

Evolution of mitochondrial-derived peptides, humanin and MOTSc and changes in insulin sensitivity during early gestation

Supplementary material

Supplementary files

Supplementary Table S1. Mitochondrial-derived peptides (MDPs) in the first and second trimesters of gestation in GDM (cases) and controls

<i>Variables</i>	<i>Total</i> <i>(n= 73)</i>	<i>GDM</i> <i>(n= 28)</i>	<i>Controls</i> <i>(n=45)</i>	<i>p value</i>
<i>First trimester Humanin (pg/mL), mean (SD)</i>	797.9±607.7	817.4±740.9	785.8±516.7	0.507
<i>First trimester Humanin (pg/mL), median (IQR)</i>	658.0 (455.0-905.0)	586.0 (422.5-980.0)	672.00 (515.0-859.0)	
<i>Second trimester Humanin (pg/ml), mean (SD)</i>	697.2±523.0	593.3±501.0	761.9±1205.9	0.910
<i>Second trimester Humanin (pg/ml), median (IQR)</i>	523.0 (276.0-771.0)	501.0 (269.5-754.0)	536.0 (276.5-798.0)	
<i>First trimester MOTSc (ng/mL), mean (SD)</i>	725.1±332.8	727.5±292.8	723±358.6	0.798
<i>First trimester MOTSc (ng/mL), median (IQR)</i>	600.0 (473.7-973.2)	592.8 (513.5-1008.7)	658.8 (436.5-919.2)	
<i>Second trimester MOTSc (ng/ml), mean (SD)</i>	592.0±250.5	554.2±236.5	615.5±258.7	0.571
<i>Second trimester MOTSc (ng/ml), median (IQR)</i>	541.5 (435.1-673.8)	530.2 (430.6-607.5)	543.6 (439.8-784.8)	
<i>First trimester Glucose (mmol/l), mean (SD)</i>	4.3±0.3	4.2±0.3	4.4±0.3	0.135
<i>First trimester Glucose (mmol/l), median (IQR)</i>	4.3 (4.0-4.6)	4.3 (4.0-4.5)	4.3 (4.1-4.6)	
<i>Second trimester Glucose (mmol/l), mean (SD)</i>	4.4±0.6	4.6±0.8	4.3±0.4	0.181
<i>Second trimester Glucose (mmol/l) median (IQR)</i>	4.4 (4.0-4.6)	4.4 (4.1-4.8)	4.3 (4.0-4.5)	
<i>First trimester Insulin (μU/mL), mean (SD)</i>	8.2±4.5	7.7±3.9	8.5±4.8	0.457
<i>First trimester Insulin (μU/mL), median (IQR)</i>	7.3 (4.7-10.6)	6.3 (4.4-10.9)	7.6 (5.3-9.9)	
<i>Second trimester Insulin (μU/mL), mean (SD)</i>	11.1±10.1	11.1±6.9	11.0±11.7	0.266
<i>Second trimester Insulin (μU/mL), median (IQR)</i>	9.4 (6.6-11.7)	9.8 (8.0-12.1)	9.0 (5.9-11.2)	
<i>First trimester HOMA-IR, mean (SD)</i>	1.6±0.9	1.5±0.8	1.7±1.0	0.380
<i>First trimester HOMA-IR, median (IQR)</i>	1.3 (0.9-2.0)	1.2 (0.7-2.0)	1.5 (0.9-2.0)	
<i>Second trimester HOMA-IR, mean (SD)</i>	2.3±2.5	2.5±2.7	2.1±2.4	0.166
<i>Second trimester HOMA-IR, median (IQR)</i>	1.8 (1.2-2.3)	1.8 (1.5-2.5)	1.7 (1.0-2.2)	
<i>First trimester HOMA-β (%), mean (SD)</i>	223.2±144.1	234.5±151.9	216.5±140.6	0.646
<i>First trimester HOMA-β (%), median (IQR)</i>	176.2 (125.1-277.9)	229.3 (113.4-278.2)	173.2 (126.2-278.8)	
<i>Second trimester HOMA-β (%), mean (SD)</i>	288.1±262.0	277.4±265.1	295.0±262.8	0.432
<i>Second trimester HOMA-β (%), median (IQR)</i>	208.0 (146.7-356.2)	195.0 (146.7-258.9)	220.0 (147.8-360.1)	

GDM: Gestational diabetes mellitus. Comparisons across groups were performed by the Mann-Whitney U-test.

