



## Article

# Decreased Fatty Acid Oxidation Gene Expression in Preeclampsia according to the Onset and the Presence of Intrauterine Growth Restriction

Alejandra Abascal-Saiz <sup>1,†</sup>, Eva Fuente-Luelmo <sup>2,†</sup>, María Haro <sup>2</sup>, Victoria Fioravanti <sup>3</sup>, Eugenia Antolín <sup>1</sup>, María P. Ramos-Álvarez <sup>2</sup>, and José L. Bartha <sup>1,\*</sup>

## SUPPLEMENTARY FILES

**Supplementary Table S1:** qPCR primers and conditions.

	Product length (pb)	Primer Forward / Primer Reverse	Annealing temperature	Efficiency (%)	R <sup>2</sup>	Slope	Intercept
GAPDH	87	TGCACCACCAACTGCTTAGC / GGCATGGACTGTGGTCATGAG	61	97.6	0.999	-3.381	25.18
LCHAD (HADHA)	195	ATCAAATCAGAACGTGCCGTCC / CTCCCAGGCAGGATCCATTG	62	96.1	0.988	-3.418	47.426
MCAD (ACADM)	146	ACCAAGTATGCCCTGGAAAGG / CGACCAGAACATCAACCTCCAA	62	99.8	0.986	-3.327	49.383
RPL30	149	AAAGCTGGCAGTTGTTAGC / AAGACGAAAAAGTCGCTGGA	60	91.2	0.991	-3.553	42.151
CPT1A	288	TTCAGTTCACGGTCACTCCG / TGACCAACGTTCTCGTCTGG	61	93.9	0.953	-3.477	51.124
CPT2	129	TTGAAGACACCATAGGAGATAACC / CCAGCTGCTCATGCAGTTCTTT	60	91.1	0.991	-3.555	51.064

Key: qPCR: quantitative PCR, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, LCHAD: long-chain 3-hydroxyacyl-CoA dehydrogenase, HADHA: Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha. MCAD / ACADM: Medium-chain acyl-CoA dehydrogenase, RPL30: Ribosomal protein L30, CPT: Carnitine palmitoyltransferase.

**Supplementary Table S2:** Relative LCHAD mRNA expression (a.u.)

	LCHAD									Comparison of placenta sides	
	Comparison of different groups										
	Global		Maternal side			Fetal side			p value		
		p value: control vs. subgroup	p value: intra subgroup		p value: control vs. subgroup	p value: intra subgroup		p value: control vs. subgroup	p value: intra subgroup	p value	
Control, n=28	1.003 (0.383)	-	-	0.908 (0.598)	-	-	1.067 (0.584)	-	-	0.733	
Preeclampsia, n=28	0.635 (0.276)	1.1 × 10 <sup>-5*</sup>	-	0.636 (0.532)	5.6 × 10 <sup>-4*</sup>	-	0.608 (0.250)	5.5 × 10 <sup>-4*</sup>	-	0.677	
EOPE, n=14	0.625 (0.263)	3.3 × 10 <sup>-4*</sup>	0.575	0.635 (0.440)	0.005*	0.512	0.583 (0.252)	0.019*	0.924	0.534	
LOPE, n=14	0.655 (0.318)	0.003*		0.681 (0.766)	0.04*		0.633 (0.334)	0.013*		0.925	
SPE, n=14	0.635 (0.271)	0.001*	0.822	0.721 (0.497)	0.017*	0.987	0.648 (0.242)	0.018*	0.998	0.272	
MPE, n=14	0.635 (0.319)	0.001*		0.574 (0.684)	0.013*		0.608 (0.419)	0.014*		0.650	
IUGR, n=10	0.635 (0.263)	0.001*	0.813	0.636 (0.608)	0.017*	0.726	0.583 (0.227)	0.028*	0.868	0.678	
NIUGR, n=17	0.635 (0.322)	0.001*		0.681 (0.514)	0.013*		0.681 (0.297)	0.009*		0.959	

Key: a.u.: arbitrary units, LCHAD: long-chain 3-hydroxyacyl-CoA dehydrogenase, EOPE: early-onset preeclampsia, LOPE: late-onset preeclampsia, SPE: severe preeclampsia, MPE: mild preeclampsia, IUGR: intrauterine growth restriction, NIUGR: Not intrauterine growth restriction. The results are expressed as the median and interquartile range (in brackets). \*There were significant differences among the groups ( $p < 0.05$ ).

**Supplementary Table S3:** Relative MCAD mRNA expression (a.u.)

	MCAD									
	Comparison of different groups								Comparison of placenta sides	
	Global		Maternal side			Fetal side				
		p value: control vs. subgroup	p value: intra subgroup		p value: control vs. subgroup	p value: intra subgroup		p value: control vs. subgroup	p value: intra subgroup	
Control, n=28	1.020 ± 0.210	-	-	1.001 ± 0.235	-	-	1.058 ± 0.286	-	-	0.361
Preeclampsia, n=28	0.755 ± 0.247	$7.67 \times 10^{-5}*$	-	0.796 ± 0.334	0.011*	-	0.719 ± 0.193	$8 \times 10^{-6}*$	-	0.172
EOPE, n=14	0.708 ± 0.325	$4.9 \times 10^{-4}*$	0.571	0.734 ± 0.409	0.021*	0.537	0.669 ± 0.233	$1.6 \times 10^{-4}*$	0.641	0.512
LOPE, n=14	0.798 ± 0.143	0.013*		0.852 ± 0.247	0.265		0.759 ± 0.152	0.02*		0.244
SPE, n=14	0.685 ± 0.286	$1.6 \times 10^{-4}*$	0.284	0.754 ± 0.398	0.037*	0.753	0.650 ± 0.217	$4.1 \times 10^{-5}*$	0.374	0.151
MPE, n=14	0.819 ± 0.194	0.024*		0.834 ± 0.270	0.193		0.783 ± 0.150	0.004*		0.709
IUGR, n=10	0.744 ± 0.344	0.004*	0.979	0.780 ± 0.464	0.155	0.998	0.659 ± 0.269	$3.1 \times 10^{-4}*$	0.634	0.371
NIUGR, n=17	0.762 ± 0.165	0.002*		0.793 ± 0.245	0.061		0.753 ± 0.133	$1 \times 10^{-4}*$		0.334

