

Increased Urinary F2-Isoprostane Concentration as an Indicator of Oxidative Stress in Overweight Cats

I. Jeusette¹
A. Salas¹
N. Iraculis¹
M. Compagnucci⁰
C. Torre¹
V. Romano¹
N. Kirschvink²

¹*Affinity-Petcare,
R&D,
Barcelona, Spain*

²*Animal Physiology,
Veterinary Department,
University of Namur
Rue De Bruxelles 61
5000 Namur, Belgium.*

KEY WORDS: Cats, obesity, aging, oxidative stress, blood lipids

ABSTRACT

The objective of this study was to assess the effect of excess body weight (BW) on blood cholesterol, triacylglycerol (TAG), acute phase proteins, and on urinary F2-Isoprostane in healthy cats. Seven lean and 20 overweight cats fed ad libitum with the same food were investigated. The results show that excess BW is associated with increased oxidative processes in cats, as indicated by a significantly increased urinary F2-Isoprostane/creatinine ratio in overweight cats. A significant and positive correlation between BW and urinary F2-Isoprostane/creatinine ratio was also detected, whereas other blood variables were similar in lean and overweight cats. An age-effect was also observed on TAG plasma concentration, TAG being higher in older cats, with the difference coming from the overweight group.

INTRODUCTION

The reported prevalence of obesity or excess body weight (BW) in cats ranges from 17 to 52 %.^{1,2,3} Similar to human patients, obesity is recognized as a health risk in cats. Indeed, even a small increase in BW leads to decreased insulin sensitivity, increased insulin resistance and increased blood lipid concentrations in felines.^{4,5} Furthermore, overweight cats bear an increased risk of developing hepatic lipidosis, hyperlipidemia, diabetes mellitus, lameness, non-allergic skin disease, lower urinary tract diseases or death at middle age.^{6,7}

In humans, obesity goes along with oxidative stress that is associated with or resulting from different conditions such as hyperglycemia, hyperleptinemia, increased tissue lipid levels, inadequate antioxidant defences, increased rates of free radical formation by endothelial cells, and chronic inflammatory processes.⁸ Rats and mice used as models of obesity-associated disorders

also provide evidence that oxidative stress and inflammation occur.⁹ Among the indicators of obesity-associated oxidative stress and inflammation are increased levels of F2-Isoprostanes¹⁰ and of acute-phase proteins¹¹ respectively.

Although oxidative and inflammatory markers are used for diagnostic and research purposes in companion animals,^{12,13} investigations about the relation between overweight and oxidative or inflammatory markers are not yet available in the scientific literature. Therefore, the objectives of this study were to assess the effects of excess BW on blood lipids and acute-phase proteins as well as on urinary F2-Isoprostane in cats.

MATERIAL AND METHODS

Animals and Diet

Twenty seven European domestic short hair cats, accustomed to *ad libitum* fed dry diets, were enrolled for this study. Seven male and female neutered cats, aged 5.1 ± 1.4 y had a maximum body condition score (BCS) of 5 (BCS ranging from 1-9, with 1-3 lean; 4-5 ideal, 6-9 overweight to obese)¹⁴ and were attributed to the group of Lean cats,

Table 1: Characteristics of lean and overweight cats investigated in the study. Data are shown as mean \pm SEM.

	Unit	Lean	Overweight
all	n	7	20
NM	n	1	9
NF	n	6	11
young adult (1y)	n	1	9
adult (3-6y)	n	4	6
senior (9-14y)	n	2	5
Mean Age	year	5.1 ± 1.4	4.6 ± 0.9
Mean BW	kg	3.6 ± 0.3 a	5.2 ± 0.2 b
Mean BCS	point/9	5 ± 0 a	8 ± 0 b
Mean CTC	cm	31 ± 1 a	37 ± 1 b
Mean PC	cm	31 ± 2 a	43 ± 1 b
Mean c. Body Fat	%	19 ± 2 a	33 ± 2 b

n number of cats; NM neutered male; NF neutered female; BW Body weight; BCS Body condition score; CTC Cranial thoracic circumference; PC Pelvic circumference; c. Body Fat Calculated body fat.

a,b Within a line, values with a different letter indicate a significant group-related difference (lean vs. overweight) ($P < 0.05$).

whereas 20 male and female neutered cats, aged 4.6 ± 0.9 y had a BCS ranging from 6 to 9 and were considered as Overweight (Table 1). Weight gain had been originally induced by allowing *ad libitum* food consumption all life long. All cats were determined to be healthy on the basis of results of physical examination and clinical laboratory data.

Cats were maintained at the Affinity Pet-care center facility, housed by groups ($n=6$ or 7) in indoor parks and given free access to water.

