



ORIGINAL ARTICLE

The effect of dietary long-chain omega-3 fatty acid supplementation on owner's perception of behaviour and locomotion in cats with naturally occurring osteoarthritis

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Summary

The aim of this randomized, double-blinded, placebo-controlled, cross-over designed study was to demonstrate the clinical effect, registered by a survey, of a 10-week period of omega-3 fatty acid supplementation of the diet (1.53 g eicosapentaenoic acid (EPA) and 0.31 g DHA, both per 1000 kcal ME, equivalent to the complete diet) of 16 cats with radiologically documented, naturally occurring osteoarthritis (OA), in comparison with a 10-week period of supplementation with corn oil (0.00 g EPA and 0.00 g DHA, both per 1000 kcal ME). Cats on the fish oil revealed higher activity level ($p = 0.07$), more walking up and down the stairs ($p = 0.07$), less stiffness during gait ($p = 0.03$), more interaction with the owner ($p = 0.07$) and higher jumps ($p = 0.03$) compared to those on corn oil supplementation. In conclusion, supplementation of long-chain omega-3 polyunsaturated fatty acids changes the owner's perception of some aspects of behaviour and locomotion in cats with naturally occurring OA.

Introduction

Osteoarthritis (OA) in cats is getting more awareness in the recent years by researchers and veterinarians. OA is initiated by trauma of joint cartilage (secondary OA) and/or shortening of the proteoglycans with concurrent increase in advanced glycation end products (primary OA). Pathogenesis of OA is characterized by a chronic inflammatory state resulting in an increase in matrix metalloproteinases (MMPs) that perpetuates the cycle of destruction of cartilage and inflammation (Allan, 2000; Anderson et al., 2004; Burns, 2006; Kerwin, 2010; Slingerland et al., 2011). OA is a common finding in older cats, with a prevalence of 22–72% in cats >6 years of age (Hardie et al., 2002; Clarke et al., 2005; Godfrey, 2005; Beale, 2005;

Lascelles, 2008). The prevalence and severity of OA increase with age (Slingerland et al., 2011). The vertebrae, elbows, hips, shoulders and tarsi are the most commonly affected joints (Slingerland et al., 2011). The behavioural changes of cats with OA are decreased mobility, less grooming and defecating outside the litter box (Hardie, 1997; Rothschild et al., 1998; Clarke et al., 2005; Clarke and Bennett, 2006; Slingerland et al., 2011). The first signs of OA are mostly subtle, so OA is commonly recognized at a progressive state of disease and often remains undiagnosed and untreated in the earlier stages (Godfrey, 2005; Lascelles, 2010; Rychel, 2010). It may be difficult to detect painful OA in cats, because cats tend to adapt their mobility to their abilities to avoid pain (Taylor and Robertson, 2004). These behavioural

adaptations are mistakenly considered normal for an ageing cat by the owner and are not related to OA. Nevertheless, Zamprogno *et al.*, (2010) and Slingerland *et al.* (2011) demonstrated that activity-related behaviour, as noted by cat owners, is significantly different between healthy cats and cats with signs of OA-associated pain. Treatment of cats suffering from OA will include guided weight reduction (Scarlett and Donoghue, 1998), non-steroid anti-inflammatory drugs (NSAIDs) (Clarke and Bennett, 2006; Gunew *et al.*, 2008) and nutritional management (Gück, 2009). Scarlett and Donoghue (1998) demonstrated a 4.9 times increased risk of lameness because of OA in obese cats vs. lean cats that were admitted to veterinary hospitals and followed up during 4.5 years. Obese cats may have more severe OA and OA-associated pain compared to lean cats, because of the chronic release of pro-inflammatory cytokines by adipose tissue, and the release of leptin from chondrocytes, as is demonstrated in men and research animals (obese/obese mouse) (Simopoulou *et al.*, 2007). Weight loss improved locomotion in overweight dogs with OA (Hardie, 1997; Laflamme, 2005; Gück, 2009; Marshall *et al.*, 2010; Rychel, 2010). Nutritional management of OA may further include modulation of the inflammatory response. Nutrients that are claimed to modulate the inflammatory response are long-chain omega-3 fatty acids (Gück, 2009). Omega-3 fatty acids sort their effect by altering the cell wall fatty acid composition (Cao *et al.*, 2006), resulting in a competition for cyclo-oxygenase enzymes between eicosapentaenoic acid (EPA), which promotes the anti-inflammatory mediators (i.e. leukotriene B5, prostaglandin E3 and thromboxane 3 series), and arachidonic acid (AA), which promotes the pro-inflammatory mediators (i.e. leukotrienes B4, prostaglandin E2 and thromboxane 2 series). The increase in anti-inflammatory mediators results in decreased production of MMPs as well as increased production of tissue inhibitors of MMPs. The effects of omega-3 fatty acid supplements on OA have been studied in different species, including human (Cao *et al.*, 2006) and dogs (Frost-Christensen *et al.*, 2006; Fritsch *et al.*, 2010; Roush *et al.*, 2010a,b). Omega-3 fatty acids have been proposed to have a beneficial effect on OA in cats (Gück (2009), and this has been investigated in several studies. They have anti-arrhythmic effects (Freeman, 2010) and also have an anti-inflammatory action on bowel disease (Trepanier, 2009). Further, a multicomponent therapeutic diet with increased EPA and DHA levels has been shown to relieve OA-associated pain in cats, when compared to a control food (Lascelles *et al.*, 2010). These effects were registered

