SERUM HIGH MOBILITY GROUP BOX 1 (HMGB1) LEVELS ARE INDEPENDENTLY ASSOCIATED WITH GLUCOSE CLAMP-DERIVED MEASURES OF INSULIN RESISTANCE IN PCOS.

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INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine disorders among women of reproductive age, and is characterised by ovulatory dysfunction and hyperandrogenism; in addition by chronic inflammation, and in most cases by insulin resistance (IR). HMGB1 is a small protein with cytokine activity that can activate nuclear factor kappa light chain enhancer of activated B cells (Nf-kB), and signals through the Receptor for advanced glycation end products (RAGEs) and through the Toll like receptor family. HMGB1 is increased in IR/hyperinsulinemia related diseases as diabetes type 2, and its circulating concentrations have been reported to be higher in PCOS women with IR by us in one previous study. We have previously shown that HMGB-1 increases in the presence of CFTR malfunction, and is lowered by insulin in an *in vitro* model. We have also shown reduced CFTR gene expression in granulosa cells from PCOS women.

AIM

The aim of the present study was to investigate HMGB1 concentrations in serum in PCOS women in relationship with insulin sensitivity, and verify whether insulin infusion used for the clamp lowers HMGB1 levels. Furthermore relationships with clinical, endocrine and metabolic parameters of PCOS patients were searched.

METHODS

Sixty women with PCOS, 30 with IR and 30 with normal insulin sensitivity (IS), and 30 healthy controls were included in the study. In these subjects, body fat was quantified by bioelectrical impedance; serum HMGB1 levels were measured using a specific ELISA method (Tecan). Serum androgens were measured by liquid chromatography/mass spectrometry and equilibrium dialysis. Hirsutism was evaluated by Ferriman - Gallwey Score. In PCOS women, IR was measured using the hyperinsulinemic-euglycemic clamp technique, combined with indirect calorimetry. Additional biochemical parameters were also evaluated. Specific features are reported in **Table 1.**

Table 1. Characteristics of women with PCOS and controls (mean ± SD).

	PCOS	Controls	p
Age (years)	24,1 ± 5,1	27,8 ± 3,2	**<0,001**
BMI (Kg/m^2)	26,2 ± 5,4	20,9 ±1,9	**<0,001**
WHR	0,83 ± 0,08	0,77 ± 0,06	**0,001**
Fat mass (Kg)	23,2 ± 10,4	12,0 ± 5,0	**<0,001**
Fat-free mass (Kg)	47,3 ± 4,8	43,8 ± 2,4	**0,003**
Ferriman-Gallwey score	7,8 ± 5,6	-	-
Total testosterone (ng/dL)	38,4 ± 14,3	28,4 ± 12,8	**0,002**
SHBG (nmol/L)	37,9 ± 16,5	60,7 ± 18,1	**0,017**
Free testosterone (ng/dL)	0,68 ± 0,29	0,32 ± 0,14	**<0,001**
Androstenedione (ng/dL)	162,2 ± 56,4	129,8 ± 48,9	**0,01**
DHEAS (µmol/L)	5,8 ± 2,5	3,9 ± 1,2	**0,042**
LH/FSH	2,04 ± 1,2	0,54 ± 0,12	**0,002**
Ovarian volume (mL)	13,3 ± 4,6	7,2 ± 1,7	**<0,001**
N° of ovarian follicules	13,7 ± 3,6	5,1 ± 2,0	**<0,001**
Systolic BP (mmHg)	117 ± 12	110 ± 10	**0,012**
Dyastolic BP (mmHg)	73 ± 10	69 ± 10	**0,063**
Total cholesterol (mg/dL)	160 ± 33	163 ± 19	0,752
HDL cholesterol (mg/dL)	53 ± 12	62 ± 15	**0,01**
Triglyceride (mg/dL)	72 ± 46	62 ± 29	0,369
Fasting glycaemia (mg/dL)	84 ± 9	77 ± 7	**0,012**
Fasting insulin (mU/L)	12,8 ± 10,1	5,2 ± 2,0	0,055
M-clamp (mg/Kg$_{FFM}$ · min)	11,0 ± 3,3	-	-

