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The erythrocyte membrane lipidome profile in healthy dogs and changes in dogs with diabetes mellitus and chronic enteropathy

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Introduction

Analysis of red blood cells membrane lipidome represents a powerful diagnostic tool in humans for assessing the quantity and quality of fatty acids and for the follow-up of the membrane remodeling under physiological and pathological conditions [1].

However a systematic study to evaluate membrane homeostasis in dogs has not yet been established.

The aim of this study was to compare RBC membrane lipidome profiles of healthy dogs (HD) with dogs newly diagnosed with diabetes mellitus (DM) and dogs with chronic signs (i.e. >3 weeks) of enteropathy) (CE). All dogs receiving dietary ω3 supplementation were excluded from the study.

Methods

Blood samples were collected from:

- HD (n=17): 5 males (1 neutered) and 12 females (3 sterilized) with median age: 38 months (2-98)
- DM dogs (n=7): 3 males and 4 females (2 sterilized), with median age: 144 months (96-158)
- CE dogs (n=6): all 6 males (2 neutered), with median age: 81 months (12-126)

The RBC membranes were isolated from EDTA-treated blood and a cluster made of 10 saturated [SFA (palmitic, stearic)], monounsaturated [MUFA (palmitoleic; oleic, vaccenic)] and polyunsaturated [PUFA (linoleic, dihomo-gamma-linolenic, arachidonic, EPA, DHA)] fatty acids was determined by Gas-Chromatography.

Results

Table 1. Fatty acid methylesters (FAME) obtained from erythrocyte's membranes phospholipids from blood samples collected from Healthy dogs, dogs with Diabetes Mellitus (DM) and dogs with Chronic Enteropathy (CE).[a]

FAME	HD	DM	CE	Dm vs CE
	n=17[b]	n=7[b] (p value)[c]	n=6[b] (p value)[d]	p value[e]
16:0.	15,61 ± 1,95	15,84 ± 4,56 (0,9907)	8,55 ± 3,00 (< 0,0001)	0,0138
9c-16:1	0,26 ± 0,08	0,70 ± 0,31 (< 0,0001)	0,16 ± 0,10 (0,0162)	0,0014
18:0.	20,09 ± 2,18	20,27 ± 3,70 (0,3378)	24,12 ± 2,53 (0,0012)	0,1005
9c-18:1	9,60 ± 0,97	11,30 ± 1,30 (0,0037)	9,45 ± 1,17 (0,7584)	0,0336
11c-18:1	2,17 ± 0,29	2,26 ± 0,47 (0,2495)	1,14 ± 1,01 (0,0008)	0,0214
LA	14,18 ± 2,08	14,43 ± 2,44 (0,8818)	14,18 ± 3,48 (0,9990)	0,9314
DGLA	1,25 ± 0,44	1,25 ± 0,10 (0,9205)	1,62 ± 0,81 (0,1721)	0,3137
ARA	35,16 ± 2,75	30,93 ± 5,36 (0,0080)	38,76 ± 5,71 (0,0516)	0,0237
EPA	0,68 ± 0,48	1,22 ± 0,87 (0,0208)	0,96 ± 1,11 (0,3643)	0,5082
DHA	1,00 ± 0,66	1,79 ± 0,48 (0,0135)	1,05 ± 0,54 (0,8612)	0,0346

[a] the values are reported as relative percentage (%rel) of the total fatty acid peak areas detected in the GC analysis, corresponding to >98% of the total peaks present in the chromatogram.
[b] The values are given as mean ± SD and n is the number of samples per group.
[c] Significance between HD and DM
[d] Significance between HD and CE
[e] Significance between DM and CE

Table 2. Total fatty acids methylesters (FAME) per family and membrane homeostasis index obtained from the data of Table 1.[a] Healthy dogs, dogs with Diabetes Mellitus (DM) and dogs with Chronic Enteropathy (CE)