by accelerometry and behavioural changes. However, it is unclear whether these effects were attributable to increased EPA and DHA levels or to concomitant additional nutritional modifications, such as green-lipped mussel extract and glucosamine/chondroitin sulphate (Beale, 2004).

The aim of this randomized, double-blinded, placebo-controlled, cross-over designed study was to demonstrate the clinical effect, registered by a survey, of a 10-week period of omega-3 fatty acid supplementation (1.53 g EPA and 0.31 g DHA, both per 1000 kcal ME, equivalent to the complete diet) of 16 cats with radiologically documented, naturally occurring OA, in comparison with a 10-week period of supplementation with corn oil (0.00 g EPA and 0.00 g DHA, both per 1000 kcal ME).

Material and methods

Animals

Owners of 35 cats, referred to the University department all with radiographically confirmed OA as part of a previously reported study (Slingerland *et al.*, 2011), were invited to participate in this study. Owners of 24 cats were willing to participate in our study. Radiographs of both shoulders [mediolateral (ML)], elbows (ML), carpi (dorsopalmar) including front leg digits, coxofemoral joints (ventrodorsal), stifles (ML and craniocaudal) and tarsi (ML) were evaluated. For each joint, OA was graded as absent, minimal, moderate or severe, according to Hardie *et al.* (2002). The inclusion criteria were >8 years of age, moderate OA [according to Hardie *et al.* (2002)] in one or more joints, being on a commercially available dry cat food and the willingness of the owners to cooperate in this study (i.e. daily supplementation of the daily ration with an exact amount of oil). Exclusion criteria were the use of NSAIDs and the use of (dietary) supplements from 2 weeks before the start of the study and during the study.

Questionnaire

A questionnaire based on the previous study in these cats was used (Slingerland *et al.*, 2011) completed with questions on behaviour and activity, according to Zamprogno *et al.* (2010). The questionnaire was tested prior to being used by interviewing cat owners in the waiting room of our clinic, and necessary adaptations were made. The questionnaire included only closed questions with predetermined and pre-coded categories (Appendix 1). The same researcher (M.M.C.B.) had telephone contact with the owner

prior to the study to ask for cooperation and completion of the survey, and after both supplementation periods, without knowing whether the cat just completed a 10-week period on oil 'A' or oil 'B'.

Supplements

The supplements (Catoils[®] joint and corn oil) were provided by Nutriceuticoids[®], Oelegem, Belgium. The test and control supplement had an equal appearance [i.e. corn oil with fish smell and fish oil [mainly derived from Anchovy (*Engraulidae*) and Sardine (*Clupeidae*)] in identical bottles and were indicated by a letter 'A' or 'B' on the 100-mL bottle. The owners were advised to provide the oil in a dosage of 1 ml per 5 kg body weight daily to their cats, (dosage syringe was provided) added to the declared given maintenance food (i.e. a commercially available dry cat food, fed dry, which meets NRC requirements for all life stages) (NRC 2006). The key was broken after statistical analyses of all study results. The amount of oil made available to the owner was sufficient for the test period and did not contain an extra volume to prevent errors of long-term administration of the product during the next test period. Oil 'A' contained 0 mg/ml eicosatetraenoic acid (ETA), 0 mg/ml EPA and 0 mg/ml DHA. Oil 'B' contained 15 mg/ml ETA, 500 mg/ml EPA and 100 mg/ml DHA. Vitamin E (alpha-tocopherol 10 mg/ml) was added to both formulations to prevent rancidity. The fatty acid compositions of the provided maintenance cat foods, as declared by the manufacturer, are shown in Table 1. The fatty acid composition of oil 'A' and oil 'B', as determined by an independent laboratory (Napro Pharma AS, Norway) using method Ph.Eur.2.4.29 (chromatography), is shown in Table 2.