RESULTS

HMGB1 levels did not differ in PCOS women with respect to healthy controls (4.11 ± 3.22 vs 3.77 ± 2.50 ng/mL, respectively; p=0.61). HMGB1 levels in PCOS IS women were not significantly different with respect to controls (3.16 ± 2.59 ng/mL vs 3.77 ± 2.50 ng/mL, respectively p=0.43) whereas PCOS IR women showed higher levels of this protein as compared with PCOS IS (5.00 ± 3.53 vs 3.16 ± 2.59 ng/mL, respectively; p=0.017) (**Figure 1**). In women with PCOS, HMGB1 levels were associated with several metabolic parameters, including IR measured by glucose utilization during the clamp (rho -0.37, p=0.005) (**Figure 2** and **Table 2**). This correlation was preserved after adjusting for potential confounding parameters, such as age, fat mass and serum free testosterone. HMGB1 levels did not change during glucose-clamp induced acute hyperinsulinemia, either in the whole cohort of patients nor in IR and IS subgroups analyzed separately. Both in the whole population under study and in PCOS women, HMGB1 levels did not correlate with anthropometric parameters, biochemical and hormonal features and ovarian morphology.

Figure 1. HMGB1 levels are increased in IR PCOS women with respect to IS PCOS women (p<0.05).

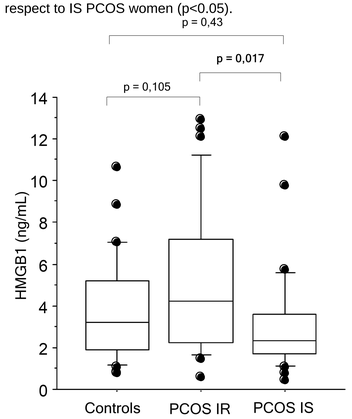

Figure 1. Box-plots of HMGB1 levels in healthy controls and in IR and IS subgroup of PCOS women.

Figure 2. Correlation between HMGB1 levels and IR measured by glucose utilization (p=0.005).

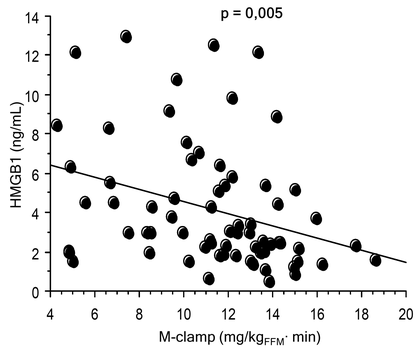

Figure 2. Correlation between HMGB1 levels and M-clamp values.

Table 2. Correlations between HMGB1 values and clinical, endocrine e metabolic parameters in PCOS women

	HMGB1	
	rho	p
Age	-0,197	0,133
BMI	0,095	0,468
WHR	0,171	0,197
Fat mass	0,029	0,824
Fat-free mass	0,018	0,891
Ferriman-Gallwey score	0,092	0,483
Total testosterone	-0,019	0,887
SHBG	-0,181	0,169
Free testosterone	0,094	0,475
Androstenedione	-0,091	0,49
DHEAS	0,284	0,067
LH/FSH	0,223	0,112
Ovarian volume	0,038	0,811
N° of ovarian follicules	-0,066	0,704
Systolic BP	0,069	0,602
Dyastolic BP	0,016	0,902
Total cholesterol	-0,052	0,694
HDL cholesterol	-0,154	0,248
Triglyceride	0,162	0,217
Fasting glycaemia	0,067	0,612
Fasting insulin	0,275	**0,036**
M-clamp	-0,37	**0,005**

CONCLUSIONS

HMGB1 blood levels showed an independent association with insulin sensitivity in women with PCOS. No changes were observed in HMGB1 concentrations during the clamp when insulin was infused. This could be due to the fact that the time of infusion was insufficient to observe any changes or increased HMGB1 could be related with the inflammatory status possibly different in the IS and IR PCOS women. This aspect is currently under investigation. No associations with other typical features of the syndrome were found.

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