Journal of Feline Medicine and Surgery (2009) **11**, 271–276
doi:10.1016/j.jfms.2008.07.004

ELSEVIER

jfms

Oxidative stress and innate immunity in feline patients with diabetes mellitus: the role of nutrition

Craig B Webb PhD, DVM, DACVIM, Assistant Professor*, **Lauren Falkowski** PVM

Clinical Sciences Department, Colorado State University, Fort Collins, CO 80523, USA

This study was undertaken to test the hypothesis that oxidative stress is increased and neutrophil function is decreased in cats with diabetes mellitus (DM). Measures of oxidative stress and neutrophil function were evaluated in 20 control and 15 diabetic cats. Cats were then fed a diet designed specifically for feline diabetics (Purina DM Dietetic Management Feline Formula) for 8 weeks, after which all assays were repeated. Cats with DM had significantly less plasma superoxide dismutase (SOD) than control cats, consistent with a greater degree of oxidative stress in the DM group. Following 8 weeks of consuming a diabetes-specific diet glutathione peroxidase, an antioxidant enzyme increased significantly in both groups. Other parameters of oxidative stress, as well as neutrophil function, were similar between groups and did not change following dietary intervention. The DM cats were significantly older and heavier than the control cats, which may have contributed to differences in parameters of oxidative stress and levels of antioxidant enzymes between these groups, but the decreased level of SOD enzyme in the diabetic group would appear to support the continued development of targeted antioxidant supplementation for this cats with this disease.

Date accepted: 1 July 2008

Oxidative stress is thought to be a key component in the pathophysiology of type 2 diabetes mellitus (DM) in humans, and it is now estimated that 85% cats with DM are type 2 diabetics.[1,2] As one source of an increase in the production of oxidative free radicals is the metabolic response to hyperglycemia, the use of high protein–low carbohydrate diets in diabetic cats may lead to a significant reduction in oxidative stress.[3] Oxidative stress may affect neutrophil lifespan, and phagocytic cell function may be negatively impacted by oxidative stress in patients with DM, resulting in a decrease in their ability to prevent or eliminate infection.[4,5]

This study was undertaken to test the hypothesis that oxidative stress is increased and neutrophil function is decreased in cats with DM. Serum fructosamine levels were used as a quantitative measure of glycemic control over time, while body weight, clinical signs, and parameters of insulin treatment provided a more clinical picture of the cats during the course of the study. Oxidative stress could manifest as an increase in lipid peroxidation, a change in the redox state of glutathione (GSH), or a decrease in antioxidant enzyme levels. An increase in erythrocyte lipid peroxidation could lead to premature red blood cell destruction, while a change in GSH or a decrease in antioxidant enzyme levels could decrease the cat's ability to metabolize or eliminate potentially harmful free radicals. Neutrophils are a key component of the innate immune system and an increase in oxidative stress could reduce their ability to eliminate bacterial pathogens, resulting in chronic or recurrent infections.

Parameters of oxidative stress, levels of antioxidant enzymes, and neutrophil function were measured in both diabetic and control cats. Cats were then fed a commercially available diet designed specifically for feline diabetics, Purina DM Dietetic Management Feline Formula (Purina DM) for 8 weeks, after which the above parameters were re-evaluated. Results were compared both between groups and within groups over time to illustrate differences in oxidative stress and neutrophil function between diabetic cats and healthy cats, as well as to try and determine what effect nutrition had on those variables.

Materials and methods

Cats

All cats were client-owned animals and all study procedures were performed at Colorado State University

*Corresponding author. Tel: +1-970-297-4431; Fax: +1-970-297-4156. E-mail: cbwebb@colostate.edu

