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POSTER

Effect of sex and age on fatty acid composition of Iberian swine foetuses exposed to maternal malnutrition

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SUMMARY

Intrauterine growth restriction (IUGR) is the consequence of inadequate placental supply of oxygen and/or nutrients during prenatal development. The adaptive response of the offspring is time-dependent, but there is also increasing evidence of a strong modulation by sex. The present study aimed to determine time-related changes and sex-related differences in fatty acid composition at non-adipose tissues involved in metabolism regulation during the development of IUGR fetuses. For that, we analyzed the liver and muscle (*longissimus dorsi*) fatty acids composition of IUGRs foetuses (obtained as consequence of maternal malnutrition) throughout pregnancy (Day 70 *vs.* 90) and sex-related effects (male *vs.* female). The final distribution of sampled foetuses was: 33 foetuses at Day 70 of pregnancy (13 females and 20 males), and 23 foetuses at Day 90, (10 females and 13 males). Both in liver and muscle a higher concentration of saturated fatty acids (SFA; P<0.005) was found in females when compared to male foetuses. A lower concentration of monounsaturated fatty acids (MUFA; P<0.05) and C18:1/C18:0 and MUFA/SFA ratios (P<0.05 and P<0.005 respectively) in liver neutral fatty acids was observed for females *vs.* males. Moreover, the fatty acid composition changed with pregnancy time. Actually, both muscle and liver fatty acids changed showing lower concentration of total PUFA and n-6 PUFA (P<0.05 and P<0.0001 respectively) and higher MUFA/SFA ratio (P<0.05 and P<0.0005 respectively) at day 70 of gestation when compared to those values of polar fatty acids at day 90, independently of sex. In conclusion, the present study shows that prenatal developmental lipid traits are primarily determined by foetal sex but also by the progression of stress caused by maternal malnutrition.

Efectos del sexo y edad gestacional sobre la composición de ácidos grasos de fetos de cerdo Ibérico expuestos a malnutrición materna

Palabras clave adicionales

Crecimiento intrauterino retardado (CIUR).
Desarrollo prenatal.

RESUMEN

El crecimiento intrauterino retardado (CIUR) es debido a un inadecuado aporte de oxígeno y/o nutrientes durante el desarrollo prenatal. La respuesta adaptativa de la descendencia es tiempo dependiente, pero además están aumentando las evidencias de que el sexo fetal ejerce un fuerte efecto modulador sobre dicha respuesta. El presente estudio pretendió determinar los efectos de la edad gestacional y el sexo fetal sobre la composición de ácidos grasos en tejidos no adiposos involucrados en la regulación metabólica durante el desarrollo de fetos con CIUR. Para ello, se analizó la composición de ácidos grasos en hígado y músculo (*longissimus dorsi*) de fetos con CIUR (obtenidos como consecuencia de una malnutrición materna) durante la gestación (día 70 *vs.* 90), así como el efecto del sexo (machos *vs.* hembras). La distribución final de los fetos muestreados fue de: 33 fetos a día 70 de gestación (13 hembras y 20 machos), y 23 fetos a día 90 (10 hembras y 13 machos). Tanto en hígado como en músculo, una mayor concentración de ácidos grasos saturados (SFA; P<0.005) fue encontrada en fetos hembra con respecto a fetos macho. Fue observada una menor concentración de ácidos grasos mono-insaturados (MUFA; P<0.05) y de los ratios C18:1/C18:0 y MUFA/SFA (P<0.05 y P<0.005 respectivamente) en la fracción de lípidos neutros en hembras con respecto a machos. Además, la composición de ácidos grasos cambia con la edad gestacional. De hecho, la composición lipídica de músculo e hígado cambió, mostrando menor concentración de PUFA y PUFA n-6 (P<0.05 y P<0.0005 respectivamente) y mayor ratio MUFA/SFA (P<0.05 y P<0.0005 respectivamente) a día 70 de gestación que a día 90 en la fracción de lípidos polares, independientemente del sexo. En conclusión, el presente estudio muestra que las características lipídicas durante el desarrollo prenatal son determinadas por el sexo fetal pero también por la progresión del estrés causado por la malnutrición materna.

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INTRODUCTION

Inadequate placental supply of oxygen and/or nutrients is the cause of the process named *intrauterine growth restriction* (IUGR), which is highly prevalent in swine either by inadequate maternal nutrition or, mainly, by placental insufficiency (Ashworth et al. 2001; Wu et al. 2006) and constitutes one of the main

factors limiting farm profitability (Gonzalez-Bulnes et al. 2016). Numerous studies have been focused on the effects of prenatal nutritional programming on developmental traits and phenotype of the individual (reviewed by Gonzalez-Bulnes et al. 2012). The results obtained from these studies indicated that consequences of prenatal nutritional programming are influenced both by the timing, degree and duration of the nutritional challenge and by the adaptive response of the mother and the conceptus. Nevertheless, there is also increasing evidence of a strong modulation by sex on adaptive response of the offspring (Moritz et al. 2010; Aiken & Ozanne 2013).

