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The Influence of Long-chain Polyunsaturated Fatty Acids on Total Lipid Fatty Acid Composition of a Canine Mastocytoma Cell Line

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Summary

Cutaneous mast cells are considered as key immune effectors in the pathogenesis of canine atopic dermatitis (CAD). These cells release immediate-phase and late-phase mediators of inflammation. Dietary fatty acids are incorporated in cellular membranes and seem to influence mediator production and release. A dietary intervention with n6- and n3-fatty acids is thought to alleviate clinical symptoms in atopic dogs. The purpose of this study was to examine the effects of n6- and n3-fatty acids on the fatty acid composition of canine mastocytoma cells (C2) as a possible model for CAD. The C2 was cultured in a basic medium called Dulbecco's modified Eagle's medium (DEH) or with additional 14 μM linoleate (C18:2n6, DEH–LA), γ-linolenate (C18:3n6, DEH–GLA), arachidonate (C20:4n6, DEH–AA), α-linolenate (C18:3n3, DEH–LnA), eicosapentaenoate (C20:5n3, DEH–EPA) or docosahexaenoate (C22:6n3, DEH–DHA). Cell growth was examined for 11 days in all media. Cell growth increased from days 1 to 8 and decreased thereafter in all media conditions. The fatty acids supplied did not influence cell growth. The cells were harvested after 8 days for fatty acid analysis. The fatty acid composition was determined by gas chromatography after extraction and trans-esterification of the lipids. The added fatty acids increased the concentration of these fatty acids in C2 differently (LA 4.9-fold, GLA 6.9-fold, AA 6-fold, LNA 9.3-fold, EPA 6.5-fold and DHA 8.4-fold). Furthermore, elongated and Δ6-desaturated products of the corresponding fatty acids were significantly elevated. However, Δ5-desaturated products were not measurable. These results let us assume that C2 has no measurable activity of the Δ5-desaturase. In case the low activity of Δ5-desaturase is one of the mechanisms underlying the pathogenesis of CAD, C2 seems to be an adequate model for investigations in CAD.

Introduction

An increased incidence of atopic dermatitis in humans was observed during the last 30 years in industrialized nations. In dogs, a comparable syndrome called canine atopic dermatitis (CAD) appeared, which settles 8–10% of the skin diseases in this species (Scott et al., 1995). As causes for both diseases, immunological and environmental factors like keeping or feeding conditions next to genetic defects are discussed (Hillier and Griffin, 2001).

Clinical symptoms of the CAD were described for the first time by Halliwell and Schwartzman (1971). Today CAD is defined as a genetically predisposed, chronic allergic disease of the skin, characterized by a hypersensitivity to environmental antigens (Olivry et al., 2001a). Hillier and Griffin (2001) assumed that increased in-house keeping and prophylactic measures like vaccinations and anti-parasitical treatments possibly enhance the incidence of atopic diseases. The main symptom of CAD is strong itching, besides leaking, scouring and biting. In addition, skin lesions like erythema and papules appear on head, body and limbs (Willemse, 1991). Mediators released from mast cells especially seem to promote the clinical characteristics of atopic diseases. Therefore, cutaneous mast cells play a key role in the pathogenetic events of CAD (Olivry et al., 1997).

Dietary fatty acids of the n6- and n3-family are incorporated into cellular membranes directly or after metabolism and influence membrane properties (Grammatikos et al., 1994). In mast cells, altered membrane lipids affect the exocytosis of preformed mediators (e.g. histamine) and the release of newly synthesized lipid mediators, e.g. eicosanoids (Gueck et al., 2003, 2004), which are formed out of membrane phospholipids.

In atopic dermatitis of man, an interference of the fatty acid metabolism because of decreased desaturase activity was described earlier (Manku et al., 1982). However, in CAD this mechanism is disputed. In the plasma of atopic dogs Saevik et al. (2002) found slightly lower concentrations of linoleate (LA) and increased concentrations of the long-chain n3-fatty acids eicosapentaenoate (EPA) and docosapentaenoate (DPA), however, the LA metabolites di-homo-γ-linoleate (DGLA) and arachidonate (AA) were unchanged. However, Taugbol et al. (1998) observed an increased concentration of DGLA and a lower concentration of C22:4n6 in plasma of diseased dogs. The therapeutical effects of n3- and n6-fatty acids in CAD are not completely understood. After dispensation of a diet rich in zinc and LA to healthy older dogs, Marsh et al. (2000) observed a positive effect on skin and hair. Rees et al. (2001) also showed, that healthy dogs receiving a fatty acid-rich diet, had an