The cats received *ad libitum* a high quality maintenance diet with pork fat as main source of lipids (40 % protein, 32 % starch, 17 % fat, 5 % total dietary fibre, 4,570 kcal/kg measured metabolisable energy, dry matter basis). Diet exceeded FEDIAF (European Pet food industry federation) guidelines for a maintenance diet for adult cats. The cats were fed the diet for 8 weeks and were tested after the 8-week time period. Mean Food intake by group was recorded every day.

All animal handling procedures were carried out following the recommendations of the Law on Animal Welfare 22/2003 published in Spain in the "Diari Oficial de la Generalitat de Catalunya" (DOGC, 2003).

Experimental Protocol

BW (expressed in kg), BCS (score ranging from 1-9), cranial thoracic circumference (CTC, expressed in cm), pelvic circumference (PC, expressed in cm), body length (L, expressed in cm) and right forelimb length (RF, expressed in cm) were measured and body fat was calculated using the following morphometric equation: (Body fat % = $-0.02 * L/BW - 4.12 * RF + 1.48 * PC - 1.16 * CTC + 92.93$).¹⁵

Blood samples were collected from the jugular vein after 20 hr-fast and immediately placed into dry, EDTA-,

and heparin-coated tubes on ice for serum or plasma collection. After collection, blood samples were spun and serum or plasma was frozen before being sent on dry ice to the laboratory. Samples were stored at -20°C, until assays were performed. The following blood parameters were analysed by a veterinary laboratory (Idexx laboratory, Barcelona, Spain): cholesterol, triacylglycerol (TAG) by spectrophotometry; acute phase proteins (haptoglobin and serum amyloid A [(SAA)] by enzyme-linked immuno-assay; α -1 glycoprotein by radial immunodiffusion). Plasma leptin was also determined by use of the Multispecies Leptin RIA Kit (Linco Research Inc., Missouri, USA) that is validated in cats.^{16,17}

Urinary samples were taken by use of a sterile urinary catheter when the animals were sedated (100 μ g/kg; Domitor, Pharmacia Animal Health). Urine samples were centrifuged and supernatant was frozen before being sent on dry ice to the laboratory. Samples were stored at -80°C until assay. Urinary F2-Isoprostane concentration was determined by enzyme immunoassay (Cayman, Ann Arbor, USA).

Briefly, urine samples were defrosted and pH adjusted to 2-2.5 with 2 normal hydrochloric acid (2N HCl). Samples were centrifuged for 10 minutes at 3,600 rpm and 4° C. One ml of supernatant was run through a C18 1ml•100 mg-1 column (Bond Elut, Varian, Harbor City, CA, USA). Column was rinsed with 1 ml water and 1 ml hexane. Elution was performed with 2 ml (4 x 500 μ l) ethyl-acetate/methanol (95:5 V/V). Eluate was vacuum dried and restituted with buffer provided by the manufacturer. The kit was consequently used according to manufacturer's instructions. With-in assay coefficient of variation of the assay was

Table 2: Blood parameters assessed in healthy lean cats (n=7) and overweight cats (n=20). Data are shown as mean \pm SEM. Significant (P<0.05) group effects (lean vs. overweight) are indicated

Parameters	Unit	Lean cats	Overweight cats	Group effect (Lean vs. Overweight)	Reference range
Cholesterol	mg/dL	109 \pm 17	97 \pm 7	NS	<265
TAG	mg/dL	44 \pm 5	48 \pm 4	NS	<133
Leptin	ng/mL	2.8 \pm 0.3	3.5 \pm 0.4	NS	-
SAA	g/dL	2.14 \pm 0.08	2.14 \pm 0.10	NS	<37.5
α 1-Glycoprotein	μ g/mL	177 \pm 25	301 \pm 85	NS	<830
Haptoglobin	mg/dL	0.68 \pm 0.06	0.80 \pm 0.07	NS	<2
Urinary F2-Isoprostane	pg/mL / mg/dL	3.40 \pm 0.89 ^a	7.80 \pm 1.24 ^b	S (P<0.05)	-

TAG: triacylglycerol, SAA : serum amyloid A

a,b Within a line, values with a different letter indicate a significant group-related difference (lean vs. overweight) (P <0.05).

below 7 %; between-assay coefficient of variation was below 17 %. Urinary creatinine was determined by spectrophotometry and F2-Isoprostane concentrations were standardised for urine dilution by expressing data as an F2-Isoprostane/urinary creatinine ratio.¹²

Statistical Analysis

Data were tested for normality. For each continuous variable, one general linear model (SAS proc GLM) was performed. The model included the effect of group (lean versus overweight), age, sex and their interactions, except for food and energy intake, which model only included the group effect. Data are expressed as mean \pm SEM. Correlations between variables were tested by use of linear regression. The limit of statistical significance of the tests performed was defined as P \leq 0.05 while trend was defined as P \leq 0.10.