Study protocol

Before treatment with either oil 'A' or oil 'B', a questionnaire was completed by the owners. The cats were then randomly given either oil 'A' or oil 'B' for 10 weeks. After this 10-week period, the questionnaire was completed by the owners. Then, another 10-week period of supplementation with the other oil was performed, followed by the final questionnaire. No wash-out period was introduced between the two periods.

Control group

From the Department's colony, 20 healthy cats (all neutered) were used to investigate the blood fatty

Table 1 Fatty acid composition of the maintenance cat foods

Cat number	EPA+DHA	Total n-6	Total n-3	n-6:n-3
1	0.26	na	na	na
2	0.05	na	na	na
3	0.42	3.22	0.8	4
4	0.05	na	na	na
5	0.05	na	na	na
6	0.2	3.8	0.54	7
7	0.32	4.62	0.81	6
8	0.26	3.45	0.6	6
9	na	3.22	0.39	8
10	na	3.22	0.39	8
11	na	3.22	0.39	8
12	0.05	na	na	na
13	0.7	2.88	1.08	3
14	0.05	na	na	na
15	na	2.78	0.7	4
16	0.05	na	na	na
17	0.26	3.45	0.6	6
18	na	2.78	0.7	4
19	0.26	3.45	0.6	6
20	0.05	na	na	na

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; n-6, omega-6 polyunsaturated fatty acids; n-3, omega-3 polyunsaturated fatty acids; na, not available.

Data in % on dry matter basis, obtained from manufacturer.

acid concentrations before and after a 10-week period on one of the two investigated supplements, given randomly (i.e. daily supplementation with oil 'A' or oil 'B' respectively). The animals were fed a dry diet which was supplemented daily with either oil 'A' or oil 'B'. Blood samples were taken, before and after the 10-week period, by jugular venipuncture and collected in heparinized tubes. The blood was immediately centrifuged and the plasma harvested and stored at -20°C until assay. These samples served to determine the fatty acid profile of the cholesteryl ester (CE) fraction of plasma. The investigators were blinded to the oils the cats were given during this 10-week period.

Analysis of plasma fatty acid composition

Cholesteryl esters in plasma of day 0, day 70 and day 140 were isolated by a modified 'Bligh & Dyer' extraction according to Retra *et al.* (2008), followed by a solid-phase extraction method according to Hamilton and Comai (1988). Thereafter, the CEs were saponified according to the modified method described by Kates (1986), where petroleum ether was replaced by hexane. Polyunsaturated fatty acid analysis was performed by high-pressure liquid chromatography/mass spectrometry (HPLC/MS)

Table 2 Fatty acid composition of the oils

Fatty acid	Fish oil g/100g	Corn oil g/100g
Omega-6	4.60	51.80
C18:2 (LA)	1.30	51.80
C20:4 (AA)	2.10	0.00
Omega-3	63.60	1.00
C18:3 (ALA)	0.60	1.00
C20:4 (ETA)	1.60	0.00
C20:5 (EPA)	43.90	0.00
C22:5 (DPA)	2.20	0.00
C22:6 (DHA)	11.30	0.00
Other		
C16:0 (Sat.)(PA)	0.60	10.23
C16:1 (n-7)(PO)	0.20	0.10
C18:0 (sat.)(ST)	4.60	1.80
C18:1 (n-9)(OL)	9.70	28.60
Total PUFA	69.50	52.80
Total MUF	16.40	29.50
Total SAF	6.40	12.80
Total unknown FA	2.50	0.00

Data in % on dry matter basis, obtained from Napro Pharma AS, Norway. Peroxide value: maximum 5 meq/kg. Anisidine value: maximum 15 meq/kg.

LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; ETA, eicosatetraenoic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; PA, palmitic acid; PO, palmitoleic acid; ST, stearic acid; OL, oleic acid; PUFA, polyunsaturated fatty acids; MUF, monounsaturated fatty acids; SAF, saturated fatty acids; FA, fatty acids.

according to Retra *et al.* (2008) in which the Synergi 4 μm MAX-RP 18A column was replaced by a Kinetex 2.6 μm C18 100A column (150 \times 3 mm; Phenomenex, Torrance, CA, USA). Internal standards were used for comparison.