Previous studies of our group regarding IUGR processes in Iberian pigs have shown that maternal malnutrition compromises adequate developmental and metabolic traits of the foetuses, causing growth-retardation and adaptive changes for enhancing offspring viability (Gonzalez-Bulnes et al. 2016). Hence, the present study aimed to determine time-related changes and sex-related differences in fatty acid composition at non-adipose tissues involved in metabolism regulation (muscle and liver) during the development of foetuses exposed to maternal undernutrition.

MATERIAL AND METHODS

A total of 56 foetuses were obtained from 9 multiparous purebred Iberian sows, which were fed with a standard grain-based food diet. The amount of food was adjusted to fulfill individual daily maintenance requirements from the start of the experimental period to Day 35 of pregnancy, based on data from the British

Table I. Effects of age and sex on foetal fatty-acid composition of *longissimus dorsi* muscle. Mean values for neutral and polar lipids in the *longissimus dorsi* muscle 70 and 90 Days of gestation female (F) and male (M) foetuses exposed to maternal malnutrition (Efectos del sexo y edad en la composición en acido grasos fetales del musculo *longissimus dorsi.* Valores medios para lípidos neutrales e polares en el musculo *longissimus dorsi* en día 70 y 90 de gestación de fetos hembra (F) y macho (M) expuestos a malnutrición materna.)

	70		90		MSE[a]	*P*-value				
						Same age F *vs.* M		Same Sex 70 *vs.* 90		Age*Sex
Foetal neutral fatty-acid composition. Fatty acid (g/ 100g total fatty acid)	F	M	F	M		70	90	F	M	
SFA[1]	46.45	43.66	45.55	44.81	0.407	0.029	0.345	0.566	0.166	0.063
MUFA[2]	39.14	40.51	42.12	42.35	0.394	0.231	0.842	0.027	0.054	0.016
PUFA[3]	15.01	15.83	12.34	12.84	0.492	0.568	0.307	0.085	0.021	0.027
UI[4]	0.98	1.01	0.87	0.88	0.021	0.546	0.37	0.123	0.008	0.021
PUFAn-3[5]	3.27	2.48	1.67	1.67	0.232	0.3	0.686	0.143	0.001	0.057
PUFAn-6[6]	2.59	3.05	2.37	2.49	0.108	0.173	0.249	0.558	0.037	0.084
∑n-6/∑n-3	0.98	1.24	1.45	1.54	0.044	0.006	0.517	0.003	<0.001	<0.001
C18:1/C18:0	2.08	2.24	2.43	2.62	0.05	0.158	0.388	0.009	0.009	0.001
MUFA/SFA	0.85	0.93	0.93	0.95	0.013	0.046	0.618	0.135	0.488	0.052
Foetal polar fatty-acid composition. Fatty acid (g/ 100g total fatty acid)										
SFA[1]	37.94	37.63	38.39	37.87	0.692	0.447	0.261	0.216	0.568	0.467
MUFA[2]	37.12	37.63	36.15	36.03	0.681	0.256	0.427	0.002	0.002	0.904
PUFA[3]	24.95	24.74	25.47	26.09	0.477	0.401	0.215	0.219	0.002	0.195
UI[4]	1.39	1.39	1.37	1.39	0.025	0.879	0.257	0.24	0.815	0.655
PUFAn-3[5]	4.15	4.15	4.27	4.11	0.083	0.993	0.268	0.329	0.725	0.568
PUFAn-6[6]	20.23	19.96	20.56	21.42	0.399	0.283	0.118	0.448	0.001	0.123
∑n-6/∑n-3	4.9	4.84	4.84	5.24	0.064	0.647	0.081	0.769	0.016	0.085
C18:1/C18:0	0.45	0.43	0.48	0.47	0.004	0.168	0.908	0.001	0.001	<0.001
MUFA/SFA	0.98	1	0.94	0.95	0.008	0.298	0.733	0.008	0.031	0.013

[a]MSE = Mean square error
[1]SFA = Saturated fatty acids; Includes: C14:0, C16:0, C17:0 and C18:0
[2]MUFA = Monounsaturated fatty acids; Includes: C16:1n-9, C16:1n-7, C17:1. C18:1n-9, C18:1n-7 and C22:1n-9
[3]PUFA = Polyunsaturated fatty acids: Includes: C18:2n-6, C18:3n-3, C20:3n-9, C20:3n-6, C20:4n-6, C20:5n-3, C22:4n-6, C22:5n-6, C22:5n-3 and C22:6n-3
[4]UI = Conversion index
[5]Includes: C18.2n-6, C20:4n-6 and C22:4n-6
[6]Includes: C18:3n-3, C20:5n-3 and C22:6n-3