improved state of hair and skin. In a prior study with healthy dogs, the authors already had assessed, that a linoleate (LNA)-rich diet led to an increase of plasma n3-fatty acids, especially EPA (Bauer et al., 1998). Some authors assume an interference of the transformation from LA to GLA (Scott et al., 1997), others negate that (Saevik et al., 2002). Hansen et al. (1998) showed an increase of DHA and EPA in the plasma of dogs on dietary fish oil. Bauer et al. (2002) also assessed, that the variations of fatty acids in plasma and granulocytes depend upon the diet. An influence of dietary fat on the fatty acid composition of the skin was described by Campbell and Dorn (1992) as well as Campbell et al. (1995). In order to produce these alterations, the diet should be applied over 12 weeks at least (Olivry et al., 2001b).

The connection between the ratio of dietary n3- to n6-fatty acids, the plasma concentrations of these fatty acids and the release of inflammatory mediators was examined by Wander et al. (1997) feeding a fish oil-rich diet to healthy dogs. These animals showed a significant increase in plasma concentrations of the n3-fatty acids EPA and DHA as well as a clear decline of the pro-inflammatory prostaglandin E_2 (PGE2). Another study showed a significant increase of less inflammatory PGE3 in monocytes of healthy dogs after supplementation of n3-fatty acids (Kearns et al., 1999). The production of the leucotrienes B4 or B5 also depends on the fatty acid composition of the diet (Vaughn et al., 1994; Byrne et al., 2000). Scott et al. (1997) made a dietary intervention with n6- and n3-fatty acids (5:1) in 18 dogs suffering with CAD. Clinical symptoms improved in eight dogs (responders). Considering the fatty acid concentrations in both groups (Scott et al., 1997) the authors assume a defective fatty acid metabolism in responders (Δ5-desaturase) and in non-responders (Δ6- and Δ5-desaturase). This could explain the different responses of the dogs to fatty acid supplementation. In contrast to that, Taugbol et al. (1998) and Saevik et al. (2002) reported, that atopic dogs in comparison with healthy ones had no differences in the fatty acid composition of subcutaneous fat and plasma.

Subject of the experiments described here is the influence of different fatty acids on cellular fatty acid pattern of the canine mastocytoma cell line C2. Part the data presented in this work was published earlier. However, in these publications, they made for the explanation of the results of the other parameters measured and were not compared against each other. We made a synopsis of the data of the fatty acid analysis to demonstrate the adequacy of our cell culture system as an *in vitro* model of CAD.

Materials and Methods

All chemicals and reagents were obtained from Sigma-Aldrich (Taufkirchen, Germany) unless noted otherwise. The characteristics of the cell line used (C2) are comparable with other mast cell preparations like human lung mast cells, peritoneal or the bone marrow-derived mast cells of rats (DeVinney and Gold, 1990). C2 were cultured in Dulbecco's modified Eagle's medium/HAM's F12 (DEH) containing 0.14 μM LA (Biochrome KG, Berlin, Germany), supplemented with 2 mM glutamine, 25 mM HEPES (pH 7.4), 1.6 mM histidine, 100 U/ml penicillin, 100 μg/ml streptomycin and 5% FCS. The LNA (0.14 μM; Biotrend, Köln, Germany) was added in ethanol (0.1% v/v). The fatty acid concentration in DEH was 0.94 μM C14:0. 0.49 μM C15:0, 10.5 μM C16:0, 0.44 μM C16:4n1, 5.3 μM C18:0, 8.8 μM C18:1n9, 2.5 μM C18:1n7; 3.1 μM C18:2n6; 0.25 μM C18:3n3, 0.9 μM C20:3n6, 3.3 μM C20:4n6, 0.4 μM C20:5n3, 0.2 μM C22:0, 1.8 μM C22:5n3, 1.8 μM C22:6n3, 0.17 μM C24:0, 0.21 μM C24:1n9; total fatty acids 41.2 μM.

For investigation of the influence of the different fatty acids, C2 were cultured in DEH or in DEH with 14 μM of the following fatty acids using ethanol (0.1% v/v) as a vehicle. The final concentration of ethanol was 0.2%.