1098-612X/08/040271+06 $34.00/0

(CSU) Veterinary Medical Center. Inclusion criteria for the DM group were simply a previous diagnosis and ongoing treatment for DM with no known concurrent disease. Inclusion for the control group was simply a healthy cat with no history of significant disease. Owners signed an informed consent form, and all procedures were performed in accordance with the CSU Animal Care and Use Committee Guidelines. Owners completed a questionnaire regarding their cat's medical history, date of initial diagnosis with DM, previous and current treatment including diet, type, amount and frequency of insulin administration. All of the cats in the DM group had been previously diagnosed with diabetes between 1 month and 7.5 years prior to entry into the study (mean $\pm$ standard deviation, 2.4 ± 2.1 years). All of the DM cats were being treated with insulin at a mean dose of 2.25 ± 0.96 U twice-daily for a mean duration of 10 ± 14.1 months (range 0.25–48 months). A history, complete physical examination, body weight, complete blood count (CBC) and fructosamine level was performed for all DM cats. Cats in the control group were recruited predominantly from CSU veterinary students, faculty and staff and a similar evaluation to that for the DM group was performed, including fructosamine levels, prior to entry into the study. All of the DM and control cats were under the care and supervision of a veterinarian and we relied on the regular and more complete (ie, feline leukemia virus and feline immunodeficiency virus status, fecal analysis, de-worming and vaccinations, biochemical profile and urinalysis, thyroid hormone level and other ancillary testing where appropriate) assessment of the cat by that veterinarian to confirm that none of the cats had any other concurrent disease at the time of entry into the study. No remarkable abnormalities were noted in the history or seen on physical examination or CBC results to suggest ongoing pathology other than DM in any of the cats.

Study design

Upon entry into the study all DM and control cats had their body weight recorded and a 6 ml blood sample drawn for determination of a CBC, fructosamine level, and for use in the assays of oxidative stress and neutrophil function. Superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), the reduced glutathione-to-oxidized glutathione (GSH:GSSG) ratio, and neutrophil (PMN) phagocytosis and oxidative burst were evaluated in 20 control and 15 diabetic cats.

Treatment

Following the initial sample collection all DM and control cats were fed Purina DM for 8 weeks. The owners were given the choice of feeding dry, canned, or both types of food to their cats, but were asked to strictly adhere to the use of the diet exclusively for the entire 8-week duration of the study. Owners were otherwise instructed to continue or modify the treatment regimen of their diabetic cat based on the recommendations of their clinician. Following 8 weeks on Purina DM the cats were again weighed and the sample acquisition and assays were repeated.

Sample preparation

Peripheral blood samples were divided into an ethylenediaminetetraacetic acid (EDTA)-preserved sample (2 ml) for the CBC, a serum tube for fructosamine (1 ml), and a heparin-preserved sample (3 ml) for neutrophil function and oxidative stress assays. The samples for the oxidative stress assays were prepared immediately following collection, stored at -70°C, and these assays were run as a single batch within 25 days of collection. The samples for the neutrophil function assays were stored on ice and completed within 6 h of collection.

Complete blood counts

CBCs were run on a Siemens Advia 120. One hundred cell differentials were performed manually along with a morphology review to determine the absolute neutrophil number for use in calculating the appropriate number of *Escherichia coli* for use in the neutrophil function assay.

SOD and GPx

The SOD enzyme catalyzes the reaction that takes the superoxide free radical to hydrogen peroxide (reaction 1). The GPx enzyme catalyzes the reaction that takes hydrogen peroxide to water (reaction 2). During this reaction reduced GSH is converted to GSSG, and the GSH:GSSG ratio is used as a measure of oxidative stress (described later in this section).

$$\underset{\text{Superoxide dismutase}}{O_2^{\bullet -} \rightarrow H_2O_2} \qquad \text{(reaction 1)}$$

$$\underset{\text{Glutathione peroxidase}}{H_2O_2 \rightarrow H_2O + \tfrac{1}{2}O_2} \qquad \text{(reaction 2)}$$

$$GSH \rightarrow GSSG$$

Commercially available colorimetric kits for the measurement of erythrocyte SOD and whole blood GPx were previously validated for use in the domestic cat (Ransod and Ransel, Randox Laboratories, Oceanside, CA).[6] In that study, intra- and interassay coefficients of variation were determined ($\sim$10%), and it was shown that freezing samples at -80°C did not affect results measured at day 28.

