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Familial Diabetes in Obese PCOS Predisposes Individuals to Compensatory Hyperinsulinemia and Insulin Resistance (IR) Also for Reduced Hepatic Insulin Extraction (HIE)

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Abstract: Background: Polycystic ovary syndrome (PCOS) is a frequent reproductive disease characterized by hyperandrogenism, oligo /anovulation, and polycystic aspects at ultrasound. In these last years, a body of evidence disclosed the frequent occurrence in PCOS patients of insulin resistance (IR) and compensatory hyperinsulinemia. **Aim:** To evaluate whether any relationship exists between IR, compensatory hyperinsulinemia and familial predisposition to diabetes. **Methods:** A group of overweight/obese PCOS patients (n = 84) was selected from our Clinic database according to the Rotterdam criteria and the following parameters were extracted from the database: insulin, C Peptide, aspartate amino transferase (AST), alanine amino transferase (ALT), HOMA (Homeostasis Model Assessment) index, total cholesterol, LDL (Low Density Lypoprotein), HDL (High Density Lypoprotein), and body mass index (BMI). The presence and absence of diabetes among first grade relatives (parents and/or grandparents) were also considered. The Hepatic Insulin Extraction (HIE) index was computed as a ratio between insulin and C-Peptide plasma levels. **Results:** PCOS patients with familial diabetes showed higher levels of ALT, AST, HOMA index, and HIE. Baseline insulin levels above 12 µU/mL were more frequently observed in PCOS with familial diabetes. HIE index, ALT, and AST were higher in these latter PCOS patients than in PCOS without diabetic first grade relatives, sustaining the hypothesis of an impaired liver clearance of insulin in the case of familial diabetes. **Conclusions:** According to our study, the presence of anamnestic evidence of familial diabetes together with baseline levels of insulin higher that 12 µIU/mL and elevated transaminase levels should be considered as a consistent clinical suspect of liver impairment that might trigger compensatory hyperinsulinemia and lead to NAFLD and liver steatosis.

Keywords: HIE index; PCOS; insulin resistance; familial diabetes; liver function; insulin degrading enzyme

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1. Introduction

Polycystic ovary syndrome (PCOS) is a very common clinical situation that affects up to 25% of women of reproductive age [1,2]. The diagnosis of PCOS relies on the presence of two out of three of the criteria established at the consensus meeting in Rotterdam [3]; however, in recent years, the dismetabolic state, insulin resistance (IR), and the correlated compensatory hyperinsulinemia gained attention and consideration and a variable spectrum of clinical manifestations has been highlighted [3,4]. In addition to the well-known clinical symptoms, PCOS is also characterized by the frequent occurrence of metabolic problems, such as obesity (up to 50% of the patients) and hyperinsulinemia that occurs in a high percentage of subjects. Indeed, extensive literature has shown that insulin resistance is very common in PCOS independently from BMI [5].

Insulin resistance is a compensatory event that elevates insulin plasma levels in response to a metabolic load and/or in baseline conditions to manage glycemic values within the normal range. Such compensatory hyperinsulinemia has been observed in up to 50–70% of women with PCOS and obesity and in 15–30% of PCOS with normal weight [4,5]. Although compensatory hyperinsulinemia represents a metabolic attempt to adjust and control glucose profiles, women with PCOS and IR have a greater risk of incurring in metabolic complications such as metabolic syndrome (MS) [6,7] and non-alcoholic fatty liver disease (NAFLD) and/or liver fibrosis [8].

Recently, a new index, HIE (Hepatic Insulin Extraction), has been evaluated in obese PCOS subjects, and it has been reported to be positively correlated with IR [9,10]. The HIE index is the ratio between insulin and C-peptide plasma levels and reflects the balance between the kinetics of synthesis and the clearance of the two peptides. HIE is usually computed as the ratio between the area under the curve (AUC) of insulin and the AUC of C-peptide (AUC Ins/AUC C-Pept) [9,11]. Recent studies clearly demonstrated that HIE is associated with muscle and adipose tissue IR. Moreover, a reduced liver function and the ability to clear insulin from the blood participates in improving IR [9,12]. On such basis, HIE might represent a relevant clinical index that may help in preventing the risks of NAFLD and hepatic steatosis as recently reported [8,9].

From the physiological point of view, HIE has been demonstrated to reflect insulin kinetics as a balance between pancreatic insulin synthesis and its hepatic clearance. Since C-peptide almost exclusively reflects pancreatic synthesis because hepatic C-peptide clearance is almost negligible [9,10], HIE represents a simple-to-compute index for linking hepatic functions with PCOS dismetabolic conditions. On such basis, we aimed to compute HIE in early morning fasting conditions in a larger group of overweight/obese PCOS patients than what was tested previously [13] to evaluate whether a single HIE computation might be a simpler index than performing computations with OGTT [13].