Linoleate n6	DEH–LA
α-Linolenate n3	DEH–LNA
Arachidonate n6	DEH–AA
Eicosapentaenoate n3	DEH–EPA
γ-Linolenate n6	DEH–GLA
Docosahexaenoate n3	DEH–DHA

The C2 were seeded at a density of 0.5×10^6 cells/ml (75 cm^2 flasks) in the corresponding medium and incubated at 37°C in a humidified atmosphere with 5% CO_2. On day 4 cells were fed again with the corresponding medium. After 8 days replicate aliquots were harvested for measurement of fatty acid content.

For measurement of C2 fatty acid composition, cells were washed three times in phosphate-buffered saline (PBS) and 10^7 cells were dried under nitrogen and stored at −25°C until analysis. The cellular lipids were prepared according to Sonnichsen and Muller (1999). Cell pellets were trans-esterified with 500 μl methanolic HCl, 250 μl *n*-hexane and 500 μl internal standard (IS; 0.8 mg L-phosphatidylcholine-C17:0 in 1 ml methanol with 0.2% butylhydroxytoluol as antioxidant). After cooling-off, 500 μl *n*-hexane and 1 ml Aqua bidest were added. The upper hexane phase was evaporated with nitrogen. The fatty acid methylesters (FAME) were taken up in 60 μl *n*-hexane. An aliquot of 1 μl was injected on-column on a Varian CP 3800 gas chromatograph (Varian, Darmstadt, Germany) equipped with an Omegawax™ 320 column (Supelco, Bellefonte, PA, USA), 0.32-mm internal diameter, 30-m length. The column temperature was 200°C.

The analysis of the chromatogram took place by means of the program STAR 5.0 (Varian) using the IS as a reference peak. To identify and evaluate the single fatty acids, the FIM-FAME-6 mix (Matreya, Pleasant Gap, PA, USA) was used containing 32 FAME in a concentration of 1 mg/ml heptane. Furthermore three single substances (C20:4n6, C20:5n3 and C22:5n3) were used as standards and the RF-values determined. C20:4n6 was used as a pure substance to determine the sequence of C20:4n6 and C20:3n3 unambiguously. For the identification of further FAME, the Omegawax Column test mix (Supelco) was used including the FAME C16:2n4, C18:2n4, C20:4n3, C16:1n4, C18:3n4, C23:0, C18:1n7, C18:4n3 and C24:0. This is a qualitative standard only, so the RF-values based on recognizable variations of the calibrated RF-values were diverted. Moreover C22:4n6 was identified by gas chromatography–mass spectrometry at the Institute for Analytical Chemistry of the Faculty for Chemistry and Mineralogy of the University of Leipzig under the following conditions:

Column	SPB 1701 (30 m × 0.25-mm diameter × 0.25 μm film; Supelco)
Injection	280°C, 1 μl sample, split 1:20
Carrier gas	Hydrogen, total flow 50 ml/min
Head pressure	70 kPa
Oven	Start 150°C, rate 3°C/min, end 250°C
Detection	Mass spectrometer, solvent delay 5 min, scan-mode 35–450 amu, ionization 70 eV

Because of unavoidable contaminations of reagents and solvents with some fatty acids (C10:0, C14:0, C16:1n7, C18:0, C22:2n6, C24:1n9) a blank was subtracted. Data are shown as mean values ± SD. Fatty acid data of C2 cells were subjected to analysis of variance. Group mean values of the different media were tested for differences by Student's *t*-test in case of normal distribution. When the values were not normally distributed, the Mann–Whitney rank sum test was used. Both tests were carried out by means of the program SigmaStat 2.0 of JANDEL.

Results

Supplementation of LA led to a significantly higher insertion of LA and its metabolites (C18:3n6, C20:2n6 and C20:3n6, sum of the n6-FA). An increase of AA could not be assessed. The supplementation with LNA caused a significant increase of all n3-FS measured with the exception of DHA. The strongest increase was reached in C20:4n3. The n6- to n3-ratio changed significantly corresponding to the supplement, in LA from 1.3 to 3.6 and in LNA from 1.3 to 0.4 (Table 1). The other fatty acids of C2 remained unaffected. The supplement of AA to the culture medium led to an increase of the n6 fatty acids C20:3, C20:4 and C22:4 in C2. The sum of the n6-fatty acids and the n6- to n3-ratio also changed (Table 2). Further variations of the fatty acid composition of the C2 were not observed. The EPA supplementation changed the n3-fatty acids of C2. EPA itself and the elongation and desaturation products increased (Table 3). Further variations were not observed. The supplementation of GLA to the culture medium led to a rise of the n6-fatty acid C20:4, further major variations of the fatty acid pattern were not observed (Table 4). The addition of DHA to the medium caused a significant increase of the n3-fatty acids C20:5n3, C22:5n3 and the sum of the n3-fatty acids (Table 5). By that a decline of the n6- to n3-ratio in comparison with C2 cultivated in DEH was observed. The