Statistics

A Kolmogorov–Smirnov test was used to test for normality. Because most values were not normally distributed, nonparametric tests were used. Because no wash-out period was introduced, a Grizzle test for carryover effect was performed on all parameters tested. A *p*-value of <0.10 was considered significant, because the study design is a two-way crossover. A Mann–Whitney *U*-test has been performed to determine the differences between treatment groups regarding answers to the questions in the client-owned cats, followed by a Bonferroni correction for multiple comparisons. A Mann–Whitney *U*-test has been performed to determine the differences between fatty acid patterns of the cholesteryl fraction of plasma in the cats from the Department's colony.

A *p*-value of <0.05 was considered significant. A *p*-value of <0.10 was set as trend.

All statistical analyses were carried out using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

All studies described in this article were approved by the Committee of Experimental Committee Use of Animals of Utrecht University and performed with written consent from the owners of the investigated 24 privately owned cats and from the owner of the 20 cats from the Department's colony.

Results

From the 35 owners contacted, 24 owners approved. Three cats were lost to follow up for different reasons (1 owner did not want to participate anymore after 2 weeks, 1 cat did not like the taste of the oil 'A' from day 1 on, and 1 cat started vomiting when fed the oil 'B' on day 3), so we completed the study with 21 cats, 11 of which first received oil 'A' and 10 first received oil 'B'. The 20 client-owned cats included 2 Abyssinians, 16 Domestic shorthairs and 2 Persians. The age was 13.0 ± 2.9 years. All the cats were neutered, 11 cats were females and nine were males. Most cats accepted the oil well, two cats occasionally vomited, and three cats did not like the taste of the oils, but the owners managed to provide the oils orally to the cat by a syringe followed by a meal. After 2 days, one cat died and was censored. This cat was on oil 'A'. After the first 10-week period, four cats were lost to follow up, so the first period was completed with 20 cats and the second period was completed with 16 cats. None of the included cats were in need of any medication during the study period. The data of the 4 cats that did not complete the second period were censored.

The owner's perception of cat's behaviour and well-being, in cats on the oil 'A' compared with those on the oil 'B', has been evaluated: the results are shown in Table 3. After a 10-week period on oil 'B', the cats revealed higher activity level (*p* = 0.07), more walking up and down the stairs (*p* = 0.07), less stiffness during gait (*p* = 0.03), more interaction with the owner (*p* = 0.07) and higher jumps (*p* = 0.03) when compared with a 10-week period on oil 'A'. Compared with the study of Slingerland *et al.* (2011), we did not demonstrate a significant difference in defecation outside the litter box, because none of the cats in this study showed this clinical sign before, during or after the study periods.

There was improvement of behaviour during play with other pets, jumping on objects, moments of grooming and grooming time with the use of both

Table 3 Questionnaire results

Question	p-value Mann–Whitney
3. time grooming	0.564
4. defecation outside litter box	1,000
5. change in usage of litter box	1,000
6. play with other pets	0.381
7. change in activity level	0.070
8. differences in jumping on objects	0.564
9. change in jumping height	0.030
11. change in walking up the stairs	0.070
12. change in walking down the stairs	0.070
13. stiffness during gait	0.030
14. change in aggression	0.780
15. change in satisfaction	0.564
16. change in interaction with people	0.070
17. change in petting time	0.780
18. change in interaction with cats	1,000
19. changes in self-grooming moments	0.138
20. changes in self-grooming time	0.138

Questions are shown in Appendix 1. Mann–Whitney compared 10-week period on oil 'A' vs. oil 'B'. $p < 0.05$ is set as the level of significance and significant results are shown in boldface. $p < 0.10$ is set as trend and results are shown in italic.

oils, and did not reach statistical significance between groups ($p = 0.381$, $p = 0.564$, $p = 0.138$ and $p = 0.138$ respectively). No carryover effects were observed ($p > 0.10$).

The 20 cats of the Department's colony were all Domestic Shorthairs. The age was 6.5 ± 3.3 years. All cats were neutered, 10 cats were males and 10 cats were females.

