A Case-Control Study on the Intake of Polyunsaturated Fatty Acids and Chronic Renal Failure in Cats

Esther A. Plantinga

Anton C. Beynen

Department of Nutrition, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

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Abstract

Background: A case-control study was carried out to determine the association between chronic renal failure (CRF) and polyunsaturated fatty acid (PUFA) intake in cats.

Methods: Thirty-six cats, newly diagnosed with CRF, were matched to 35 controls. Plasma cholesteryl-ester (CE) fatty acid composition, in combination with a food intake questionnaire, was used to assess fatty acid intake.

Results: The cases had a significantly higher relative percentage of arachidonic acid (AA) and a significantly lower percentage of linoleic acid in plasma CEs than the control cats ($P < 0.01$). Linoleic acid intake was significantly lower in cases than in controls.

Conclusions: It is suggested that high AA intake might be a risk factor of CRF in cats.

Introduction

Chronic renal failure (CRF) is a common clinical problem in cats, affecting up to 30% of all animals above 15 years of age.1 Affected cats have a poor prognosis because the renal dysfunction frequently progresses to end-stage renal failure.2 The pathogenesis of CRF in general is not yet fully understood, but in people risk factors such as systemic hypertension, high dietary protein intake, and hyperlipidemia have been identified.3

The type of polyunsaturated fatty acids (PUFA) in the diet might be important in relation to chronic renal failure. Studies with dogs and rats show that supplementation with n-3 PUFA might delay and that supplementation with n-6 PUFA might accelerate the progression of CRF.4-6 The possible protective effect of polyunsaturated fatty acids might relate to the role of these fatty acids as precursors of eicosanoids. A predominance of n-6 fatty acids in the diet, linoleic acid being the most important n-6 fatty acid, will lead to a higher percentage of arachidonic acid (AA) in the cellular membranes, which can result in a proinflammatory status as a result of the production of prostaglandins of the 2 series and leukotrienes of the 4 series. As the relative dietary intake of n-3 fatty acids increases, more prostaglandins of the 3 series and leukotrienes of the 5 series are produced, these eicosanoids are being considered to reduce inflammation.7-9 The antiinflammatory effects of n-3 fatty acids can also be explained by another mechanism as the principles, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are competitive inhibitors of AA conversion into eicosanoids.10

One important difference between cats and other mammalian species is that cats are not or are only marginally capable of desaturating and elongating linoleic acid into

AA, because they lack the necessary enzymes.11 This means not only that AA in tissues reflects the intake of this fatty acid, but also that feeding of extra linoleic acid to cats will not lead to an increase in arachidonic acid and thus might not result in an increase of proinflammatory eicosanoids.

To find out whether PUFA play a role in the development of renal disease in cats, a case-control study was carried out. The intake of the major n-6 PUFA, linoleic acid, was assessed on the basis of food intake questionnaires. In addition, the percentage of linoleic acid in plasma cholesterol esters (CEs) was used as a biomarker of the intake of this fatty acid. A controlled dietary trial with cats has shown that an increased intake of linoleic acid is associated with an increase in its content in the plasma CEs.12 The intake of relevant n-3 fatty acids (?-linolenic acid, EPA, DHA) cannot be assessed using a food questionnaire, because the amount of these fatty acids in commercial cat foods is essentially unknown. Thus, the intake of these fatty acids was estimated using their concentrations in plasma CEs. In cats, an increased intake of the these n-3 fatty acids is reflected by an increase in their contents in plasma CEs.12

Materials and methods

Cats

From May to September 2001, 36 cats, newly diagnosed with CRF, and 35 healthy control animals were obtained with the help of various Dutch veterinary clinics and the Faculty of Veterinary Medicine, Utrecht University. Control cats were matched to the cases on the basis of age, breed, and gender. The characteristics of the cats are given in Table 1. The majority of the cats (93%) were European Shorthairs. The rest consisted of Siameses and Persian cats.