Malondialdehyde

MDA is an end-product of free radical-induced cell membrane lipid peroxidation. Heparinized whole

blood (250 μl) was transferred to an EDTA tube and maintained at −70°C until analysis. The whole blood sample was thawed and pipetted into a solution of 0.9 mM butylated hydroxytoluene in absolute alcohol. A 0.03 mM solution of thiobarbituric acid in 50% glacial acetic acid was added, and the resulting solution heated for approximately 1 h. The reaction mixture was cooled and acidified prior to adding *n*-butanol. The MDA was extracted into the *n*-butanol and the fluorescent emission at 550 nm compared to that of standardizing solutions to determine the micromoles MDA per liter of whole blood.

Determination of whole blood GSH:GSSG ratio

The ratio of GSH:GSSG has been used previously as one measure of oxidative stress in cats (reaction 3).[7,8]

$$\mathrm{GSH} \xrightarrow[\text{Glutathione peroxidase}]{} \mathrm{GSSG}$$

$$\mathrm{GSSG} \xrightarrow[\text{Glutathione reductase}]{} \mathrm{GSH} \qquad \text{(reaction 3)}$$

GSH was quantified from EDTA-preserved blood using an enzymatic assay. The assay specifications demonstrate linearity to a sample concentration of 2400 μM GSH, recovery of 98%, a lower limit of detection (sensitivity) of 0.54 μM and no interfering effects with tested analogs (specificity).[9] The assay was run twice, once with and once without a GSH scavenging reagent, so that both GSSG and total glutathione (GSH + GSSG) can be quantified. For oxidized glutathione, a thiol-scavenging reagent, 1-methyl-2-vinyl-pyridium trifluoromethane sulfonate (M2VP) was added to rapidly scavenge GSH and eliminate continued oxidation of GSH to GSSG after sample acquisition. GSH reductase, nicotinamide−adenine dinucleotide phosphate (NADPH), and the chromagen 5,5-dithiobis-(2-nitrobenzoic acid) were added sequentially to the sample. GSH reductase converts the oxidized glutathione to GSH, which reacts with the chromagen to form a product which absorbs visible light (412 nm). The change in absorbance was measured spectrophotometrically. The rate of this reaction is proportional to the resultant GSH concentration. Total glutathione, GSH + GSSG, was quantified in EDTA-preserved blood without the addition of M2VP. The concentration of GSH was determined by the difference between the total glutathione (GSH + GSSG) concentration and the oxidized glutathione (GSSG) concentration. The GSH:GSSG ratio may then be calculated.

Neutrophil phagocytosis and oxidative burst

Intracellular reactive oxygen species convert the nonfluorescent compound dihydrorhodamine 123 (DHR; Molecular Probes division of Invitrogen Corporation, Carlsbad, CA) to a green fluorescent molecule rhodamine 123, which can be measured by flow cytometry.[10,11] From a working stock of 0.05 mM DHR, 10 μl was added to 100 μl heparin-preserved whole blood for a final concentration of 5 μM DHR and incubated for 45 min at 37°C.

Lyophilized *E coli* (strain K 12) conjugated to Alexa Fluor 488 (a fluorescent molecule detected by flow cytometry: Molecular Probes division of Invitrogen Corporation, Carlsbad, CA) was reconstituted with 100 μl of 2 mM sodium azide/phosphate-buffered saline (PBS) solution to produce a working concentration of 2 mg/100 μl (6×10^8 *E coli* particles/100 μl). *E coli* was added to the DHR-treated sample for the final 30 min of incubation at a ratio of 30 *E coli* per neutrophil. Immediately following incubation the erythrocytes were lysed with NH_4Cl (5 min incubation at room temperature followed by two washes in HBSS (Hanks Balanced Salt Solution, Sigma−Aldrich, St Louis, MO)) and the leukocyte pellet resuspended in FACS (Fluorescence-Activated Cell Sorting solution, Sigma−Aldrich, St Louis, MO) buffer. Ten microliters of trypan blue was added to this cell suspension. Trypan blue is a vital stain that does not cross the membrane of intact cells and quenches fluorescent emissions (ie, the Alexa Fluor 488 signal associated with *E coli* adhered to the cell surface but not having undergone phagocytosis). The difference in the fluorescence emission spectrum for Alexa Fluor 488 and DHR can be distinguished and measured by flow cytometry such that the two signals, corresponding to *E coli* phagocytosis (Alexa Fluor 488) and the oxidative burst (DHR) can be measured from the same cell at the same time. Control samples included a leukocyte suspension with no additions, a suspension treated with DHR only, and a suspension treated with *E coli* only, were prepared in the same manner as described.

