[3] and reducing body fat mass, either through dietary energy restriction or bariatric surgery, can reverse many of the associated clinical and nephropathologic manifestations.[4]

Obesity is a common medical disorder in dogs, and can predispose to associated diseases such as osteoarthritis, respiratory disease, neoplasia, and insulin resistance.[5] However, it is less clear as to whether there is an association between excess adiposity and renal functional or disease alterations in this species. When monitoring renal disease, clinicians rely primarily on serum creatinine and urea concentrations, urine specific gravity (USG), and urine protein:creatinine ratio (UPCR). Further, microalbuminuria is considered to be an excellent marker of early kidney disease,[6] being a sign of mildly altered glomerular permeability.[7] However, all of these tests are insensitive, with experimental studies suggesting that azotemia and impaired urine concentrating ability are not seen until functional nephron mass is reduced by at least two thirds.[8] Non-invasive and simple methods that have the ability to detect renal damage in CKD prior to functional nephron impairment (eg, inadequate urine concentrating ability or azotaemia) are limited. Given that there is a high risk of progression to irreversible renal damage in patients with CKD, there is a current need both to develop markers that enable early detection of renal dysfunction,[9] as well as to identify causal factors that might predispose to such dysfunction. In response, new serum biomarkers for early renal injury have started to gain attention in human and veterinary medicine, and examples include homocysteine (Hcy), cystatin C (CysC), and clusterin (Clu).[10]

Homocysteine is an amino acid, the majority of which (98%) circulates in an oxidized form bound to

Abbreviations:

BCS	body condition score
BW	body weight
CKD	chronic kidney disease
CLU	clusterin
CysC	cystatin C
DEXA	dual-energy X-ray absorptiometry
Hcy	homocysteine
MER	maintenance energy requirement
UAC	urine albumin corrected by creatinine
UPCR	urine protein:creatinine ratio
USG	urine specific gravity

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protein.[11] In humans, total plasma homocysteine (tHcy) concentration is inversely correlated with the glomerular filtration rate (GFR),[12] and positively correlated with circulating creatinine concentration.[13] In dogs, significantly greater tHcy concentrations are reported in dogs with cardiac and renal diseases.[14] CysC is a 13 kDa, 122-amino acid, cysteine protease inhibitor, and is produced at a constant rate by all nucleated cells in the body.[15,16] As a result, circulating concentrations correlate with GFR.[17] Dogs appear to be similar to humans, whereby circulating CysC concentration might be a better marker of renal functional impairment than serum creatinine concentration.[10,18,19] Clu is a glycoprotein which is composed of two 40 kD subunits (NA1, NA2) bound by disulfide groups.[20] Reports in human medicine indicate that Clu is upregulated and released into the urine after nephron damage.[21] Serum clusterin is also increased after renal injury,[22] possibly because it is upregulated.[21] Therefore, circulating clusterin concentrations can also be used as an early marker of renal injury.

The aim of this study was to investigate the possible influence of weight loss, in dogs with naturally occurring obesity, on biomarkers of renal status, as described in humans.[4] As a result, we chose to examine the behavior of three novel biomarkers of renal functional impairment and/or disease (tHcy, CysC, and CLU), in addition to traditional markers of CKD (serum urea and creatinine, USG, UPCR, and urine albumin:creatinine [UAC]) before and after weight loss in dogs with naturally occurring obesity.

Material and Methods

Animals

Dogs were referred to the Royal Canin Weight Management Clinic, University of Liverpool UK, for investigation and management of obesity and associated disorders. Sixty-five dogs were recruited between February 2005 and August 2010, and those successfully losing weight had completed by January 2011. Eligibility criteria included confirmation of obesity (based upon body fat measurement by dual-energy X-ray absorptiometry; DEXA) and availability of sufficient surplus plasma and urine for analysis. The study protocol adhered to the University of Liverpool Animal Ethics Guidelines, and was approved by both the University of Liverpool Research Ethics Committee and the WALTHAM ethical review committee. Owners of all participating animals gave informed written consent.