Table 1. Fatty acids in nmol/10^7 cells of C2 grown in basal (DEH), linoleate (DEH–LA) or α-linolenate medium (DEH–LNA)

Fatty acid	DEH ($n = 7$)	DEH–LA ($n = 7$)	DEH–LNA ($n = 8$)	*P*-value
C18:3n3	0.3 ± 0.07	0.2 ± 0.05	2.8 ± 1.0	$P < 0.001$
C18:4n3	0.1 ± 0.07	0.1 ± 0.03	0.3 ± 0.07	$P < 0.001$
C20:3n3	0.2 ± 0.08	0.1 ± 0.05	1.0 ± 0.4	$P < 0.001$
C20:4n3	3.9 ± 1.3	3.4 ± 0.7	50.0 ± 3.5	$P < 0.001$
C20:5n3	7.6 ± 3.5	6.8 ± 1.1	9.0 ± 1.9	$P < 0.01$
C22:5n3	3.7 ± 1.7	3.2 ± 0.7	4.4 ± 1.3	$P < 0.01$
C22:6n3	16.2 ± 6.7	14.6 ± 2.9	13.7 ± 2.5	NS
Sum of n3	32 ± 13.0	29 ± 5.1	81 ± 19	$P < 0.001$
C18:2n6	9.4 ± 3.3	46.1 ± 8.1	9.0 ± 1.6	$P < 0.001$
C18:3n6	1.2 ± 0.5	3.5 ± 0.9	0.8 ± 0.2	$P < 0.001$
C20:2n6	0.2 ± 0.1	0.7 ± 0.2	0.1 ± 0.03	$P < 0.001$
C20:3n6	12.0 ± 5.0	37.6 ± 3.5	9.6 ± 1.8	$P < 0.001$
C20:4n6	16.3 ± 5.9	14.7 ± 2.6	13.8 ± 2.5	NS
C22:4n6	0.6 ± 0.3	0.5 ± 0.1	0.4 ± 0.1	NS
Sum of n6	41 ± 15	104 ± 23	34 ± 6.2	$P < 0.001$

Mean ± SD; *P*-values give the significance level of differences between basal and supplemented media; NS, not significant.

Table 2. Fatty acids in nmol/10^7 cells of C2 grown in basal (DEH) or arachidonate medium (DEH-AA)

Fatty acid	DEH ($n = 6$)	DEH–AA ($n = 7$)	*P*-value
C20:3n6	10.6 ± 3.1	16.8 ± 2.3	$P = 0.002$
C20:4n6	13.8 ± 2.2	82.7 ± 12.9	$P = 0.001$
C22:4n6	0.4 ± 0.06	4.9 ± 1.5	$P = 0.001$
Sum of n6	38.1 ± 9.1	117 ± 17.4	$P = 0.001$
Ratio n6/n3	1.4 ± 0.1	4.1 ± 0.3	$P = 0.001$

Mean ± SD; *P*-values give the significance level of differences between basal and supplemented medium.

Table 3. Fatty acids in nmol/10^7 cells of C2 grown in basal (DEH) or eicosapentaenoate medium (DEH–EPA)

Fatty acid	DEH ($n = 6$)	DEH–EPA ($n = 6$)	*P*-value
C20:5n3	5.3 ± 1.7	34.3 ± 5.2	$P = 0.002$
C22:5n3	4.0 ± 1.1	14.4 ± 2.5	$P < 0.001$
C22:6n3	13.3 ± 3.6	12.6 ± 2.2	$P = 0.7$ NS
Sum of n3	26.4 ± 6.6	65.3 ± 9.6	$P < 0.001$
Ratio n6/n3	1.4 ± 0.1	0.5 ± 0.03	$P = 0.002$

Mean ± SD; *P*-values give the significance level of differences between basal and supplemented medium; NS, not significant.