RESULTS

Overweight cats had a significantly (P<0.05) higher BW, BCS, CTC, PC, and % cal-

culated body fat than lean cats (Table 1). Compared to lean cats, mean excess BW and calculated body fat (%) of overweight cats achieved 44% and 74 %, respectively. No significant age effect was observed on BW or morphometric measurements. A significant ($P<0.05$) gender effect was observed on BW, neutered male having higher BW than neutered females, but not on BCS or morphometric measurements.

The mean food intake by day was similar during the study with 81 ± 1 g/day (349 ± 6 kcal/cat/day) in lean versus 77 ± 2 g/day (335 ± 8 kcal/cat/day) in overweight cats. However, lean cats consumed significantly more food when expressed in kcal/kg BW/day (Lean 96 ± 2 versus overweight 66 ± 2 kcal/kg BW/d) ($P<0.05$). The modification of BW observed at the end of the investigation period was not significant (overweight +1%, lean +3% BW) ($P>0.05$).

Cholesterol, TAG, Leptin, haptoglobin, α 1-glycoprotein, and SAA concentrations (Table 2) were within laboratory reference range and not significantly different between lean and overweight cats. However, blood TAG levels were positively and significantly correlated with BW ($r=0.44$, $P<0.05$). Regarding urinary F2-Isoprostane/creatinine ratio, a significant difference between lean and overweight cats was observed, overweight cats having higher values than lean cats (Table 2). Urinary F2-Isoprostane/creatinine ratio was significantly and positively correlated with BW ($r=0.40$; $P<0.05$), but not with blood parameters.

A significant age effect was observed on TAG concentration and a positive correlation existed between TAG concentration and age ($r=0.56$, $P<0.05$) (Table 3). A significant gender effect was observed on cholesterol concentration ($P<0.05$), cholesterol concentration being higher in neutered males (119 ± 3 mg/dl) than in

neutered females (90 ± 2 mg/dl).

DISCUSSION

The objective of this study was to assess in cats the effect of excess BW on blood parameters and urinary F2-Isoprostane in order to assess whether feline obesity is associated with hyperlipidemia, oxidative stress, and/or inflammation as described in human patients.⁸

Despite *ad libitum* feeding, the cats' BW during this 8 weeks study was not significantly changed (Overweight + 1% and lean, +3 % BW), suggesting that cats were in a steady phase of BW maintenance. Lean cats with a maximum BCS of 5 on the 9-point scale, which is considered as ideal, had an estimated body fat ranged within previously reported reference values for lean cats,¹⁴ but a real evaluation with accurate methods as DEXA-Scan or deuterium injection would have to be used to obtain more exact body fat measurement. Despite significantly higher BW and calculated body fat in overweight than in lean cats, leptin concentrations did not differ significantly between groups. This could be justified because neither extremely lean nor extremely obese cats had been compared in this study. Furthermore, cats were

Table 3: Cholesterol and triacylglycerol plasma concentration in young, adult and senior cats. Significant age effects are indicated.

Groups	n	Cholesterol (mg/dL)	TAG (mg/dL)
Young (1y)	9	76 ± 5	37 ± 2
Adult (3-6y)	10	114 ± 10	46 ± 4
Senior (9-14y)	7	112 ± 14	61 ± 9 *
Age effect		NS	S
Lean Adult	4	108 ± 9	44 ± 3 a
Lean Senior	2	111 ± 34	43 ± 4 a
Overweight Young	9	76 ± 2	37 ± 1 a
Overweight Adult	6	119 ± 4	47 ± 2 a
Overweight Senior	5	113 ± 6	69 ± 5 b

*within a column, indicates a significant ($P<0.05$) age effect

a,b within a column, values with a different letter are significantly different ($P<0.05$).

ad libitum fed with a mean energy intake for both groups higher than recommended by NRC (2006)¹⁸ while in other published studies, cats were energy-restricted to maintain BW.^{19,20}

No significant differences were observed between lean and overweight cats for cholesterol or TAG concentrations, which is in disagreement with previous works reporting increased concentrations of TAG^{19,20} and cholesterol¹⁹ in obese cats. Overweight cats investigated in this study had similar TAG values than those previously reported in obese insulin-resistant cats, but the lean cats had higher TAG values than reported by others.^{19,20} Regarding cholesterol concentration, values in overweight cats were lower than reported in the literature.^{19,20} Chronically obese cats could have similar cholesterol values than lean cats, but with abnormalities of LDL and HDL lipoprotein fractions,²⁰ but in the current study, lipoprotein separation was not performed. Despite no significant difference between lean and overweight animals, blood TAG levels were positively and significantly correlated with BW, which is in favour of increased TAG with weight gain.