Supplementary Table S2. Crude and adjusted odds ratio and 95% CI

according to median mitochondrial-derived peptides on the risk of high HOMA-IR in the first (1T) and second trimesters (2T) of gestation.

Biomarkers	Cut-of points	HOMA-IR low	HOMA-IR high	Crude OR	(95% CI)	aOR ^a	(95% CI)	
		N=48 (1T) N=49 (2T)	N=24 (1T) N=23 (2T)					
First trimester								
Humanin								
(pg/mL)								
(Median)								
High (reference)	659+	27	8	1.00		1.00		
Low	≤ 658	21	16	2.57	0.92 7.15	1.85	0.57 5.98	
First trimester								
MOTSc (ng/ml)								
(Median)								
High (reference)	600.1+	25	11	1.00		1.00		
Low	≤ 600.0	23	13	1.28	0.48 3.43	2.47	0.72 8.52	
Second trimester								
Humanin								
(pg/mL)								
(Median)								
High (reference)	524+	30	6	1.00		1.00		
Low	≤ 523	19	17	4.47	1.50 13.36	2.12	0.60 7.46	
Second								
trimester								
MOTSc (ng/ml)								
(Median)								
High (reference)	541.6+	27	8	1.00		1.00		
Low	≤ 541.5	22	15	2.30	0.82 6.42	3.82	1.02 14.24	

Note: One case was excluded because of missing insulin values in the first trimester, and one control was excluded because of missing glucose values in the second trimester.

High HOMA-IR was considered ≥ 70th percentile of its distribution (≥ 2.28).

^aaOR= Odds ratios adjusted for maternal age, BMI and smoking habit.

Supplementary Table S3. Spearman rank correlation bivariate analysis of variables associated with HOMA-IR in the first and second trimesters of gestation separately.

	<i>First Trimester</i>		<i>Second Trimester</i>	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p value</i>
<i>BMI</i>	0.513**	<0.0001	0.433**	0.0001
<i>Age</i>	0.009	0.942	0.179	0.133
<i>Humanin</i>	-0.324**	0.005	0.186	0.118
<i>MOTSc</i>	0.037	0.759	-0.086	0.471
<i>Humanin Change</i>	0.189	0.113	-0.059	0.622
<i>MOTSc Change</i>	0.165	0.166	0.262*	0.026

*2-tailed correlation significance, $p < 0.05$. ** 2-tailed correlation significance, $p < 0.01$.

Supplementary table S4. Association between changes in levels of mitochondrial-derived peptides throughout pregnancy and the HOMA-IR index.

MDPs Change	Cutoff points	HOMA-IR low	HOMA-IR high	Crude OR	(95% CI)	aOR ^a	(95% CI)
		N=49	N=23				
Change in Humanin (pg/mL)							
Low (reference)	≤ 122.00	25	11	1.00		1.00	
High (above median)	123.00+	24	12	1.14	0.42 3.06	1.71	0.51 5.73
Change in MOTSc (pg/mL)							
Low (reference)	≤ 52.80	28	8	1.00		1.00	
High (above median)	52.81+	21	15	2.50	0.89 6.99	3.73 ^b	1.03 13.50

Note: One control was excluded because of missing glucose values in the second trimester.

High HOMA-IR was considered ≥ 70th percentile of its distribution (≥ 2.28).

^a aOR= Odds ratios adjusted for maternal age, BMI and smoking habit.