Weight Loss Regimen

Full details of the weight loss regimen have been previously described.[23,24] Briefly, dogs were determined to be systemically well, and without significant abnormalities on complete blood count, serum biochemical analysis, and urinalysis. Throughout weight loss, patients were weighed on electronic weigh scales,[a] which were regularly calibrated using test weights.[b] Body composition was analyzed by fan-beam DEXA,[c] both before and after weight loss, and results used to estimate both target weight and the changes in both fat and lean mass during weight loss.[24,25]

A weight management protocol was then instigated,[23,24] using either a high protein high fiber[d] (35 dogs) or high protein moderate fiber[e] (2 dogs) weight loss diet (Table 1). The initial food allocation for weight loss was determined by first estimating maintenance energy requirement (MER = 440 kJ [105 kcal] × body weight [kg]$^{0.75}$/day[26]) using the estimated target weight. The exact level of restriction for each dog was then individualized based upon gender and other factors (i.e. presence of associated diseases), and was typically between 50 and 60% of MER at target weight.[24] Owners also implemented lifestyle and activity alterations to assist in weight loss. Dogs were reweighed every 7–21 days and changes made to the dietary plan if necessary.[23,24]

Analysis

Plasma tHcy concentration was measured with a commercial kit[f] and an automated analyzer[g] following the instructions of the manufacturer. Plasma CysC and Clu concentrations were measured with species-specific commercially available ELISAs assays.[h] For plasma tHcy, intra- and interassay coefficients of variation (CV) were 3.4% and 6.2%, respectively; for plasma CysC, intra- and interassay coefficients of variation (CV) were 7.5% and 11.1%, respectively; and for plasma Clu, intra- and interassay coefficients of variation (CV) were 8.4% and 14.3%, respectively. Dilution of canine serum samples, in each of the

Table 1. Composition of diets fed to dogs to achieve weight loss.

Criterion	HPHF Diet		HPMF Diet	
ME content*	2900 kcal/kg		3275 kcal/kg	
Moisture	9.0		9.0	
	Per 100 g DM	g/1000 kcal (ME)	Per 100 g DM	g/1000 kcal (ME)
Moisture	8	28	9	27
Crude protein	30	103	34	104
Crude fat	10	34	10	31
Crude fiber	17.5	60	11.5	35
Total dietary fiber	28	97	18.5	56
Ash	5.3	18	7.9	24
Fiber sources	Cellulose, beet pulp, FOS, psyllium husk, diet cereals		Cellulose, beet pulp, diet cereals	

HPHF = high protein high fiber diet.[d] HPMF = high protein medium fiber.[e] ME = metabolizable energy content, as measured by animal trials according to the American Association of American Feed Control Officials protocol[58]; DM = dry matter; FOS = fructo-oligosaccharides.

assays, resulted in linear regression equations with correlation coefficient close to 1.0. For each analyte, all samples (before and after weight loss samples from all dogs) were analyzed in a single batch.

Urine specific gravity was measured by a hand refractometer.[i] Serum urea and creatinine and urine protein and creatinine assays were performed on the automated clinical chemistry analyzer.[g] In the cases of serum urea and creatinine, commercially available reagents were used. [g]Urine protein was determined by the pyrogallol method using a commercially available reagent[j], while creatinine was measured using the modified Jaffe method using a commercially available reagent[k] on samples diluted 1:20 with deionized water. The UPCR was then calculated with the formula: UPCR = protein (mg/dL)/creatinine (mg/dL). Urine albumin was determined by human immunoturbidometric assay[l] in an automated clinical chemistry analyzer[g] previously validated for use in canine urine samples.[27] Urinary albumin levels (mg/L) were adjusted for urinary albumin concentration (mg/L) and expressed as micrograms per gram of creatinine for statistical analysis as previously described.[28]

Statistical Analysis

Data are expressed as median (range) except where indicated. Statistical analyses were performed with computer software (Stats Direct version 2.6.8; Stats Direct Ltd.), with the level of significance set at $P < .05$ for two-sided analyses. Given that there were no known previous studies examining our chosen novel renal biomarkers in obese dogs undergoing weight loss, a meaningful power calculation could not be performed. Instead, the number of dogs enrolled was based upon the prior experience from a previous study of plasma biomarkers from the same clinic.[29]

The Shapiro-Wilk test was first used to assess whether or not data were normally distributed, and either parametric or non-parametric tests were used, as appropriate. These included simple and multiple linear regression, paired and unpaired Student's *t* test, Mann-Whitney *U* test, and Kendall's rank correlation.

