CORRELATED RESPONSE IN PLASMA FATTY ACIDS PROFILE IN RABBITS SELECTED FOR ENVIRONMENTAL SENSITIVITY

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ABSTRACT

Two rabbit lines were divergently selected for increasing and decreasing environmental variability of litter size at birth. Decreasing litter size variability generates more resilient females with less sensitivity to stress and diseases, being a useful selection criterion to improve environmental sensitivity. Fatty acids modulate the immune cell function. Saturated fatty acids (SFAs) have an inhibitory effect on lymphocyte proliferation, monounsaturated fatty acids (MUFAs) exerts a protective and anti-inflammatory effect on macrophages, and n-3 polyunsaturated fatty acids (PUFAs) affect response of lymphocytes by mean of IL-1, IL-2, IL-6, TNF as well as prostaglandin E2 and leukotriene B4. Plasma fatty acids profile was assessed in 10 females from the homogeneous line and 12 females from the heterogeneous line from the 12th generation of selection. The homogeneous line showed higher levels of SFAs (+3.98 ng/ml P=0.90 for C14:0; +2.30 ng/ml P=0.98 for C15:0; +54 ng/ml P=0.90 for C16:0 and +29 ng/ml P=0.90 for C18:0) and MUFAs (+12.0 ng/ml P=0.98 for C16:1 and +53 ng/ml P=0.90 for C18:1n9c) than the heterogeneous line. Besides, this line had also a higher amount of n-3 PUFAs (+2.18 ng/ml P=0.90 for C18:3n3 and +1.91 ng/ml P=0.90 for C20:5n3) and a lower amount of n-6 PUFAs (-3.66 ng/ml P=0.96 for C20:3n6 and -0.28 ng/ml P=0.90 for C20:4n6) than the heterogeneous one. In conclusion, selection for environmental sensitivity shows a correlated response in the plasma fatty acids profile.

Key words: Environmental Sensitivity, MUFAs, n-3 PUFAs, n-6 PUFAs, Selection for Resilience.

INTRODUCTION

A divergent selection experiment for environmental sensitivity has been performed successfully in rabbits at the Universidad Miguel Hernández de Elche (Blasco *et al*., 2017). This trait was measured as environmental variability of litter size at birth. Selection to reduce litter size variability can be a useful way to improve doe's resilience, which is defined as the capacity of the does to be minimally affected by disturbances, or its cope to rapidly return to the state before exposure to a perturbation (Colditz and Hine, 2016). In this regard, the line selected to reduce litter size variability showed less sensitivity to stress and diseases than the heterogeneous one (Argente *et al*., 2019). Different susceptibility to illness between lines would be related to a different immune response. Fatty acids are known to play diverse roles in immune cells, modulating the outcome of immune responses; for example, they have been found to regulate phagocytosis, reactive oxygen species production, cytokine production and leukocyte migration, also interfering with antigen production by macrophages (Yaqoob and Calder, 2007). Therefore, the divergent lines for environmental sensitivity can show differences in the plasma fatty acids levels.

The objective of this study was to evaluate the correlated response to selection for litter size variability in the fatty acids profile in plasma, in order to identify specific biomarkers for environmental sensitivity.

MATERIALS AND METHODS

Animals and experimental design

Rabbits used in this study come from the 12th generation of a divergent selection experiment for environmental sensitivity. The selection was based on the phenotypic variance of the litter size of each doe, after correcting the litter size for both year-season and parity-lactation status (Blasco *et al*., 2017). All animals were reared in the farm of the Universidad Miguel Hernández de Elche (Spain). The rabbits were fed a standard commercial diet (Nutricun Elite Gra®, de Heus Nutrición Animal, La Coruña, Spain). Food and water were provided ad libitum. Does were housed in individual cages (37.5 × 33 × 90 cm^3) under a constant photoperiod of 16 h continuous light: 8 h continuous darkness, and with controlled ventilation throughout the experiment. Does were first mated at 18 weeks of age.