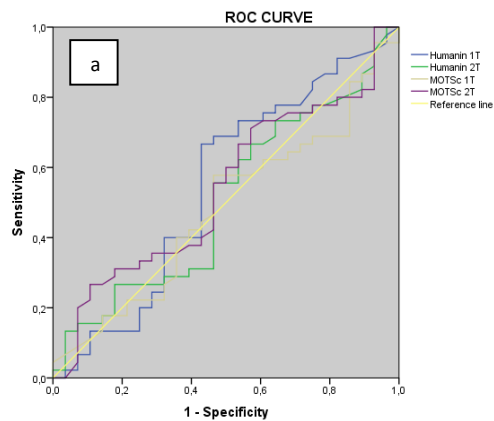
^b *p* value=0.045.

Supplementary table S5. Association between changes in levels of mitochondrial-derived peptides throughout pregnancy and the occurrence of gestational diabetes mellitus (GDM).

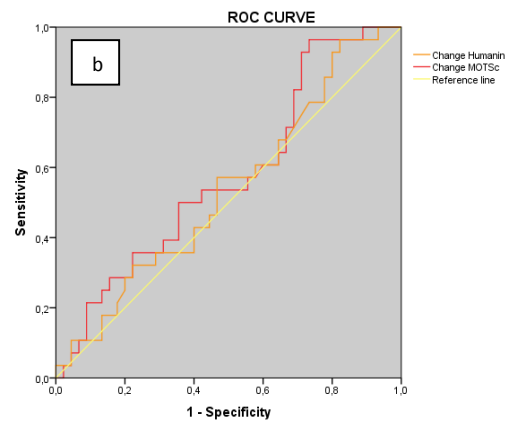
MDPs	Cutoff points	Controls	GDM	Crude OR	(95% CI)	aOR _a	(95% CI)		
		N=45	N=28						
First trimester									
Humanin (pg/mL) (Median)									
High (reference)	659+	24	12	1.00		1.00			
Low	≤ 658	21	16	1.52	0.59 3.94	1.44	0.54 3.85		
First trimester MOTSc (ng/ml)(Median)									
High (reference)	600.1+	23	13	1.00		1.00			
Low	≤ 600.0	22	15	1.21	0.47 3.10	1.35	0.50 3.63		
Second trimester									
Humanin (pg/mL) (Median)									
High (reference)	524+	23	13	1.00		1.00			
Low	≤ 523	22	15	1.21	0.47 3.10	1.23	0.46 3.28		
Second trimester MOTSc (ng/ml)(Median)									
High (reference)	541.6+	23	13	1.00		1.00			
Low	≤ 541.5	22	15	1.21	0.47 3.10	1.23	0.46 3.28		
Change in Humanin (pg/mL)									
Low (reference)	≤ 122.00	24	13	1.00		1.00			
High (above median)	123.00+	21	15	1.32	0.51 3.40	1.34	0.50 3.56		
Change in MOTSc (pg/mL)									
Low (reference)	≤ 52.80	24	13	1.00		1.00			
High (above median)	52.81+	21	15	1.32	0.51 3.40	1.34	0.50 3.56		

^aaOR= Odds ratios adjusted for maternal age, BMI and smoking habit.

Supplementary Figure S1. Receiver operating characteristic (ROC) curve for mitochondrial-derived peptides (MDPs): (a) in the first (1T) and second trimesters (2T), and (b) changes across gestation built on gestational diabetes mellitus (GDM).



Humanin 1T: AUC 0.546; 95%CI (0.403-0.690);
Humanin 2T: AUC 0.508; 95%CI (0.370-0.646)
MOTSc 1T: AUC 0.482; 95%CI (0.346-0.618)
MOTSc 2T: AUC 0.540 (0.403-0.676)



Change Humanin: AUC 0.537; 95%CI (0.402-0.673)
Change MOTSc: AUC 0.576; 95%CI (0.442-0.710)