Differences in the concentrations of the various biomarkers, prior to and after weight loss, were assessed with either the paired Student's *t* test or the Wilcoxon signed rank sums test. Linear regression was used to determine factors associated with outcomes of weight loss (ie, percentage change in lean tissue and rate of weight loss). Initially, simple regression was used to determine associations between with outcome variables and both baseline parameters (eg, age at enrollment, sex, percentage body fat) and plasma biomarker concentrations before weight loss. A multiple linear regression model was then constructed, which initially included any variables identified as $P < .2$ on univariable analysis.[30] The model was subsequently refined by backward-stepwise elimination of the least significant variable at each round, with variables retained in the final model if they were significant ($P < .05$).[30]

Results

Baseline Characteristics of the Dogs and Details of Weight Loss

The median age of the 37 dogs was 72 months (12–132 months), and a range of breeds was represented (Table 2); 21 were male (20 neutered) and 16 were female (14 neutered) (Table 2). Median body weight prior to weight loss was 35.0 kg (5.4–77.0 kg), and body fat mass was 45.0% (30.0–54.7%).

Median duration of the weight loss period was 250 days (91–674 days), and median weight loss during this time was 28% (10–44%) (Table 2). Thus, the median rate of weight loss was 0.8%/week (0.2–1.4%/week). The median change in body fat mass was −52% (−78 to −18%), whilst median change in lean tissue mass was −7% (−21% to 14%). Finally, the median energy intake (in metabolizable energy) during weight loss was 60 kcal/kg$^{0.75}$/day (44–74 kcal/kg$^{0.75}$/day).

Table 2. Summary of weight loss in the study dogs.

Criterion	Result
Age	72 month (12–132)
Sex	1 M, 20 NM, 2 F, 14 NF
Breed	Akita, Border Collie, Cairn Terrier, CKCS (3), Cocker Spaniel, Corgi, Dachshund, Doberman, English Bull Terrier, Golden Retriever, Irish Setter, Labrador (10), Lhasa Apso, Miniature Schnauzer, Mixed Breed (5), Pug (3), Samoyed, Schipperke, Yorkshire Terrier (2)
Body weight BEFORE	35.0 kg (5.4–77.0)
Body weight AFTER	25.8 kg (4.4–51.4)
Body fat mass BEFORE	13400g (1600–37700); 45% (30–55)
Body fat mass AFTER	6700g (700–37700); 29% (11–45)
Lean tissue mass BEFORE	17700g (3500–36600); 52% (43–68)
Lean tissue mass AFTER	17700g (3300–33400); 68% (53–86)
Duration	250 days (91–674)
Rate of weight loss[a]	0.8%/week (0.2–1.4)
Body weight change[b]	−28% (−10 to −44)
Change in fat mass[b]	−52% (−78 to −18)
Change in lean mass[b]	−7% (−21 to 14)
EI during weight loss[c]	251 (184–310) [60 (44–74)]

All data are expressed as median (range). M: male; NM: neutered male; F: female; NF: neutered female; CKCS: Cavalier King Charles Spaniel.

[a]Rate of weight loss expressed as percentage of starting body weight lost per week.

[b]Refers to the percentage change in starting mass calculated as follows: ([start mass − end mass] ÷ start mass) × 100%.

[c]EI: energy intake expressed as metabolizable energy (in kJ [Kcal]) per kg of metabolic body weight (BW$^{0.75}$) per day.

Associations between Baseline Parameters Renal Biomarkers before Weight Loss

There was no association between baseline parameters (including age, sex, breed, neuter status, body weight, and body fat percentage) and renal biomarkers before weight loss (plasma Hcy concentration $P = .082$, all other results $P > .2$).