One blood sample from the ear vein was taken at second mating in 10 non-lactating females of the homogeneous line and 12 non-lactating females of the heterogeneous line for litter size variability. The second mating is a key and representative point in the doe's reproductive life. Samples were collected into tubes containing K3-EDTA. All samples were immediately centrifuged at 4000 rpm for 15 min, and plasma was stored at -80 ºC until required for lipid analyses. All experimental procedures involving animals were approved by the Miguel Hernández University of Elche Research Ethics Committee (Reference number 2017/VSC/PEA/00212), in accordance with Council Directives 98/58/EC and 2010/63/EU.

Chemical Analyses

A 200 µl of plasma sample was taken in a scrum cap glass tube. The plasma sample was processed following the method by Shirai *et al.* (2005). In the last step, after isooctane was evaporated under a stream of nitrogen gas, 200 µl of hexane was added, and the sample was injected into the GC system. The fatty acids were measured using a gas chromatograph (GC-17A, Shimadzu, Kyoto, Japan) with Flame ionization detector (FID), equipped with a CP-Sil 88 for FAME capillary column (100 m x 0.25 mm x 0.36 mm; 0.20 µm film thickness; Agilent technologies, Madrid, Spain). The carrier gas was helium (flow 1.2 ml/min) with a split injection of 1:1. The temperature profiles were as follows: initial temperature, 45 ºC during 4 min; with a first rate, 13 ºC/min until 175 ºC; and a second rate, 4 ºC/min until 215 ºC held for 30 min; injector temperature, 250 ºC; and detector temperature, 260 ºC. The fatty acids were identified by comparing the retention times with FAME MIX standard (CRM47885, Supelco, Spain).

Statistical Analysis

The model used to analyse plasma fatty acids profile included the effects of line (homogeneous and heterogeneous line) and month (March, April and May). All analyses were performed using Bayesian methodology. Bounded uniform priors were used for all effects. Residuals were a priori normally distributed with mean **0** and variance $\mathbf{I}\sigma_e^2$. The priors for the variance were also bounded uniform. Features of the marginal posterior distributions for all unknowns were estimated using Gibbs sampling. Inferences were derived from the marginal posterior distributions. Mean and the highest posterior density region at 95% (HPD95%) were provided (Blasco, 2017). When $|D_{H-L}|>0$, we consider there to be enough evidence of a difference between the heterogeneous (H) and homogeneous (L) lines if the probability (P) of $|D_{H-L}|$ is greater than 0.90. We used a chain of 60,000 samples, with a burn-in of 10,000. Only one out of every 10 samples was saved for inferences.

RESULTS AND DISCUSSION

Compared with the heterogeneous line (Table 1), the homogeneous one showed a greater amount of saturated fatty acids (SFAs) (+3.98 ng/ml P=0.90 for C14:0; +2.30 ng/ml P=0.98 for C15:0; +54 ng/ml P=0.90 for C16:0 and +29 ng/ml P=0.90 for C18:0) and monounsaturated fatty acids (MUFAs) (+12.0 ng/ml P=0.98 for C16:1 and +53 ng/ml P=0.90 for C18:1n9c). In relation to polyunsaturated fatty acids (PUFAs), the homogeneous line exhibited also a higher amount of n-3 PUFAs (+2.18 ng/ml P=0.90 for C18:3n3; +1.91 ng/ml P=0.90 for C20:5n3). Regarding to n-6 PUFAs, the homogeneous line had a higher amount of C22:2n6 (+0.55 ng/ml P=0.96) and lower amount of C20:3n6 (-3.66 ng/ml P=0.96) and C20:4n6 (-0.28 ng/ml P=0.90 for C20:4n6) than the heterogeneous one.

Table 1. Plasma fatty acids profile at mating in the heterogeneous and homogeneous lines for litter size variability.