The diagnosis of CRF was made on the basis of clinical signs of CRF (polyuria/polydipsia, vomiting, weight loss, anorexia) and an elevation of the plasma urea and creatinine concentrations. Only cases with a plasma creatinine concentration above 175 ?mol/L were considered eligible for this study. CRF diagnosis was based on a single measurement of urea and creatinine, which might have led to some of the cats being wrongly categorized.13 The observed contrast between cases and controls could thus be smaller than the true contrast.

Food Intake Questionnaire

A questionnaire was used to estimate the dietary intake of the cats. Part of the questionnaire had questions about the brand and quantity of the commercial cat foods that were given to the cats. The remainder of the questionnaire consisted of 26 food items that are frequently used to feed cats, such as milk, fish, fresh meat, and cooked rice. The owners were asked to fill out whether they used each food item, and if they did, the amounts they used. The food intake data were converted into nutrients using cat food analysis data as provided by the various manufacturers, and standard food tables were used for the composition of the other foods.14 The intake of linoleic acid was expressed as percentage of total fatty acids or as percentage of dietary metabolizable energy. Total dietary fatty acids were calculated as 95% of total dietary crude fat. It was assumed that, on a weight basis, linoleic acid and crude fat provide identical amounts of metabolizable energy. To calculate the metabolizable energy content of the diets, the following conversion factors were used: 1 g crude protein = 17 kJ, 1 g carbohydrates (nitrogen-free extract) = 16 kJ, and 1 g crude fat = 37 kJ.

Blood Sampling and Analysis

Blood samples were taken after the cats had been fasted for 8 to 12 hours before sampling. Sampling was undertaken by jugular venipuncture into tubes containing lithium heparin. The blood was immediately centrifuged and the plasma harvested and stored at -20˚ C for further analysis. Plasma urea and creatinine were measured at the clinical laboratory of the Faculty of Veterinary Medicine in Utrecht, The Netherlands, using standard autoanalyzer techniques (Beckman).

Fatty acid analysis was performed by capillary gas chromatography using a flame ionization detector, a Chromopack column (Fused Silica, no. 7485, CP.FFAPCB 25 m ∞ 0.32 mm., Chromopack, Middelburg, The Netherlands) and H_2 as a carrier gas. Plasma total lipids were extracted according to the method of Wang and Frank.15 The

CEs were isolated with prepacked silica Sep-Pak columns (3 ml/500 mg, Varian Bond Elut 1210–2041, Allech Associates Inc., Deerfield, IL) using the method of Hamilton and Comai.16 The cholesteryl-ester methylation occurred as described by Metcalfe et al.17

Statistical Analysis

Pearson's correlation coefficients between the fatty acid intake estimated by the food intake questionnaires and the CE fatty acid measurements were computed with the help of the SPSS 9.0 for Windows statistical program (SPSS Inc., Chicago, IL). Furthermore, the statistical significance of the differences between the cases and controls were evaluated with an independent sample t test.

Results

The fatty acid compositions of plasma CE in the cases and the controls are expressed in Table 2. The cases had a significantly lower content of linoleic acid (C18:2 n-6) and higher level of AA (C20:4 n-6). Table 3 shows the nutrient intake of both cases and controls. The cases had a significantly lower intake of linoleic acid. There was a significant correlation between linoleic acid intake and the linoleic acid content of the plasma CE: the linear correlation coefficient was 0.376 for the cases and 0.362 for the controls ($P < 0.05$). The relationship between the linoleic acid content of the diet and that of the plasma CE of controls and cases combined is shown in Figure 1.

Discussion

The purpose of this study was to determine whether there exists an association between PUFA intake and the risk of CRF in cats. The CE fatty acid composition was used to assess the dietary fatty acid intake. However, CE fatty acid composition only reflects the previous intake of fatty acids for a period up to 1 month.18,19 A food intake questionnaire was also used as an estimate of the fatty acid intake. However, the PUFA content of most commercial cat foods is unknown, with the exception of linoleic acid. Figure 1 shows a statistically significant correlation between linoleic acid in CEs and linoleic acid intake, which supports our controlled feeding trial12 and indicates that the CE composition can be used as a biomarker for fatty acid intake.