A limitation of this study is the small sample size and lack of age- and gender-matched controls. That is why group, age, and sex effects were tested together in the statistical model. We found a significant effect of age on blood TAG concentration, the difference coming from the overweight group, and a significant effect of gender on cholesterol concentration that overlapped group (lean versus overweight) and age effects. A previous study with lean cats showed that age influences lipoprotein lipase, hepatic lipases, and lecithin:cholesterol acyl transferase enzymatic activity, but no significant increase in blood lipids with age was reported.²¹ In humans, it has been shown that ageing is associated with impaired-cell sensitivity to glucose and impaired-cell compensation to insulin resistance²² and that muscle insulin resistance - due to decreased muscle glycogen synthesis - promotes hepatic de novo

lipogenesis, resulting in an increased plasma triglyceride concentrations.²³ In cats as well, risk factors for diabetes mellitus include obesity, age, male gender, inactivity, and being neutered.^{23,24} It remains to be determined if this increase in blood TAG concentration could be a sign of decreased muscle insulin sensitivity. A gender effect had also previously been shown, neutered males having higher blood cholesterol than intact males or intact females.²¹

Urinary F2-Isoprostanes are markers of *in vivo* lipid peroxidation and are used as an index of oxidative status.¹⁰ In human patients, increased F2-Isoprostanes concentration in a variety of diseases (cardiovascular, pulmonary, neurological, renal, and liver) suggests an excess of free radicals and oxidant injury. Although the association between increased oxidative stress and disease does not necessarily imply a causative link, the fact that the F2-Isoprostane levels increase is an early event in pathologies like asthma, hepatic cirrhosis, scleroderma, and Alzheimer's disease suggests a causative role for oxidative stress at least in these diseases.¹⁰ A significant increase of urinary F2-Isoprostane was observed in overweight cats, as it has been observed in obese humans, suggesting that obesity in cats could be associated with an increased pro-oxidative burden or increased cell oxidation susceptibility. However, a complete urinalysis might be performed to rule out lower urinary tract inflammation as a source of F2-isoprostanes in the urine of these obese cats. It remains also to be determined if an oxidative status modification is a predisposing factor for frequently observed diseases in obese cats (e.g. hepatic lipidosis, hyperlipidemia, diabetes mellitus, lameness, renal, or urinary diseases). It has already been shown that cats with diabetes mellitus or with renal insufficiency undergo oxidative stress, as indicated by decreased plasma superoxide dismutase, increased serum 8-OHdG or comet assay parameters.^{25,26}

Acute-phase proteins were not significantly modified in overweight cats, sug-

gesting that the inflammation associated with excess BW was limited or that these markers were not sensitive enough to detect a slight modification. Indeed, in another study performed in obese cats, expression of inflammatory cytokine TNF α (tumor necrosis factor alpha) in fat tissue was shown to be increased, suggesting an inflammatory status in overweight cats.⁵ In this study, fat biopsies were not performed for reasons of animal well-being.

In conclusion, this preliminary study shows that ad libitum fed overweight cats undergo increased oxidative processes that can be evidenced by F2-Isoprostanes in urine samples. Also, age is a predisposing factor to increased TAG blood concentration in overweight cats.

ACKNOWLEDGEMENTS

The authors thank Dr Sonia Fernandez and Ramon Vilanova for daily animal care, Laetitia Wiggers (Namur University, Belgium) for sample purification and F2-Isoprostane analyses and the laboratory of Nutrition and Endocrinology of the veterinary faculty of the University of Nantes for leptin analysis.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

REFERENCES

1. 1. Lund EM, Armstrong PJ, Kirk CA, Kolar LM, Klausner JS. Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. *J Am Vet Med Assoc* 1999;214:1336-1341.
2. 2. Allan FJ, Pfeiffer DU, Jones BR, Esslemont DH, Wiseman MS. A cross-sectional study of risk factors for obesity in cats in New Zealand. *Prev Vet Med* 2000;46:183-196.
3. 3. Russell K, Sabin R, Holt S, Bradley R, Harper EJ. Influence of feeding regimen on body condition in the cat. *J Small Anim Pract* 2000;41:12-17.
4. 4. Biourge V, Nelson RW, Feldman EC, Willits NH, Morris JG, Rogers QR. Effect of weight gain and subsequent weight loss on glucose tolerance and insulin response in healthy cats. *J Vet Int Med* 1997;11:86-91.
5. 5. Hoenig M, McGoldrick JB, de Beer M, Demacker PNM., Ferguson DC. Activity and tissue-specific expression of lipases and tumor-ne-