Statistical analyses

Following confirmation of a Gaussian distribution using the Kolmogorov−Smirnov test, a comparison of the means for determination of statistically significant differences between groups was performed using either a paired or an unpaired, two-tailed Student's *t*-test where appropriate, assuming unequal variance with a Welch correction. Statistical analysis was undertaken using commercially available computer software (GraphPad Prism software, San Diego, CA). A P value < 0.05 was considered significant for all statistical analyses performed in this study. Results are presented as means ± standard deviation where appropriate.

Results

Age, body weight, serum fructosamine, insulin treatment and diet

The DM group (11.6 ± 3.9 years) was significantly older than the control group (3.0 ± 2.4 years)

($P < 0.001$). The DM cats were significantly heavier than the control group at the start of the study (6.2 ± 0.98 kg and 5.2 ± 0.83 kg, respectively) ($P < 0.02$), as well as at the completion of the study (DM at 6.3 ± 1.20 kg and control at 5.4 ± 0.92 kg; $P < 0.02$). There was a small but statistically significant increase in mean body weight for the control group of cats (5.2–5.4 kg; $P < 0.005$) during the study. The increase in body weight in the DM group (6.2–6.3 kg; $P = 0.48$) was not found to be statistically significant.

The serum fructosamine level was significantly greater in the DM group than the control group both before (411.7 ± 164.4 and 264.3 ± 40.6 μmol/l, respectively; $P < 0.005$) and after (353.4 ± 131.5 and 245.5 ± 32.0 μmol/l, respectively) consumption of the Purina DM diet for 8 weeks. Serum fructosamine levels decreased significantly in both the DM (411.7–353.4 μmol/l; $P < 0.05$) and control groups (264.3–245.5 μmol/l; $P < 0.005$).

Prior to enrollment in the study only four of the DM cats were being fed a diet specifically designed for diabetic cats exclusively (two cats were being fed Purina DM Dietetic Management Feline Formula and two cats were being fed Hill's Prescription Diet m/d) while none of the control cats were being fed a diet designed for diabetic cats. Eight of the DM cats were on glargine insulin, four were on PZI, and three were on Humulin Neutral Protamine Hagedorn (NPH) insulin. Two cats were being administered their insulin once-daily while 12 cats received insulin injections twice-daily. The average twice-daily dose of insulin for the DM cats was 2.25 ± 0.96 U/injection. The DM cats had been on this dose of insulin for a mean duration of 10 ± 14.1 months (range 0.25–48 months) prior to the start of the study. The amount of insulin was reduced in two of the DM cats by 1.0 and 0.5 U during the course of the study, while the insulin dose was increased by 1 U in the DM cat with acromegaly.

Measures of oxidative stress

The mean SOD level was significantly less in the DM group (211.4 ± 143.5 U/ml) than in the control group (352.2 ± 200.4 U/ml; $P = 0.02$). This difference was no longer significant by the end of the study. Neither group showed a statistically significant change in their SOD level over that 8-week time period, but the mean SOD level increased in the DM group and decreased in the control group (255.4 and 280.5 U/ml, respectively).

The other parameters of oxidative stress measured in this study, MDA, GPx, and the GSH:GSSG ratio, were not significantly different between the control and the DM groups either at the beginning or at the end of the study. The mean GPx levels increased significantly by the end of the study in both groups, from $47{,}083 \pm 15{,}913$ to $68{,}839 \pm 15{,}042$ in the control group and from $41{,}081 \pm 19{,}334$ to $67{,}128 \pm 11.066$ U/l in the DM group ($P < 0.005$).