FAME	HD	DM	CE	DM vs CE
	n=17[a]	n=7[a] (p value)[b]	n=6[a] (p value)[c]	p value[d]
SFA[e]	35,69 ± 2,23	36,11 ± 4,93 (0,3520)	32,67 ± 2,73 (0,1733)	0,1075
MUFA[f]	12,04 ± 1,09	14,26 ± 1,46 (0,0010)	10,75 ± 1,35 (0,0293)	0,0021
PUFA ω3[g]	1,68 ± 0,92	3,02 ± 1,13 (0,0045)	2,01 ± 1,00 (0,4645)	0,0956
PUFA ω6[h]	50,59 ± 2,47	46,61 ± 5,22 (0,0061)	54,57 ± 2,63 (0,0031)	0,0041
PUFA	52,27 ± 2,59	49,63 ± 4,60 (0,0396)	56,58 ± 3,02 (0,0030)	0,0070
SFA/MUFA	2,99 ± 0,34	2,57 ± 0,56 (0,2123)	3,08 ± 0,49 (0,4394)	0,1733
SFA/PUFA	0,69 ± 0,08	0,74 ± 0,18 (0,1525)	0,58 ± 0,08 (0,0099)	0,0435
ω6/ω3	40,65 ± 2.69	17,37 ± 6,09 (0,0266)	34,65 ± 2,64 (0,5978)	0,0604
PUFA balance ω3/(ω3+ω6)[i]	3,19 ± 1,69	6,22 ± 2,82 (0,0021)	3,52 ± 1,68 (0,6872)	0,0484
9c-16:1/16:0 (D9)	0,02 ± 0,01	0,05 ± 0,02 (< 0,0001)	0,02 ± 0,01 (0,5781)	0,0265
9c-18:1/18:0 (D9)	0,48 ± 0,07	0,58 ± 0,14 (0,1512)	0,40 ± 0,07 (0,0195)	0,0246
20:3 ω6/18:2 ω6 (Flo+D6)	0,09 ± 0,03	0,09 ± 0,02 (0,9671)	0,11 ± 0,05 (0,1841)	0,2902
20:4 ω6/20:3 ω6 (D5)	32,18 ± 14,18	25,01 ± 5,32 (0,0080)	30,62 ± 19,23 (0,0516)	0,0237
UI - Unsaturation Index[j]	194,17 ± 10,85	187,46 ± 16,49 (0,1379)	210,13 ± 17,67 (0,0158)	0,0297
PI - Peroxidation Index[k]	169,67 ± 12,90	162,68 ± 15,51 (0,1702)	186,90 ± 21,83 (0,0292)	0,0370

[a]The values are obtained from the values of Table 1 and given as mean ± SD and n is the number of samples per group.
[e]SFA (Saturated Fatty Acids) = %16:0 + %18:0.
[f]MUFA (Monounsaturated Fatty Acids) = %9c-16:1 + %9c-18:1 + %11c-18:1.
[g]PUFA (Polyunsaturated Fatty Acids) ω-3 = %EPA + %DHA.
[h]PUFA (Polyunsaturated Fatty Acids) ω-6 = %LA + %DGLA + %ARA.
[i]PUFA balance = [(%EPA + %DHA) / total PUFA] × 100.
[j]UI (Unsaturation Index) =(%MUFA × 1) + (%LA × 2) + (%DGLA × 3) + (%ARA × 4) + (%EPA × 5) + (%DHA × 6).
[k]PI (Peroxidation Index) =(%MUFA × 0.025) + (%LA × 1) + (%DGLA × 2) + (%ARA × 4) + (%EPA × 6) + (%DHA × 8).

The main results can be summarized as follows:

(i) In HD the SFA, MUFA and ω6 levels were close to each other, while the ω3 values showed a wider variability (mean 1.68%; SD 0.92%).

(ii) The CE dogs had decreased levels of palmitic acid (p<0.01) and higher stearic acid (p<0.01) whereas DM did not show significant changes in these values, compared to HD.