Administration of oil 'B' was reflected in the fatty acid pattern of the cholesteryl fraction of plasma of the 10 cats by significant increase (before vs. after) in eicosatrienoic acid ($p = 0.041$), ETA ($p = 0.041$), EPA ($p = 0.041$), docosapentaenoic acid ($p = 0.049$) and docosahexaenoic acid ($p = 0.041$). The 10 cats on oil 'A' demonstrated no significant differences in the fatty acid pattern of the cholesteryl fraction of plasma after 10 weeks of supplementation. At the conclusion of the investigation, the key was disclosed, where oil 'A' appeared to be the control oil and oil 'B' the test oil (Table 2).

Discussion

The aim of this study was to investigate the effect of a 10-week period on omega-3 fatty acid supplementation (1.53 g/1000 kcal ME EPA and 0.31 g/1000 kcal ME DHA) on the owner's perception of behaviour and locomotion of cats with known naturally occurring OA, in comparison with a 10-week

period on corn oil supplementation. We used a 10-week period, as described in previous studies in human being long enough to measure the effects of long-chain omega-3 polyunsaturated fatty acids (Hansen *et al.*, 1998; Cao *et al.*, 2006; Masson *et al.*, 2007). A larger dose or a longer period of the fish oil supplementation could possibly have given an even better result. However, Blonk *et al.* (1990) showed that, in humans, doses over approximately 1.2 g DHA/day (given as fish oil) saturate the plasma DHA concentration and further increase in given DHA increases the plasma concentration only incrementally, suggesting a certain maximum. We did not introduce a wash-out period because this would extend the study period and may have led to decreased compliance of the cat owners. This might have caused carryover effects, although we could not demonstrate them in our results. Compared with studies in dogs by Lascelles *et al.* (2010) (i.e. 1.88 g EPA+DHA per 1000 kcal ME), Roush *et al.* (2010a,b) (i.e. 1.00 g DHA per 1000 kcal ME) and Fritsch *et al.* (2010) (i.e. 0.34, 0.90 and 1.35 g DHA per 1000 kcal ME), the DHA content in our study is at the lower end (i.e. 0.31 g DHA per 1000 kcal ME). The EPA content in our study was at the higher end (i.e. 1.53 g EPA per 1000 kcal ME) compared to the other studies (i.e. 1.88 g EPA and DHA per 1000 kcal ME; 0.41 g EPA per 1000 kcal ME; and 0.45, 1.1 and 1.6 g EPA per 1000 kcal ME). In this study, we aimed at a certain absolute amount of EPA and DHA originating from fish oil, rather than omega-3/omega-6 ratios, because recent studies in human demonstrated that the absolute amounts of EPA and DHA are more important (Zainal *et al.*, 2009). The test oil 'B' also contained 15 mg/mL ETA which may also contribute to clinical improvement as being a precursor of EPA and DHA or by its own effects as is demonstrated by Lascelles *et al.* (2010).

We demonstrated in this cross-over designed study in the healthy control cats a reflection of the supplemented fatty acids in the plasma fatty acid composition as is demonstrated by significant increased levels of eicosatrienoic acid ($p = 0.041$), ETA ($p = 0.041$), EPA ($p = 0.041$), docosapentaenoic acid ($p = 0.049$) and docosahexaenoic acid ($p = 0.041$) after supplementation. Two client-owned cats disliked the taste of the test oil and/or the control oil. Furthermore, two client-owned cats vomited occasionally while on oil supplementation; the data of these cats were not excluded because this occurred also before administration of the oils. In addition, one-third of older cats (>6 years of age) have a decreased capacity of fat digestion (Laflamme, 2005).

All these aspects may have contributed to a less than optimal uptake of the tested oils.

Zamprogno *et al.* (2010) showed that differences in clinical signs, found in their study, are indicative of effects on OA-associated pain. This was concluded because the clinical signs were significantly different between cats with low radiographic scores and no signs of pain on manipulation and cats with high radiographic scores and signs of pain on manipulation. We demonstrated significant differences in the same behavioural signs between cats on oil 'A' and oil 'B' as Zamprogno *et al.* (2010) demonstrated between cats with OA and healthy cats. Unfortunately, the groups in the study by Zamprogno (2010) were not age matched; the cats in the healthy group were younger, so age could also influence some parameters that are not necessarily related to OA; however, the study of Slingerland *et al.* (2011) demonstrated no correlation between OA-associated behavioural changes and age. Because the cats in our study had moderate OA, we could not demonstrate a correlation between OA score and grade of improvement on omega-3 fatty acid supplementation. The effects on OA-associated pain in cats may be subjective because of owner interpretation, placebo effect and better care effect; however, owners and researchers were blinded and the study had a cross-over design, thus correcting for interobserver differences. The improvement of behaviour during play with other pets, jumping on objects, moments of grooming and grooming time with the use of both oils may be attributed to placebo effect and/or better care effect in analogy with the findings of Dobenecker *et al.* (2002) in dogs.