Changes in Renal Biomarkers with Weight Loss

Urea ($P = .043$) and USG ($P = .012$) were both greater after weight loss than before weight loss, whilst UPCR, UAC, and creatinine were less after weight loss ($P = .032$, $P = .006$, and $P = .026$, respectively) (Table 3). Based upon accepted international criteria,[31] increased UPCR (>0.5) was present in 8 dogs before and in 1 dog after weight loss ($P = .012$). UAC >30 mg/g (a cut point that has been used to define microalbuminuria in humans[32]) was present in 8 dogs before and 3 dogs after weight loss. Hcy ($P < .001$), CysC ($P < .001$), and Clu ($P < .001$) all decreased upon weight loss.

Associations between Baseline Parameters Renal Biomarkers before Weight Loss with the Outcomes of Weight Loss

Both simple and multiple linear regression analyses were used to determine the effect of baseline parameters (eg, age, sex, start weight, and body fat mass) and plasma biomarkers on 2 weight loss outcomes (eg, change in lean tissue mass and rate of weight loss). For rate of weight loss, simple linear regression did not reveal any significantly associated factors ($P > .2$), and a multiple linear regression model including all factors was also not significant ($P = .522$). In contrast, simple linear regression (Table 4) suggested associations between a number of factors and change in lean tissue mass including breed (retriever vs. non-retriever, $P = .006$), duration of weight loss ($P = .008$), percentage weight loss ($P < .001$), body fat mass before loss ($P = .018$), creatinine ($P = .122$), and Clu ($P = .080$). However, the only factors remaining on multiple regression were percentage weight loss (greater weight loss, more lean tissue loss; $r = -0.67$, $r^2 = 0.45$, $P < .001$) and plasma Clu concentration before weight loss (greater clusterin, more lean tissue loss; $r = 0.48$, $r^2 = 0.23$, $P = .003$).

Table 3. Renal biomarkers before and after weight loss in the study dogs.

Analyte	Before Weight Loss	After Weight Loss	*P*
Urea (mmol/L)	5.3 (1.6–30.6)	5.5 (3.1–8.9)	.043
Creatinine (μmol/L)	84 (37–123)	76 (8–122)	.026
USG	1.033 (1.011–1.058)	1.037 (1.017–1.052)	.012
UPCR	0.50 (0.03–5.57)	0.28 (0.05–3.30)	.032
UPCR > 0.5	Yes: 8; No: 19	Yes: 1; No: 26	.015
UAC (mg/g)	54.6 (0.3–555.8)	13.8 (0.6–157.4)	.006
UAC > 30 mg/g	Yes: 8; No: 19	Yes: 3; No: 24	
Hcy (μmol/L)	10.8 (5.7–23.2)	7.8 (1.1–18.1)	<.001
CysC (mg/L)	1.6 (1.0–2.5)	1.3 (0.7–2.0)	<.001
Clu (μg/mL)	91.4 (47.6–108.70)	72.2 (38.9–122.0)	<.001

Results are expressed as mean (range). USG: urine specific gravity; UPCR: urine protein creatinine ratio; UAC: urine albumin micrograms per gram of creatinine; Hcy: homocysteine; CysC: Cystatin C; CLU: clusterin.

Discussion

The current study has investigated putative renal biomarkers in obese dogs undergoing a weight management program, that lead to a marked reduction in body fat mass. Both conventional biomarkers in current clinical use (serum urea, serum creatinine, USG, UAC, and UPCR) and novel biomarkers (tHcy, CysC, and Clu) were assessed before and after weight loss. While it is tempting to speculate that the results might be the result of altered renal function, alternative explanations are possible for many of the changes noted. Most notably, the differences identified might have been the result of other alterations occurring concurrently during the weight loss program, for example the feeding of a high protein diet for weight loss (for urea) loss of lean tissue mass (for creatinine), as described in detail below. As a result, further studies would be required to confirm these findings and determine their significance.