Neutrophil function

There were no significant differences in neutrophil function either between groups or within groups during the course of the study.

Discussion

The diabetic cats in this study were significantly older and heavier than the control cats. The mean age of diabetic cats is reported to be around 10 years of age and obesity is recognized as a factor contributing to the development of type 2 diabetes in humans and cats.[2,12] The significant difference in age between the DM and control groups was not anticipated when cases were recruited and is an obvious flaw in the design of this study. Because oxidative stress is thought to play a role in the aging process it was possible that the significantly greater mean age of the DM group might have been an important contributing factor to the lower level of SOD in these cats.[13] However, age-related changes in nuclear and mitochondrial DNA are thought to be due to an increased sensitivity to oxidative damage rather than decreased repair capacity, and antioxidant status does not change significantly with age.[14] Cats may age differently than humans, but at this point it is far from clear which specific parameters of oxidative stress actually decrease with age in either species, and the potential effect that diseases such as DM have on antioxidant enzyme activity in older cats requires further investigation.[15] The fact that the DM group was significantly heavier than the control group is another important and potentially confounding variable as obesity is associated with an increase in oxidative stress.[16]

Following consumption of Purina DM for 8 weeks, serum fructosamine levels were significantly reduced in both the diabetic and control cats. High protein and low carbohydrate diets specifically designed for diabetic cats have been shown to improve glycemic control and increase rates of remission in cats with this disease.[17] The fact that 2 months of the prescribed diet also significantly lowered the serum fructosamine levels in healthy non-diabetic cats suggests that diet may play a role as a prophylactic intervention in those cats prone to developing adult-onset type 2 diabetes. There was a small (0.2 kg), albeit significant, amount of weight gain in the control group. If it is determined that this is a consistent finding in a larger group of cats over a more prolonged period of time, then this tendency would have to be taken into account, as obesity appears to contribute to the onset and pathogenesis of type 2 diabetes in cats. Alternatively, looking for a change in body condition score before and after dietary intervention may have been informative and is an important clinical parameter in the assessment of diabetic cats. Ideally, percent body fat would have been quantified using a Dual Energy X-ray Absorptiometry (DEXA) scan in this study. This would have identified any change in lean body mass which would

have potentially impacted glycemic control in these cats.

In this study, diabetic cats had less SOD than control cats. SOD is thought to play an important role in the development and progression of oxidative stress in human diabetic patients, and has been shown to be reduced in human diabetics compared to non-diabetics, more so in non-insulin dependent patients.[18] A decrease in SOD level appears to lead to an increase in glycated proteins, insulin resistance, and diabetic complications in humans, but these variables have not yet been investigated in diabetic cats.[19] An alternative explanation for this finding is that the difference in SOD in this study is due to the difference in age and body fat between the two groups. Ideally the control group would have been matched to the DM group for both of these variables. A third possibility is that the DM group contained a number of cats with concurrent disease that went undetected by the attending clinician or our screening process but involved significant levels of oxidative stress. Regardless of the cause, a low SOD level in older, heavier diabetic cats raises the question as to whether antioxidant supplementation with a SOD enzyme complex would raise those levels, and if so, what effect that would have on clinical parameters or complications of this disease in cats. In contrast to the findings of this study, there are reports that have identified an increased SOD level in human diabetics, highlighting the complex interplay of patient, disease, and the multitude of parameters that combine to result in oxidative stress.[20]