Table 4. The *n*6 fatty acids in nmol/10^7 cells of C2 grown in basal (DEH) or γ-linolenate medium (DEH–GLA)

Fatty acid	DEH ($n = 8$)	DEH–GLA ($n = 8$)	*P*-value
C18:3n6	1.4 ± 0.3	10.0 ± 1.2	$P < 0.001$
C20:2n6	0.2 ± 0.1	0.3 ± 0.1	$P = 0.01$
C20:3n6	13.9 ± 2.2	95.8 ± 5.8	$P < 0.001$
C20:4n6	16.2 ± 3.1	19.4 ± 1.3	$P = 0.03$
C22:2n6	1.4 ± 0.5	1.5 ± 0.6	$P = 0.66$ NS
C22:4n6	0.7 ± 0.1	1.0 ± 0.1	$P < 0.001$
Sum of n6	45.3 ± 8.3	141 ± 8.2	$P < 0.001$
Ratio n6/n3	2.0 ± 0.1	5.3 ± 0.2	$P < 0.001$

Mean ± SD; *P*-values give the significance level of differences between basal and supplemented medium; NS, not significant.

Table 5. Fatty acids in nmol/10^7 cells of C2 grown in basal (DEH) or docosahexaenoate medium (DEH–DHA)

Fatty acid	DEH ($n = 8$)	DEH–DHA ($n = 7$)	*P*-value
C20:5n3	3.8 ± 0.8	8.4 ± 0.6	$P < 0.001$
C22:5n3	4.3 ± 1.1	7.5 ± 1.3	$P < 0.001$
C22:6n3	10.3 ± 1.9	87.0 ± 13.6	$P < 0.001$
Sum of n3	22.2 ± 4.2	107 ± 40.7	$P < 0.001$
Ratio n6/n3	2.0 ± 0.1	0.4 ± 0.03	$P < 0.001$

Mean ± SD; *P*-values give the significance level of differences between basal and supplemented medium; NS, not significant.

Table 6. Incorporation of the added fatty acids into the mastocytoma cell line C2

Fatty acid added	Increase of the fatty acid added	Increase of the respective fatty acid family
n6		
LA	4.9	2.5
AA	6.0	3.1
GLA	6.9	3.1
n3		
LNA	9.3	2.5
EPA	6.5	2.2
DHA	8.4	4.8

growth in DEH–DHA led to no further significant variations of the fatty acid composition of C2.

The factors for incorporation of the fatty acids tested into C2 are shown in Table 6.

Besides the changes of n6- and n3-fatty acid composition through supplementation of fatty acids, we noticed some smaller changes. The LA, LNA and EPA led to a significant decrease of C14:1n5 (1.8-fold). With the addition of DHA and GLA a small, but significant increase of C18:3n4 (1,3- and 1.4-fold respectively) occurred. With GLA a significant increase of C24:0 (1.4-fold) and C24:1n9 (1.5-fold) in comparison with the basal medium was measurable.

Discussion

Fatty acids play an important role in the pathogenesis of CAD in different ways. On one hand they have an influence on the condition of the skin; on the other hand the polyunsaturated fatty acids (PUFA) influence the immune system (Olivry et al., 2001b). A few studies in dogs showed good success in the therapy of the CAD with diets rich in PUFA. Obviously the n6- to n3-ratio has an influence on the therapy success (Bond and Lloyd, 1992; Scarff and Lloyd, 1992; Logas and Kunkle, 1994; Sture and Lloyd, 1995; Scott et al., 1997; Harvey, 1999). Furthermore, it has been shown in studies with healthy dogs that fatty acids influence pro-inflammatory lipid mediators (Vaughn et al., 1994, Kearns et al., 1999; Byrne et al., 2000).

Intracellular metabolism of fatty acids includes incorporation into complex lipids, β-oxidation, desaturation, elongation, retroconversion and formation of lipid mediators. In our *in vitro* study the total fatty acids concentration in DEH medium was 41.2 μM and in the supplemented media 55.2 μM. The amount of the fatty acids added are comparable with specifications found in the literature (Hrelia et al., 1999; Gonzalez et al., 2000).

The highest increase of the fatty acid added is observed in LNA, followed by DHA. The lowest increase was found in LA. According to Rosenthal (1987), fatty acid-specific mechanisms for the uptake of the fatty acids into cellular phospholipids exist. The author states, that AA and other C20 fatty acids are esterified more than C18 fatty acids into phospholipids of thrombocytes and endothelial cells. This preferred installation of C20 fatty acids was proven for AA in thrombocytes, lymphocytes and endothelial cells and is based on the existence of an AA-specific acyl-CoA-synthetase (Whatley et al., 1990). A higher incorporation of AA in comparison with LA was found in rats also (Whelan et al., 1992). The authors attribute this to a high AA-specificity of the enzymes of phospholipids biosynthesis (acyl-CoA-synthase, acyl-CoA:1-acyl-sn-glycero-3-phosphocholin-acyltransferase). In C2, no preferred incorporation of the AA was detected.