The observed changes in urine biomarkers used in routine clinical practice (USG, UPCR, and UAC) with weight loss could imply improved renal function, through an increase in tubular concentrating ability (increased USG) and a decrease in protein filtered by the glomerulus (decreased UPCR and UAC). Experimentally-induced obesity in dogs is known to alter renal function (eg, glomerular hyperfiltration with an associated increase in GFR) and cause 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.[33] Similar changes could be the reason why USG was less (eg, due to an increase in GFR) and UPCR and UAC more commonly abnormal (eg, due to the glomerular lesions causing protein leakage) for the dogs of the current study, when in an obese state. Other diagnostic modalities such as kidney biopsy could have helped to determine the significance of the changes in this study. However, since this study was performed under clinical conditions it was not ethically possible to perform invasive procedures such as serial kidney biopsy in client-owned dogs.

Although the concentrations of urea and creatinine were within laboratory reference intervals, an increase in serum urea and a decrease in serum creatinine were observed after weight loss. The increase in plasma urea concentration in obese dogs undergoing weight loss has been previously reported in some,[34] but not all[35] previous studies. The reason for such an increase is not clear, but it might either have resulted from a decrease in GFR as a result of weight loss, or from

Table 4. Associations between baseline parameters and renal biomarkers before weight loss with the change in lean tissue mass.

	Regression Coefficient	r	r^2	P
Simple regression				
Age	0.02	0.102	0.010	.540
Sex	−1.02	−0.062	0.004	.713
Neuter status	−1.98	−0.066	0.004	.696
Breed[a]	5.39	0.438	0.192	.006
Starting weight	0.04	0.121	0.015	.469
Body fat percentage	−0.53	−0.380	0.145	.018
Weight loss diet[b]	−11.6	−0.349	0.122	.032
Duration of weight loss	−0.02	−0.424	0.180	.008
Rate of weight loss	3.27	0.126	0.016	.449
Percentage weight loss	−0.65	0.589	0.344	<.001
Energy intake during weight loss	0.14	0.112	0.013	.487
Serum urea[c]	−0.09	−0.051	0.002	.763
Serum creatinine[c]	0.12	0.255	0.065	.122
Urine specific gravity[c]	−124.60	−0.188	0.035	.294
Urine protein:creatinine ratio[c]	0.33	0.044	0.002	.828
Urine albumin:creatinine	−0.00	−0.10	0.01	.602
Plasma homocysteine[c]	−0.26	−0.147	0.022	.385
Plasma cystatin C[c]	0.24	0.010	0.000	.950
Plasma clusterin[c]	−0.08	−0.291	0.085	.08
Multiple regression				
Final model		0.708	0.501	<.001
Percentage weight loss	−0.71	−0.674	0.454	<.001
Plasma clusterin	−0.10	−0.484	0.234	.003

[a]Breed based upon a dummy variable where dogs of retriever breed were assigned a value of 1, and dogs of other breeds were assigned a value of 0

[b]Diet based upon a dummy variable where dogs fed the HPMF diet were assigned a value of 1, and those fed the HPHF diet were assigned a value of 0.

[c]Refers to biomarker results before weight loss.

feeding a high-protein weight loss diet. Increased urea concentration after weight loss has also been described in obese humans consuming a moderate protein diet for weight loss, but not those receiving a high carbohydrate diet.[36,37] Measurement of GFR before and after weight loss might have helped to differentiate between these possibilities. The decrease in serum creatinine concentration is contradictory to previous studies in obese dogs, whereby increased serum creatinine concentration has been documented following weight loss.[34,35] This decrease could be the result of loss of muscle mass during weight management. If a genuine decrease in GFR were to have occurred with weight loss, as suggested by the increase in urea concentration, it could counteract the effect of lean tissue loss on serum creatinine concentration.

Systolic blood pressure (SBP) is another clinical parameter of critical importance in CKD, and is used in an internationally accepted clinical staging scheme.[31] Whilst canine obesity is associated with hypertension,[38,39] the effect is relatively minor and does not usually warrant antihypertensive therapy. Although the focus of the current study was to assess urinary and plasma biomarkers, SBP has been measured in another study from our clinic, which included dogs from the current study.[40] As with previous work, SBP is often marginally increased in the obese state, and decreases significantly after successful weight loss. These findings support the possibility of renal structural and functional changes in canine obesity.