Consumption of Purina DM diet for 8 weeks resulted in a significant increase in GPx activity in both diabetic and control cats. One potential explanation for the apparent effect of diet on GPx levels would be the presence of selenium in the diet used in this study.[21] Selenium, a trace element, is a co-factor for the GPx enzyme. Significant selenium deficiency is considered unlikely in most cats consuming commercial pet food, but the selenium content of pet foods can be highly variable.[22] The selenium content of the different diets fed to the study cats prior to Purina DM was not quantified. One report of selenium supplementation in humans with type 2 diabetes found a tendency to increase blood GPx activity as well as decrease other parameters of oxidative stress such as MDA levels.[23] MDA levels, however, did not change significantly in the cats in this report. GPx levels may have changed in response to parameters of oxidative stress that were not measured in this study. The fact that the GSH:GSSG ratio did not change significantly would suggest that levels of the GSH reductase enzyme changed in concert with GPx to maintain the equilibrium of this redox pair. Low GPx activity has been associated with thrombosis and cardiovascular complications in human diabetics.[24] Although these complications are not appreciated in the feline diabetic population an increase in GPx activity may be otherwise beneficial in this species as well.

MDA, a measure of lipid peroxidation was not significantly different between the DM and control cats, and did not change with consumption of Purina DM. MDA is a metabolic end-product of free radical-induced lipid peroxidation, a deleterious consequence of oxidative stress on cell membranes. The lack of a change in MDA levels may be a reflection of the fact that MDA is only one of many lipid peroxidation end-products, or it may reflect disease severity. None of the diabetic cats progressed to diabetic ketoacidosis during the course of the study.

Neutrophil function, as quantified in this study, was not different between cats in the DM and control groups, and did not change following consumption of Purina DM. Human diabetics appear predisposed to infections due, in part, to a decrease in neutrophil function.[25] Studies suggest that urinary tract infections are more common and complicated in humans with DM[26] and there appears to be an increase in the prevalence and severity of periodontal disease in people with DM associated with an ill-defined abnormal host response to infectious organisms.[27] It would appear that the cats with diabetes also present with an increase in urinary tract infections, although this is not yet supported by a prevalence study.[28] Clearly a large number of factors in addition to the parameters measured in this study contribute to the function of the innate immune system in diabetic patients, and it is certainly possible that there are deficits in those phagocytic cells at the site of infection that would go undetected through examination of circulating neutrophils.

The findings of this study support the continued development and evaluation of targeted antioxidant supplementation and dietary intervention in diabetic cats. DM may be associated with an increase in oxidative stress in cats, and diet appears to have significantly impacted a parameter of oxidative stress, GPx activity, in both affected and healthy cats. Early dietary intervention may also be beneficial in cats predisposed to developing DM, although the potential for weight gain needs to be considered. Further work to clarify the impact of age, obesity, and other diseases on levels of oxidative stress and antioxidant enzyme activity in cats needed. The increase in the commercial availability of veterinary-specific antioxidant supplements and the apparent increasing popularity of these products amongst clients make verification of product safety and identification of product efficacy of timely importance for veterinarians faced with myriad potentially beneficial choices.

Acknowledgments

The authors wish to acknowledge Nestlé Purina PetCare for their generous support of research into oxidative stress, nutrition and veterinary diseases. Nestle Purina sponsored a portion of the current study and provided the diet (Nestle Purina DM) free-of-charge.