*Key:* a.u.: arbitrary units, MCAD: medium-chain acyl-CoA dehydrogenase, EOPE: early-onset preeclampsia, LOPE: late-onset preeclampsia, SPE: severe preeclampsia, MPE: mild preeclampsia, IUGR: intrauterine growth restriction, NIUGR: Not intrauterine growth restriction. The results are expressed as the mean ± standard deviation. \*There were significant differences among the groups ( $p < 0.05$ ).

**Supplementary Table S4:** Relative CPT1A mRNA expression (a.u.)

	CPT1A									
	Comparison of different groups								Comparison of placenta sides	
	Global		Maternal side			Fetal side				
		p value: control vs. subgroup	p value: intrasubgroup		p value: control vs. subgroup	p value: intrasubgroup		p value: control vs. subgroup	p value: intrasubgroup	p value
Control, n=28	1.330 ± 0.760	-	-	1.346 ± 0.643	-	-	1.251 ± 0.722	-	-	0.526
Preeclampsia, n=28	1.379 ± 0.798	0.818	-	1.382 ± 0.823	0.859	-	1.293 ± 0.615	0.822	-	0.623
EOPE, n=14	1.629 ± 0.617	0.482	0.241	1.508 ± 0.700	0.793	0.672	1.475 ± 0.517	0.619	0.456	0.884
LOPE, n=14	1.147 ± 0.895	0.476		1.265 ± 0.934	0.940		1.150 ± 0.665	0.889		0.353
SPE, n=14	1.496 ± 0.751	0.804	0.735	1.551 ± 0.734	0.687	0.488	1.411 ± 0.702	0.772	0.680	0.582
MPE, n=14	1.270 ± 0.852	0.970		1.225 ± 0.896	0.870		1.184 ± 0.527	0.953		0.528
IUGR, n=10	1.197 ± 0.694	0.880	0.575	1.290 ± 0.744	0.975	0.856	1.217 ± 0.600	0.990	0.892	0.997
NIUGR, n=17	1.504 ± 0.861	0.757		1.445 ± 0.892	0.906		1.343 ± 0.640	0.905		0.306

*Key:* a.u.: arbitrary units, CPT: carnitine palmitoyltransferase, EOPE: early-onset preeclampsia, LOPE: late-onset preeclampsia, SPE: severe preeclampsia, MPE: mild preeclampsia, IUGR: intrauterine growth restriction, NIUGR: Not intrauterine growth restriction. The results are expressed as the mean ± standard deviation. \*There were significant differences among the groups ( $p < 0.05$ ).

**Supplementary Table S5:** Relative CPT2 mRNA expression (a.u.)

	CPT2									
	Comparison of different groups								Comparison of placenta sides	
	Global		Maternal side			Fetal side				
		p value: control vs. subgroup	p value: intrasubgroup		p value: control vs. subgroup	p value: intrasubgroup		p value: control vs. subgroup	p value	
Control, n=28	1.028 ± 0.247	-	-	1.051 ± 0.354	-		1.175 ± 0.332	-	-	0.139
Preeclampsia, n=28	0.745 ± 0.250	1.1 × 10⁻⁴*		0.782 ± 0.335	0.006*	-	0.854 ± 0.301	5.7 × 10⁻⁴*	-	0.391
EOPE, n=14	0.725 ± 0.166	0.002*	0.908	0.753 ± 0.312	0.036*	0.899	0.841 ± 0.198	0.014*	0.983	0.456
LOPE, n=14	0.766 ± 0.319	0.008*		0.813 ± 0.365	0.113		0.864 ± 0.370	0.013*		0.687
SPE, n=14	0.690 ± 0.172	4.8 × 10⁻⁴*	0.494	0.687 ± 0.292	0.007*	0.336	0.849 ± 0.237	0.013*	0.997	0.205
MPE, n=14	0.801 ± 0.307	0.023*		0.878 ± 0.359	0.297		0.858 ± 0.360	0.013*		0.801
IUGR, n=10	0.655 ± 0.247	2.4 × 10⁻⁴*	0.251	0.652 ± 0.352	0.005*	0.224	0.803 ± 0.245	0.007*	0.796	0.271
NIUGR, n=17	0.812 ± 0.274	0.021*		1.051 ± 0.354	0.259		0.888 ± 0.337	0.019*		0.880

*Key:* a.u.: arbitrary units, CPT: carnitine palmitoyltransferase, EOPE: early-onset preeclampsia, LOPE: late-onset preeclampsia, SPE: severe preeclampsia, MPE: mild preeclampsia, IUGR: intrauterine growth restriction, NIUGR: Not intrauterine growth restriction. The results are expressed as the mean ± standard deviation. \*There were significant differences among the groups ( $p < 0.05$ ).