The current study also investigated 3 novel biomarkers putatively associated with renal functional impairment or disease: tHcy, CysC, and Clu. Increased tHcy concentration has been associated with renal disease in dogs[14] and humans[11] whilst, in humans, moderate hyperhomocysteinemia has been noted in early stages of chronic renal failure, becoming more prominent as renal function deteriorates.[11] Furthermore, other human studies have identified greater tHcy concentrations in overweight and obese patients when compared with normal weight patients.[41] Thus, the decrease in tHcy concentration after weight loss in the obese dogs of the current study might indicate altered renal structure or function in the obese state with subsequent improvement with weight loss. However, given that dietary folate intake can influence plasma tHcy concentration, folate should ideally have been measured in all study dogs. This is a limitation of the current study and further assessment is required. However, in a previous human study, changes in folate concentration did not influence serum tHcy concentration in weight loss programs.[42]

Dogs are similar to humans in that circulating CysC concentration is reportedly a better and more accurate marker of renal function than creatinine or creatinine-based equations. This is thought to be because the influence of nonrenal factors (such as body composition,

age, gender, or dietary protein intake) on circulating CysC concentration is less than for creatinine concentration.[10,15,16,19,43,44] Further, given that increases in circulating CysC concentration with progressive renal compromise parallel one another in obese and nonobese humans, changes in CysC concentration are thought to reflect renal function whatever the degree of obesity.[45,46] However, circulating CysC is consistently increased in obese subjects independent of GFR, and adipose tissue expression of CysC is increased in the obese state.[46] This suggests that adipose tissue might contribute directly to circulating CysC concentration through increased adipose tissue synthesis in the obese state. Given that it is not currently known whether canine adipose tissue can synthesize CysC, it is feasible that the decrease in plasma CysC concentration is either the result of improved renal function or to decreased synthesis of CysC by adipose tissue after weight loss. Also, with regard to CysC, it should be emphasized that the median change from baseline concentrations was only 18%, and it is not known as to whether or not such a change would be clinically important. Further studies would be recommended to determine the reasons for and significance of such changes.

Weight loss also resulted in a decrease in plasma Clu concentration, which could have been the result of 2 different mechanisms: (1) improvement in renal injury, given that increased serum Clu concentration is seen after nephron damage in humans[22]; (2) improvement in lipid profile after successful weight loss, since circulating Clu reportedly acts as an apolipoprotein, by partially associating with high density lipoprotein (HDL).[47] In dogs, HDL is the predominant lipoprotein[48] and this decreases after weight loss.[49] Furthermore, a positive correlation between changes of Clu with body fat mass has been described in humans independent of age, gender, HbA1c, and fasting plasma insulin concentrations.[50] It is again advisable to consider further studies to determine the true significance of this finding.

The official National Institute of Health definition of a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".[51] Since obesity is easy to diagnose, requiring only a physical examination and body condition score to be performed, the authors believe that biomarkers are applied to assessing either disease associations or in predicting outcomes of weight loss. Such information might help when advising owners of likely weight loss outcomes, and might, ultimately, enable the clinician to tailor their weight loss therapy accordingly. For this reason, we used simple and multiple regression analysis to determine whether the biomarkers studied, when measured prior to weight loss, could predict either rate of weight loss or change in lean tissue. In this respect, preservation of lean tissue mass during weight loss is a key outcome for successful obesity management and, in humans, loss of skeletal muscle mass is correlated with physical impairment and disability[52] as well as being associated with an increased incidence of death.[53] In the present study, Clu concentration before weight loss correlated with amount of lean tissue lost during subsequent weight management (greater Clu, more lean tissue loss). The reasons for this association are not clear but, in humans, widespread upregulation of Clu gene expression and protein synthesis is seen in diseases where either abnormal cell death or proliferation occurs, including atherosclerosis, myocardial infarction, and muscle damage.[54,55] Thus, it could be hypothesized that Clu acts as a surrogate marker of deranged metabolic function in canine obesity, and this improves after successful weight loss. Whatever the reason, this intriguing study finding raises the possibility that Clu concentration before weight loss could be used as a biomarker to identify those dogs at risk of excessive loss of lean tissue mass.