References

1. Shah S, Iqbal M, Karam J, Salifu M, McFarlane SI. Oxidative stress, glucose metabolism, and the prevention of type 2 diabetes: pathophysiological insights. *Antioxid Redox Signal* 2007; **9**: 911–29.
2. Rand JS, Marshall RD. Diabetes mellitus in cats. *Vet Clin North Am Small Anim Pract* 2005; **35**: 211–24.
3. Nishikawa T, Edelstein D, Brownlee M. The missing link: a single unifying mechanism for diabetic complications. *Kidney Int* 2000; **77**(Suppl): S26–30.
4. Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunol Med Microbiol* 1999; **26**: 259–65.
5. Watson RW. Redox regulation of neutrophil apoptosis. *Antiox Redox Signal* 2002; **4**: 97–104.
6. Marshall MD, Wattman SL, Skinner ND, Charlton CJ, Harper EJ. Feline antioxidant enzyme activity: the effect of sample storage on stability. *J Nutr* 2002; **132**: 1733S–4.
7. Webb CB, Twedt DC, Fettman MJ, Mason G. S-adenosylmethionine (SAMe) in a feline acetaminophen model of oxidative injury. *J Feline Med Surg* 2003; **5**: 69–75.
8. Center SA, Randolph JF, Warner KL, et al. The effects of S-adenosylmethionine on clinical pathology and redox potential in the red blood cell, liver, and bile of clinically normal cats. *J Vet Intern Med* 2005; **19**: 303–14.
9. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: application to mammalian blood and other tissues. *Anal Biochem* 1969; **27**: 502–22.
10. Walrand S, Valeiz S, Rodriguez C, Ligot P, Chassagne J, Vasson MP. Flow cytometry study of polymorphonuclear neutrophil oxidative burst: a comparison of three fluorescent probes. *Clin Chim Acta* 2003; **331**: 103–10.
11. Kampen AH, Tollersrud T, Lund A. Flow cytometric measurement of neutrophil respiratory burst in whole bovine blood using live *Staphylococcus aureus. J Immunol Methods* 2004; **289**: 47–55.
12. Henson MS, O'Brien TD. Feline models of type 2 diabetes mellitus. *ILAR J* 2006; **47**: 234–42.
13. Rahman K. Studies on free radicals, antioxidants, and co-factors. *Clin Interv Aging* 2007; **2**: 219–36.
14. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007; **39**: 44–84.
15. Voss P, Siems W. Clinical oxidation parameters of aging. *Free Radic Res* 2006; **40**: 1339–49.
16. Vincent HK, Innes KE, Vincent KR. Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. *Diabetes Obes Metab* 2007; **9**: 813–39.
17. Bennett N, Greco DS, Peterson ME, Kirk C, Mathes M, Fettman MJ. Comparison of a low carbohydrate-low fiber diet and a moderate carbohydrate-high fiber diet in the management of feline diabetes mellitus. *J Feline Med Surg* 2006; **8**: 73–84.
18. Abou-Seif MA, Youssef AA. Evaluation of some biochemical changes in diabetic patients. *Clin Chim Acta* 2004; **346**: 161–70.
19. Maritim AC, Sanders RA, Watkins 3rd JB. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 2003; **17**: 24–38.
20. Cimbaljević B, Vasliijević A, Cimbaljević S, et al. Interrelationship of antioxidant status, lipid peroxidation, and lipid profile in insulin-dependent and non-insulin dependent diabetic patients. *Can J Physiol Pharmacol* 2007; **85**: 997–1003.
21. Allan CB, Lacourciere GM, Stadtman TC. Responsiveness of selenoproteins to dietary selenium. *Annu Rev Nutr* 1999; **19**: 1–16.
22. Simcock SE, Rutherford SM, Wester TJ, Hendriks WH. Total selenium concentrations in canine and feline foods commercially available in New Zealand. *N Z Vet J* 2005; **53**: 1–5.
23. Skripchenko ND, Sharafetdinov KhKh, Plotnikova OA, Meshcheriakova VA, Mal'tsev GIu. Effect of selenium enriched diet on lipid peroxidation in patients with diabetes mellitus type 2 [article in Russian]. *Vopr Pitan* 2003; **72**: 14–7.
24. Faure P. Protective effects of antioxidant micronutrients (vitamin E, zinc and selenium) in type 2 diabetes mellitus. *Clin Chem Lab Med* 2003; **41**: 995–8.
25. Calvet HM, Yoshikawa TT. Infections in diabetes. *Infect Dis Clin North Am* 2001; **15**: 407–21.
26. Hoepelman AI, Meiland R, Geerlings SE. Pathogenesis and management of bacterial urinary tract infections in adult patients with diabetes mellitus. *Int J Antimicrob Agents* 2003; **22**(Suppl 2): 35–43.
27. Ryan ME, Carnu O, Kamer A. The influence of diabetes on the periodontal tissues. *J Am Dent Assoc* 2003; **134**: 34S–40.
28. Bailiff NL, Nelson RW, Feldman EC. Frequency and risk factors for urinary tract infection in cats with diabetes mellitus. *J Vet Int Med* 2006; **20**: 850–5.