Society of Animal Science. At Day 35 of pregnancy, all the sows were weighed and the amount of food offered to each sow was adjusted to fulfill 50% of their daily maintenance requirements. Such diet has been previously found to affect foetal development and to induce lower body weight in the offspring (Gonzalez-Bulnes et al. 2012). The sows were weighed again at Days 70 (group 70; n=5 sows) or 90 (group 90; n=4 sows) of pregnancy, when foetuses were obtained and sampled in brief. Samples from *longissimus dorsi* muscle and intramuscular fat and fat liver was extracted using the *Ball-mill* P*rocedure* described by Segura et al (Segura, J & Lopez-Bote, C J, 2014). Fatty acids (FA) of the total lipid extracts were identified and quantified by gas-chromatography after methylation as previously described (Lopez-Bote et al. 1997; Olivares et al. 2009). Assessment of neutral and polar lipid fractions was also performed by gas-chromatography as described by Ruiz et al. (Ruiz et al. 2004). Data were analyzed using SPSS Version 22.0. Effects of time of pregnancy and sex on fatty-acid composition of the conceptuses were assessed by analysis of variance (two-way ANOVA), and results were expressed as mean ± MSE and statistical significance was accepted from P<0.05.

RESULTS

The fatty acid composition of the foetal *longissimus dorsi* muscle, regarding both, the neutral and polar fractions, was affected by both gestational age and sex (**Table I** and **Table II**). At Day 70 of pregnancy, the neutral fraction of female foetuses showed more total SFA

Table II. Effects of age and sex on foetal fatty-acid composition of liver. Mean values for neutral and polar lipids in the liver 70 and 90 Days of gestation female (F) and male (M) foetuses exposed to maternal malnutrition (Efectos del sexo y edad en la composición en acido grasos del hígado de fetos. Valores medios para lípidos neutrales e polares en el hígado en día 70 y 90 de gestación de fetos hembra (F) y macho (M) expuestos a malnutrición materna)**.**

	70		90		MSE[a]	P-value				
						Same age F vs. M		Same Sex 70 vs. 90		Age*Sex
Foetal neutral fatty-acid composition. Fatty acid (g/ 100g total fatty acid)	F	M	F	M		70	90	F	M	
SFA[1]	47.23	43.28	43.89	42.85	0.467	0.009	0.029	0.009	0.726	0.002
MUFA[2]	35.84	38.63	37.07	40.83	0.564	0.04	0.107	0.445	0.138	0.014
PUFA[3]	16.93	18.09	19.04	16.32	0.469	0.314	0.215	0.157	0.164	0.234
UI[4]	1	1.08	1.08	1.01	0.016	0.087	0.253	0.093	0.128	0.145
PUFAn-3[5]	1.75	1.99	1.8	1.7	0.063	0.155	0.793	0.697	0.138	0.267
PUFAn-6[6]	14.89	15.81	16.88	14.3	0.422	0.362	0.204	0.151	0.179	0.220
∑n-6/∑n-3	8.58	8.23	9.41	8.77	0.199	0.463	0.453	0.108	0.357	0.225
C18:1/C18:0	1.12	1.47	1.57	1.93	0.063	0.011	0.103	0.003	0.006	<0.001
MUFA/SFA	0.77	0.91	0.85	0.95	0.019	0.007	0.067	0.096	0.321	0.002
Foetal polar fatty-acid composition. Fatty acid (g/ 100g total fatty acid)										
SFA[1]	45.66	44.72	45.92	45.87	0.305	0.321	0.873	0.797	0.157	0.405
MUFA[2]	31.27	30.76	27.13	26.97	0.461	0.64	0.822	0.002	0.002	<0.001
PUFA[3]	23.07	24.51	26.95	27.16	0.376	0.148	0.576	0.001	0.003	<0.001
UI[4]	1.21	1.27	1.32	1.33	0.012	0.108	0.462	0.007	0.047	0.002
PUFAn-3[5]	2.76	2.96	2.95	2.9	0.072	0.366	0.779	0.373	0.772	0.752
PUFAn-6[6]	20.01	21.25	23.61	23.86	0.332	0.134	0.492	0.001	0.001	<0.001
∑n-6/∑n-3	7.4	7.62	8.19	8.3	0.221	0.749	0.714	0.126	0.33	0.446
C18:1/C18:0	1.23	1.22	1.02	0.98	0.033	0.85	0.623	0.026	0.005	0.004
MUFA/SFA	0.69	0.69	0.59	0.59	0.014	0.922	0.895	0.019	0.007	0.515