The high incorporation of DHA we found in C2 might occur by a selective incorporation of this fatty acid, as described for mastocytoma cells of rats into the ether phospholipids. These phospholipids represent a reservoir of PUFA for the cyclooxygenase and lipoxygenase (Masuzawa et al., 1986).

A different metabolism of the fatty acids in β-oxidation could be a possible explanation for the varying revenue of the fatty acids added. The C18 fatty acids are more oxidized than C20, and C24 fatty acids are better than these (Hiltunen et al., 1986; Reubsaet et al., 1989). The higher incorporation of LNA in comparison with LA can be explained by the preferred β-oxidation of LA over LNA (Hiltunen et al., 1986). The DHA seems to be metabolized only very slightly through β-oxidation (Osmundsen and Bjornstad, 1985; Madsen et al., 1998), so the high incorporation of this fatty acid in C2 is explainable. In a study of Madsen et al. (1998) the metabolism of DHA and EPA in cultivated hepatocytes was examined in more detail. It was assessed, that EPA goes into β-oxidation much more than DHA (EPA 16% and DHA 1%). Only 27% of the added EPA was recovered in phospholipids, in DHA it was 59%. This corresponds to our results in C2.

The supplementation with DHA caused a significant increase of DPA (1.7-fold) and EPA (2.2-fold) in C2. DHA probably arises from retroconversion and saturation of EPA, which was elongated subsequently to DPA. Moreover, in the AA-supplemented medium an increased concentration of DGLA (1.6-fold) was assessed. A contamination of the added fatty acid substrates by other fat acids was excluded by gas chromatographic analysis of the substrates. A retroconversion and saturation of DHA–EPA was assessed earlier by Rosenthal et al. (1991) in endothelial cells and fibroblasts. These cells were able to form AA from C22:4n6 through retroconversion.

Scott et al. (1997) stated that in CAD interferences of the fatty acid metabolism are of pathogenetic meaning. In diseased animals they observed only a slight transformation from DGLA to AA, referring to a low activity of the Δ5-desaturase. Animals not improving after dietary intervention, showed a low transformation of LA to GLA in comparison with animals treated successfully. Therefore, the authors suggested a diminished Δ6-desaturase activity of these dogs in addition. In C2, the Δ5-desaturase product AA was unchanged after addition of the precursor LA and weakly elevated after the addition of GLA. Therefore the Δ5-desaturase activity for n6-fatty acids seems to be absent.

In clinical studies the ratio of LA to its metabolites was used to estimate the activity of Δ6- and Δ5-desaturases, in which an increased ratio points to a decreased desaturase activity. However, in spite of the expected low desaturase activities in CAD, Saevik et al. (2002) found an increased ratio (16.1) of LA to its metabolites in normal dogs compared with atopic dogs (15.1).

With the supplementation of LNA, a minimal but significant increase of EPA and its elongation product C22:5n3 (DPA) was observed. So the Δ5-desaturase of C2 seems to act on n3-fatty acids with a low activity. Different substrate specificity of the Δ5-desaturase for n6- and n3-fatty acids were already assessed in cultivated monocytes (Anel et al., 1990).

In C2, the enrichment of the culture medium with LA led to a 3-fold higher concentration of GLA pointing to a reasonable activity of the Δ6-desaturase. The transformation of LNA to its Δ6-desaturation product resulted in a 3-fold increase of C18:4n3. So the Δ6-desaturase of C2 seems to metabolize LA and LNA approximately to the same extent. However, a preference of LNA over LA by the Δ6-desaturase, as described in endothelial cells (Rosenthal and Whitehurst, 1983; Simopoulos, 1991; Sardesai, 1992) can not be excluded.

The results argue for a considerable Δ6-desaturase activity of C2 with a preference of the n3- over the n6-fatty acids. However, the Δ5-desaturase has a diminished activity in C2. So the fatty acid metabolism of C2 is similar to atopic dogs (Scott et al., 1997). Further investigations could ascertain the

reason for a reduced Δ5-desaturase activity in atopic dogs and C2. To summarize, the use of C2 as a model for CAD to study fatty acid metabolism and production of lipid mediators seems to be justified.

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