	Heterogeneous line (H)	Homogeneous line (L)			
Traits (ng/ml)	Mean	Mean	D_{H-L}	HPD95%	P
C8:0	7.74	4.09	3.65	-4.38, 11.9	0.81
C10:0	0.87	0.16	0.71	-0.59, 2.05	0.85
C11:0	0.07	0.35	-0.28	-0.79, 0.24	0.87
C12:0	1.70	1.29	0.41	-1.06, 1.93	0.71
C14:0	12.4	16.4	-3.98	-11.16, 2.44	0.90
C15:0	2.34	4.64	-2.30	-4.43, -0.20	0.98
C16:0	244	298	-54	-169, 45	0.90
C17:0	5.25	6.46	-1.21	-3.65, 1.31	0.84
C18:0	121	150	-29	-73 17	0.90
C21:0	0.80	0.05	0.75	-0.51, 2.05	0.84
C22:0	1.07	0.10	0.97	-0.70, 2.40	0.79
C24:0	1.59	0.45	1.14	-0.70, 0.15	0.85
SFAs	400	483	-83	-249, 68	0.90
C14:1	1.76	1.55	0.21	-1.72, 2.10	0.59
C15:1	2.35	2.47	-0.12	-1.73, 1.54	0.55
C16:1	13.7	25.7	-12.0	-23.7, 0.1	0.98
C17:1	1.79	2.49	-0.70	-2.10, 0.72	0.85
C18:1n9c	176	229	-53	-135, 32	0.90
C20:1	1.16	0.94	0.22	-0.93, 1.41	0.65
MUFAs	197	262	-65	-159, 31	0.92
C18:2n6c	271	297	-26	-145, 95	0.67
C18:3n6	0.67	3.48	-2.81	-5.69, 1.17	0.84
C18:3n3	5.57	7.75	-2.18	-6.19, 0.98	0.90
C20:2	2.31	2.28	0.02	-1.46, 1.55	0.51
C20:3n6	4.44	0.76	3.66	-0.34, 7.80	0.96
C20:3n3	0.87	0.90	-0.03	-1.48, 1.47	0.51
C20:4n6	0.52	0.24	0.28	-0.12, 0.82	0.90
C22:2n6	0.36	0.91	-0.55	-1.16, 0.07	0.96
C20:5n3	1.59	3.50	-1.91	-7.59, 1.62	0.90
C22:6n3	1.77	0.36	1.41	-1.81, 3.71	0.81
n-3 PUFAs	9.87	11.97	-2.10	-9.59, 1.75	0.90
PUFAs	286	317	-31	-150, 98	0.67

D_{H-L}: median of the difference between heterogeneous and homogeneous lines. HPD95%: highest posterior density region at 95%. P: probability of the difference being > 0 when $D_{H-L} > 0$, and probability of the difference being < 0 when $D_{H-L} < 0$. SFAs: saturated fatty acids. MUFAs: monounsaturated fatty acids. PUFAs: Polyunsaturated fatty acids.

Fatty acids participate in the regulation and activation of the innate and the adaptive immune response by the production and synthesis of pro-inflammatory cytokines as well as prostaglandins, leukotrienes, thromboxanes and lipoxins (Pompéia *et al*., 2000 in humans). For example, it has been found a negative effect of palmitic acid (C16:0) and stearic acid (C18:0) on lymphocyte proliferation and a

protective effect of palmitoleate acid (C16:1) and cis-9 oleic acid (C18:1n9c) (Gorjão *et al*., 2007; Carrillo *et al*., 2012; Chan *et al*., 2015 in humans), whereas α linolenic acid (ALA, C18:3n3), eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) have been exhibited an anti-inflammatory effect by means of decreasing in production of IL-1, IL-2, IL-6, TNF as well as prostaglandin E2 and leukotriene B4 (Katayama *et al*., 2003 and Kelley *et al*., 1999 in humans; Rodríguez *et al*., 2019 in rabbits). C-reactive protein is an acute phase protein secreted by hepatocytes during inflammation, in response to the pro-inflammatory cytokines (Pepys and Hirschfield, 2003), being a useful biomarker in inflammatory processes. In a previous study, Argente *et al*. (2019) detected lower concentration of lymphocytes and C-reactive protein in the homogeneous line; therefore, this line seems to be less sensitivity to diseases and inflammatory processes than heterogeneous one. These findings agree to larger amount of palmitic acid, stearic acid, α linolenic acid, and eicosapentaenoic acid in the homogenous line.

CONCLUSIONS

Selection for environmental sensitivity shows a correlated response in the plasma fatty acids profile, corroborating lesser inflammatory response and lesser sensitivity to diseases in the homogeneous line.

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