6. 6. Scarlett JM, Donoghue S, Saidla J, Wills J. Overweight cats: prevalence and risk factors. *Int J Obes Relat Metab Disord* 1994;18:S22-28.
7. 7. Scarlett JM and Donoghue S. Associations between body condition and disease in cats. *J Am Vet Med Assoc* 1998;212:1725-1731.
8. 8. Vincent HK and Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int J Obes* 2006;30:400-418.
9. 9. de Ferranti S and Mozaffarian D. The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. *Clin Chem* 2008;54:945-55.
10. 10. Montuschi P, Barnes PJ, Roberts LJ. Isoprostanes: markers and mediators of oxidative stress. *FASEB J* 2004;18:1791-1800.
11. 11. Bays HE, González-Campoy JM, Bray GA, Kitabchi AE, Bergman DA, Schorr AB, Rodbard HW, Henry RR., Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. *Expert Rev Cardiovasc Ther* 2008;6:343-68.
12. 12. McMichael MA, Ruauux CG, Baltzer WI, Kerwin SC, Hosgood GL, Steiner JM, Williams DA. Concentrations of 15F2t Isoprostane in urine of dogs with intervertebral disk disease. *Am J Vet Res* 2006;67:1226-1231.
13. 13. McMichael MA. Oxidative stress, antioxidants, and assessment of oxidative stress in dogs and cats. *J Am Vet Med Assoc* 2007;231:714-720.
14. 14. Laflamme D. Development and validation of a body condition score system for cats: a clinical tool. *Feline Pract* 1997;25:13-18.
15. 15. Stanton CA; Hamar DW; Johnson DE; Fettman MJ: Bioelectrical impedance and zoometry for body composition analysis in domestic cats. *Am J Vet Res* 1992; 53:251-257.
16. 16. Backus RC, Havel PJ, Gingerich RL, Rogers QR. Relationship between serum leptin immunoreactivity and body fat mass as estimated by use of a novel gas-phase Fourier transform infrared spectroscopy deuterium dilution method in cats. *Am J Vet Res* 2000, 61:796-801.
17. 17. Martin L, Siliart B, Dumon H, Backus R, Biourge V, Nguyen P. Leptin, body fat content and energy expenditure in intact and gonadectomized adult cats: a preliminary study. *J Anim Physiol Anim Nutr (Berl)* 2001;85:195-199.
18. 18. NRC, 2006 : National Research Council. Nutrient Requirements of Dogs and Cats. *National Academy Press*. Washington DC.
19. 19. Hoenig M, Wilkins C, Holson JC, Ferguson DC. Effects of obesity on lipid profiles in neutered male and female cats. *Am J Vet Res* 2003;64:299-303.
20. 20. Jordan E, Kley S, Le NA, Waldron M, Hoenig M. Dyslipidemia in obese cats. *Domest Anim Endocrinol* 2008;35:290-299.
21. 21. Butterwick RF, McConnell M, Markwell PJ, Watson TD. Influence of age and sex on plasma lipid and lipoprotein concentrations and associated enzyme activities in cats. *Am J Vet Res* 2001;62:

- 331-336.
22. Chang AM, Smith MJ, Galecki AT, Bloem CJ, Halter JB. Impaired β -Cell Function in Human Aging: Response to Nicotinic Acid-Induced Insulin Resistance. *J Clin Endocrinol Metab* 2006;91:3303–3309.
 23. McCann TM, Simpson KE, Shaw DJ, Butt JA, Gunn-Moore DA. Feline diabetes mellitus in the UK: the prevalence within an insured cat population and a questionnaire-based putative risk factor analysis. *J Feline Med Surg* 2007;9: 289-299
 24. Prahl A, Guptill L, Glickman NW, Tetric M, Glickman LT. Time trends and risk factors for diabetes mellitus in cats presented to veterinary teaching hospitals. *J Feline Med Surg* 2007;9:351-358.
 25. Webb CB, Falkowski L. Oxidative stress and innate immunity in feline patients with diabetes mellitus: the role of nutrition. *J Feline Med Surg* 2009;11:271-276.
 26. Yu S, Paetau-Robinson I. Dietary supplements of vitamins E and C and beta-carotene reduce oxidative stress in cats with renal insufficiency. *Vet Res Community* 2006;30:403-413.