Slingerland *et al.* (2011) concluded that clinical examination of the larger peripheral joints had the highest sensitivity and specificity in relation to radiographically confirmed OA. As is known from previous studies (Godfrey, 2005; Clarke and Bennett, 2006; Lascelles, 2010), there is no correlation between radiographic scores and clinical signs of OA in cats, also based on the fact that many cats resist being clinically investigated (Slingerland *et al.*, 2011). The use of accelerometry to measure the activity level as described by Lascelles *et al.* (2010) has several limitations and is not validated for use in cats. Force plate analysis has its limitations as well, because only the force of the limbs is measured and client-owned cats are difficult to be taught to walk on the force plate (Zumwalt *et al.*, 2006). Radiographs in this study were used as an aid to include only cats with OA in this study; radiographs were not repeated as no meaningful changes are to be

expected within the short period of the study (Roush *et al.*, 2010a).

The sample size was not determined by a power analysis before the start of the study. Because this study was a follow-up of a previous study (Slingerland *et al.*, 2011), from which we selected cats based on previously mentioned criteria, we knew precisely the presence and severity of OA in the joints of the appendicular skeleton in the investigated group of cats.

A questionnaire that has been used before to determine the effects of OA on cat's behaviour and locomotion was used in this study and proved to be able to demonstrate subtle changes. The effects of the tested supplement containing long-chain omega-3 fatty acids, such as a higher activity level ($p = 0.07$), more walking up and down the stairs ($p = 0.07$), less stiffness during gait ($p = 0.03$), more interaction with the owner ($p = 0.07$) and higher jumps ($p = 0.03$), were visible in a randomized, double-blinded, placebo-controlled, cross-over designed clinical trial.

Conclusion

A 10-week period on long-chain omega-3 fatty polyunsaturated fatty acid supplementation (0.05 g ETA, 1.53 g EPA and 0.31 g DHA per 1000 kcal ME) changes the owner's perception of some aspects of behaviour and locomotion of cats with known naturally occurring OA in comparison with a 10-week period on corn oil supplementation.

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Appendix 1: Questionnaire

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1. Is your cat an indoor cat, an outdoor cat or both?
 2. How long is your cat outside on average: 0 h, 0–3 h, more than 3 h?
 3. Did you notice differences in time that you are allowed to groom your cat? (More, a bit more, equal, a bit less, less)
 4. Does your cat urinate or poop outside the litter box? (Yes/no)
 5. Did you notice a change in usage of the litter box? (More, a bit more, equal, a bit less, less)
 6. Did you notice behavioural changes during play with other pets? (More, a bit more, equal, a bit less, less)
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Appendix 1: (Continued)

7. Did you notice a change in activity level? (More, a bit more, equal, a bit less, less)
8. Did you notice differences in jumping on the table/couch/windowsill, etc.? (More, a bit more, equal, a bit less, less)
9. Is there a change in jumping height? (More, a bit more, equal, a bit less, less)
10. Are the spots that the cat likes best easy accessible for the cat? (More, a bit more, equal, a bit less, less)
11. Did you notice a change in walking up the stairs? (More, a bit more, equal, a bit less, less)
12. Did you notice a change in walking down the stairs? (More, a bit more, equal, a bit less, less)
13. Do you notice the cat being stiffer during gait? (More, a bit more, equal, a bit less, less)
14. Did you notice a change in aggressive behaviour? (More, a bit more, equal, a bit less, less)
15. Did you notice a change in satisfaction of your cat? (More, a bit more, equal, a bit less, less)
16. Did you notice a change in greeting people? (More, a bit more, equal, a bit less, less)
17. Did you notice a change in the time the cat wants to be petted? (More, a bit more, equal, a bit less, less)
18. Did you notice a change in the interaction with other cats? (More, a bit more, equal, a bit less, less)
19. Did you notice a change in the number of moments on which the cat is grooming itself? (More, a bit more, equal, a bit less, less)
20. Did you notice a change in grooming time? (More, a bit more, equal, a bit less, less)
21. Has your cat ever been hit by a car? (Yes/no)
22. Has the cat been lame in the past? (Yes/no)
23. Does your cat show lameness at the moment? (Yes/no)

Questions 1, 2, 21 and 22 were asked once.

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