[a]MSE = Mean square error
[1]SFA = Saturated fatty acids; Includes: C14:0, C16:0, C17:0 and C18:0
[2]MUFA = Monounsaturated fatty acids; Includes: C16:1n-9, C16:1n-7, C17:1. C18:1n-9, C18:1n-7 and C22:1n-9
[3]PUFA = Polyunsaturated fatty acids: Includes: C18:2n-6, C18:3n-3, C20:3n-9, C20:3n-6, C20:4n-6, C20:5n-3, C22:4n-6, C22:5n-6, C22:5n-3 and C22:6n-3
[4]UI = Conversion index
[5]Includes: C18.2n-6, C20:4n-6 and C22:4n-6
[6]Includes: C18:3n-3, C20:5n-3 and C22:6n-3

(P<0.05), but lower ∑n-6/∑n-3 and MUFA/SFA ratios (P<0.01 and P<0.05, respectively) than males. Regarding the changes in fatty acids along gestation, both sexes showed an increased content of ∑n-6/∑n-3 and C18:1/C18:0 ratios (P<0.01 and P<0.005, respectively, for females and P<0.001 and P<0.01, respectively, for males). Female foetuses also showed enlarged total amount of MUFA (P<0.05) with gestational time. Male foetuses showed a decrease throughout pregnancy of the total PUFA content (P<0.05), and in the conversion index (UI= the average number of double bonds per 100 fatty acids; P<0.01). Polar fatty acid composition also changed with pregnancy time with total content of PUFA, and specifically n-6 PUFA (P<0.05) and the C18:1/C18:0 ratio (P<0.0001) increasing significantly along gestation independently from sex. Males showed an increase in ∑n-6/∑n-3 ratio (P<0.05) with gestational age.

The analysis of the neutral fatty acid composition of liver, similarly to that of intramuscular fat, showed significant age- and sex-related effects. In both male and female foetuses, the C18:1/C18:0 ratio (P<0.005) increased with time of pregnancy. On the other hand, female foetuses evidenced a decrease in the relative amount of total SFA with pregnancy age (P<0.01). Moreover, female foetuses, independently of age, showed higher total SFA content (P<0.05 for both), and lower MUFA, C18:1/C18:0 (P<0.01 for both) and MUFA/SFA ratios (P<0.005) than males. The analysis of polar lipids evidenced no significant sex-related effect. Total content of PUFA and specifically n-6 PUFA (P<0.0001 for both) and UI (P<0.005) increased with foetal age in both male and female foetuses. Furthermore, both sexes showed a decreased of total MUFA content (P<0.0001) and C18:1/C18:0 and MUFA/SFA ratios (P<0.0005 for both) with gestational age.

DISCUSSION

The results of the present study give new data regarding the effects of the offspring sex, and the ontogeny of such effects on the fatty acid composition at non-adipose tissues.

Availability and composition of fatty acids are more dependent on foetal synthesis from precursors transferred from the mother (Johnston et al. 1957; Moore & Dhopeshwarkar 1980) because fatty acids do not easily cross the placenta (Pere 2003). The so-called *essential fatty acids* have to be obtained from the diet in adults animals (Leskanich & Noble 1999) or from maternal transfer in the case of foetuses (Bobiński & Mikulska 2015). In this study, female and male foetuses were littermates; hence, it can be suggested that the observed differences would be related to sex-related maternal transfer effects. However, this extent should be explored in further studies.

The main finding of the current experiment was that sex-related differences in fatty acid composition at both muscle and liver were mainly found at the neutral fraction (triglycerides, which are an essential energy source, whilst such differences were smaller at the polar fraction (phospholipids, essential for the tissue development; (Herrera 2002). Hence, these sex-related differences may have stronger postnatal effects on the energy partitioning than on the organ structures.

An adequate availability of n-6 FA is necessary for an optimal development of the offspring (Menon et al. 1981) and, the availability of n-3, may play a particular role in improving the insulin functionality (Nascimento et al. 2010). The current study showed a higher n-6/n-3 ratio in the male *vs*. female foetuses. A high n-6/n-3 ratio appears to be deleterious (Storlien *et al*. 1996), being the prodromal phase of insulin resistance (Li et al., 2015), coinciding with previous evidences addressing an early appearance of alterations in lipid metabolism and insulin regulation in males (Roden et al. 1996). Furthermore, a higher numerical MUFA/SFA and C18:1/C18:0 ratios (i.e.: a higher activity of the stearoyl-CoA desaturase), was found in male foetuses.

In conclusion, the present study remarks better adaptive response to maternal undernutrition by the females, regarding the neutral lipid fraction (triglycerides).

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