As based on the CE data, the results indicate that the cases had a significantly higher intake of AA compared with the control cats. This might imply that high intake of AA is a risk factor in the development of CRF in cats. Furthermore, the cases had a significantly lower intake of linoleic acid, suggesting that low linoleic acid intake could also be a risk factor. However, based on studies conducted with other mammalian species, it was expected that low linoleic acid intake would be protective rather than a risk factor.4–6 Cats have a limited capacity to convert linoleic acid into AA, the main precursor of proinflammatory eicosanoids in the body. Thus, in cats the source of AA is the diet. A high AA intake could lead to a higher level of this fatty acid in membranes and consequently to a relatively higher proinflammatory status.7–9

The significance of the low linoleic acid intake in the cases is unknown. High levels of linoleic acid typically occur in plant oils, whereas AA only occurs in feedstuffs of animal origin. Possibly, in the diet of the cats, the contents of linoleic acid in AA were inversely related.

The cases and controls had similar relative percentages of both ?-linolenic acid (C18:3 n-3) and EPA in plasma CEs, which points at similar intakes of each of these n-3 PUFA. Thus, the results of this study specifically indicate that a high AA intake might be a risk factor in the development of renal failure. Further controlled research in this field is necessary to clarify the role of the various PUFA in CRF in cats.

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TABLE 1. Characteristics of Cases and Control Cats

Characteristics	Control cats (n = 35)	Cases (n = 36)
Age (y)	10.17 ± 4.30	10.64 ± 4.74
Weight (kg)	4.73 ± 1.48	4.31 ± 1.32
Ratio of male:female	0.94:1.00	1.12:1.00
Plasma urea (mmol/L)	7.8 ± 2.2	18.1 ± 13.3
Plasma creatinine (?mol/L)	125.7 ± 20.6	233.5 ± 96.5

Values are means ± standard deviation.

Table 2. Plasma Cholesteryl Ester Fatty Acid Composition

Fatty acid	Control cats (n = 35) g/100 g fatty acids	Cases (n = 36) g/100 g fatty acids
Saturated		
12:0	0.00 ± 0.00	0.00 ± 0.00
14:0	0.00 ± 0.00	0.00 ± 0.00
16:0	7.41 ± 1.12	8.02 ± 2.34
18:0	1.61 ± 0.62	2.18 ± 1.70
Monounsaturated		
18:1 (n-9)	17.87± 3.10	18.43 ± 3.47
Polyunsaturated		
18:2 (n-6)	58.43 ± 6.53*	52.50± 8.69
18:3 (n-3)	0.26 ± 0.37	0.36± 0.43
18:3 (n-6)	0.00 ± 0.00	0.00 ± 0.00
20:4 (n-6)	7.60 ± 2.93†	10.75 ± 3.98
20:5 (n-3)	2.34 ± 3.13	2.73 ± 3.22
Total (n-3)	3.78 ± 3.56	4.06 ± 3.71

Values are means ± standard deviation.

*P <0.01; †P <0.001.

Table 3. Dietary Nutrient Intake of Cases and Control Cats

Nutrient	Controls (n = 35)	Cases (n = 36)
Total energy MJ	1.15 ± 0.2	1.13 ± 0.3
Protein g/day	26.1 ± 6.2	26.2 ± 6.7
Percent of energy	38.6 ± 6.4	39.4 ± 7.1
Carbohydrates g/day	18.3 ± 7.0	18.4 ± 6.2
Percent of energy	25.5 ± 8.2	26.1 ± 8.4
Fat g/day	11.1 ± 2.9	10.5 ± 2.7
Percent of energy	35.9 ± 7.2	34.5 ± 6.9
Linoleic acid g/day	1.90 ± 0.4*	1.54 ± 0.6
g/100 g FA	18.0 ± 3.8*	15.4 ± 4.3

Values are means ± standard deviation.

*P <0.05.

FIGURE 1. Scatterplot of the linoleic acid (C18:2 n-6) content of plasma CE in control cats (n) and cases (→) as a function of the dietary linoleic acid content. The linear regression equation is y = 0.73∞ + 41.31 for the controls (n = 35), and y = 0.64∞ + 46.88 for the cases (n = 36).