The main limitation of this study would be that the studied animals were a population of client-owned dogs with variable living conditions, family environment, husbandry, and medical care. This made it impossible to evaluate influence of changes in diet composition on studied analytes, to perform GFR measurements, or to perform renal biopsies in order to evaluate the weight loss on histologic changes in the kidneys. Nonetheless, the results are arguably more representative of the true clinical picture since the obesity is naturally occurring. On a related note, a 2nd limitation was the fact that a control group of healthy dogs was not included. The main reason for this was that our institution's ethics committee would not have allowed it. Although, on the face of it, blood and urine sampling a group of healthy pet dogs would be straightforward, in the UK there needs to be a clear benefit to the animal in order to justify such a procedure or it is classed as an experimental act. An alternative would have been to consider utilizing surplus frozen plasma from a 'hospital' control population of dogs, ie, dogs referred for reasons other than obesity and renal disease. Whilst this would certainly have circumvented the ethical dilemma, such an approach would have created concerns of its own. In this respect, a DEXA scan would arguably have been required to ensure that their weight status was known, which would again not have been allowed by our ethics committee. Further, it would have been difficult to guarantee that such controls were free from subclinical renal disease, since their underlying disease might arguably have caused such problems. This would then have meant that more detailed (but invasive) tests would have been required such as measurement of GFR and/or renal biopsy. Once again, such tests are invasive and would not be allowed by our ethics committee. Given these limitations, we chose to take the alternative approach of using the enrolled dogs as their own controls, ie, by assessing them before and after weight loss.

A 3rd limitation was the fact that an a priori power calculation was not performed and, as a result, raising the possibility that genuine findings might have been missed. Against this, however, a number of highly significant differences were identified. This suggests that the study had adequate power to detect the major differences of clinical importance.

A final limitation was the fact that the study mainly studied circulating biomarkers so that metabolic effects of obesity and subsequent weight loss could be assessed in addition to renal effects. Nonetheless, for future studies, it would be of interest to evaluate urinary changes of these analytes since, recently, assays for cystatin C and clusterin measurements in dog urine have been validated,[56,57] and seem to have a high sensitivity for evaluating kidney function.

In summary, the current study has demonstrated changes in a variety of renal function biomarkers in obese dogs undergoing weight loss. Although these results might suggest possible subclinical alterations in renal function in canine obesity, other explanations are possible for many of the changes observed. Therefore, further studies are now necessary to determine whether renal functional changes or injury occur in canine obesity and whether or not they improve after weight loss. Finally, the reason for the association between plasma clusterin before weight loss and subsequent lean tissue loss during weight management is intriguing, and warrants additional investigation.

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Conflict of Interest Disclosures: The following conflicts of interest apply: AJG's Senior Lectureship is funded by Royal Canin; the diet used in this study is manufactured by Royal Canin; PJM is an employee of WALTHAM, while VB is employed by Royal Canin.

Footnotes

[a] Soehnle Professional, Murrhardt, Germany
[b] Blake and Boughton Ltd, Thetford, UK
[c] Lunar Prodigy Advance; GE Lunar, Madison, WI
[d] Satiety Support, Royal Canin, Aimargues, France
[e] Obesity Management, Royal Canin
[f] Diazyme Laboratories, Diazyme Europe GMBH, Dresden, Deutschland
[g] Biochemistry analyzer, Olympus AU2700, Olympus Diagnostica GmbH, Hamburg, Germany
[h] Canine Cystatin C ELISA Kit and Canine Clusterin ELISA Kit; BioVendor–Laboratorni medicina, Brno, Czeck Republic
[i] ATAGO Company Ltd, Tokyo, Japan
[j] Protein u&csf, Spinreact SAU, Sant Esteve de Bas, Spain
[k] Creatinine-J, Spinreact SAU
[l] Microalbumin OSR6167, Olympus system reagent, Olympus Diagnostica GmbH

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