(iii) The MUFA levels were interestingly diverse in the two health conditions: higher in DM (p<0.01) and lower in CE (p<0.05) compared to HD. In particular, CE dogs had lower levels of palmitoleic (p<0.05) and vaccenic acids (p<0.01), while DM dogs showed an increased content of palmitoleic (p<0.01) and oleic acids (p<0.01).

(iv) As regards of ω6-PUFA, only in DM arachidonic acid levels differed if compared to the HD: lower levels were observed (p<0.01).

(v) ω3-PUFA levels were increased only in DM dogs in comparison to HD, both for EPA (p<0.05) and DHA

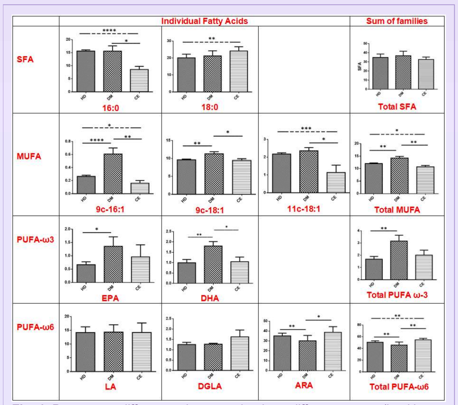

Fig. 1. Percentage differences between the three different groups (healthy dogs, dogs with Diabetes Mellitus-DM and dogs with Chronic Enteropathy-CE) for each type of fatty acid in the red blood cell membranes.
The values are given as mean ± SD.
Each member of the fatty acid family is given in a row, the last column being the sum of the corresponding fatty acid family.
Values significantly different when compared to with each other: (*) p < 0.05, (**) p < 0.01, (***) p < 0.001, (****) p < 0.0001;
For specific values see Table 2 and Table 3.

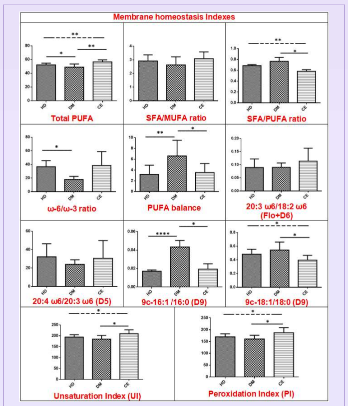

Fig. 2. Percentage differences between the membrane homeostasis indexes in the three different groups (healthy dogs, dogs with Diabetes Mellitus-DM and dogs with Chronic Enteropathy-CE).
Upper row: total PUFA, ω-6/ω-3 ratio and PUFA balance;
Middle row: SFA/MUFA, SFA/PUFA and EPA/ARA ratios;
Lower row: unsaturation and peroxidation indexes.
The values are given as mean ± SD.
Values significantly different when compared to with each other: (*) p < 0.05, (**) p < 0.01, (***) p < 0.001, (****) p < 0.0001; For specific values see Table 3.

Conclusions

These preliminary data have limitations as for the sample size, the lack of data in geriatric healthy dogs and the lack of retrospective diagnosis of disorders associated with the chronic enteropathy.
The variability of ω3 values found in erythrocyte membranes of healthy dogs, can be probably due to the individual dietary variations.

However, it can be preliminarily observed that:

- The RBC fatty acid-based membrane lipidome profiles in DM and CE dogs compared to HD showed different trends connected to metabolic transformations along the fatty acid pathways
- SFA-MUFA pathway shows significant involvement in canine diabetes mellitus, with a higher palmitic-palmitoleic and palmitic-oleic transformations due to an accelerated delta-9 desaturase enzymatic activity.
- On the other hand, CE dogs showed increased levels of stearic, and decreased palmitoleic and vaccenic acids suggesting an activation of elongation pathway, leading to profound changes of membrane fluidity and permeability properties.

In conclusion, erythrocyte membrane lipidome of dogs may be successfully applied in veterinary medicine, providing important information of different profiles under normal and pathological conditions.

Reference:
1. Ferreri C. Chatgilialoglu C. Role of fatty acid-based functional lipidomics in the development of molecular diagnostic tools. Expert Rev Mol Diagn, 12(7):767–780, 2012