2. Materials and Methods

2.1. Subjects

A total of 84 overweight/obese patients with PCOS s (23.8 $\pm$ 1.2 years, mean $\pm$ standard error of the mean (SEM)) were selected from the outpatients database of the ambulatory of the Gynecological Endocrinology Centre in our department during January 2020–December 2021. All these patients attended our clinical services for their PCOS condition and responded to the criteria established by the American Society for Reproductive Medicine and the European Society for Human Reproduction and Embryology for diagnosing the presence of PCOS [14], and at least two of the following criteria were present: (a) oligomenorrhea with inter-menstrual intervals longer than 45 days, (b) clinical (acne, hirsutism) or biochemical signs of hyperandrogenism, and (c) presence of micro-polycystic ovaries at ultrasound. As noted from the database, all these patients had the following: (1) absence of enzymatic adrenal deficiency and/or other endocrine disease, including diabetes, (2) prolactin (PRL) levels within the normal range (range 5–25 ng/mL), (3) no hormonal treatment and/or no assumption of insulin sensitizing agent at least 6 months prior to the study, and (4) body mass index above 27. Finally, the diabetic condition was excluded when performing an oral glucose tolerance test (OGTT), sampling 15 min before and 30, 90, 120, and 180 min after the oral glucose load of 75 g diluted in water.

Anamnestic history was also considered from the database and the presence or absence in the family of first-degree relative (parents and/or grandparents) with diabetes. Fortyfour out of the eighty-four patients (52.4%) reported first-degree diabetic relatives. Informed consent was obtained at the moment of access to the out-patients ambulatory from all individual participants, as a standard procedure of the Gynecological Endocrinology Center of the University of Modena and Reggio Emilia, Italy.

Only the following data were used from the database: insulin, C Peptide, aspartate amino transferase (AST) and alanine amino transferase (ALT), HOMA (Homeostasis Model Assessment) index as the index of sensitivity to insulin [15], total cholesterol, LDL, HDL,

and body mass index (BMI). Consideration was also given to insulin baseline plasma levels since an insulin plasma level of 12 μU/mL or higher is considered an index of insulin resistance [16,17].

After the endocrine evaluation, the patients underwent the most appropriate treatment to solve the clinical issue of their PCOS condition, and it has not been taken into consideration for the present study.

According to the occurrence of familial diabetes, the entire set of PCOS patients was considered according to the presence (n = 44) or absence of familial diabetes (n = 40) and also for their fasting insulin plasma levels, which is insulin $\leq$ 12 μU/mL (n = 34) and insulin >12 μU/mL (n = 50), as previously reported [16,18]. The study was approved as a retrospective observational study by the Human Investigation Committee of the University of Modena and Reggio Emilia, Italy. The study had no funding support.

2.2. Assay

All samples from each subject were assayed in the same assay. Plasma insulin and C peptide concentrations were determined using an immunoradiometric assay (Biosource Europa S.A., Nivelles, Belgium). Based on two quality control samples, the average within- and between-assay coefficients of variation were 4.0% and 10.2%. Glycemic and lipid profiles were assayed as a routine procedure by the Modena Hospital Central Laboratory.

2.3. Statistical Evaluation

After an analysis of variance (one-way ANOVA), data were tested for statistically significant differences between the groups by means of Student's t-test for paired and unpaired data where appropriate.

The Chi-square test was computed to test whether there are any statistically significant differences between the expected and observed frequencies in one or more categories of our PCOS patients under evaluation.

The HOMA index was computed to estimate sensitivity to insulin [14] since it is considered the main index of the insulin resistance and metabolic syndrome and is a common link between the coexisting abnormalities; it can be calculated by using the homeostasis model assessment of IR (HOMA-IR) as (fasting insulin mU/l) × (fasting glucose mmol/l)/22.5 [5]. We used the previously established cut-off value of 2.7 [15].

The HIE was computed as the ratio between insulin and C peptide plasma concentrations (insulin/C peptide) [19]. Data are expressed as mean ± SEM.

3. Results

Table 1 summarizes the metabolic parameters considered for the present study. Out of the total of 84 patients selected for this study, familial diabetes was recorded in 44 patients (52.4%). Considering PCOS patients according to this familial predisposition, subjects with familial diabetes showed significantly higher HIE, insulin, HOMA index, AST, and ALT plasma levels.

Table 2 summarizes the parameters when patients were compared according the presence or absence of familial diabetes and to fasting insulin levels below or above 12 μIU/mL. Interestingly, only PCOS patients with familial diabetes showed higher AST and ALT levels (23.7 ± 1.9 and 27.5 ± 1.9 vs. 19.9 ± 0.7 and 18.5 ± 0.9, $p < 0.003$ and $p < 0.0001$, respectively) than PCOS without diabetic relatives, independently from the presence or absence of baseline insulin levels above or below the cut off of 12 μIU/mL. Moreover, HOMA as an index of insulin resistance resulted in significantly higher numbers: 3.5 ± 0.5 and 2.02 ± 0.1, $p < 0.05$ (Table 2). Notably, although C-peptide levels were similar in both groups with insulin above 12 μIU/mL, the insulin levels were higher, although not significantly, in the group with familial diabetes (Table 2).

Table 1. Metabolic parameters of women with PCOS and overweight/obesity selected for the study (n = 84).

	Glucose mg/dL	Insulin µUI/mL	Total Chol mg/dL	HDL mg/dL	LDL mg/dL	Trigliceride mg/dL	AST U/L	ALT U/L	HOMA Index	C-Peptide mg/dL	BMI	HIE
All PCOS n = 84	88.0 ± 1.4	13.0 ± 0.9	176.6 ± 3.3	50.3 ± 1.4	129.4 ± 5.8	101.9 ± 5.5	21.9 ± 0.6	23.2 ± 1.2	3.0 ± 0.3	2.2 ± 0.1	32.8 ± 1.1	5.6 ± 0.2
Familial diabetes (n = 44)	89.8 ± 2.5	14.5 ± 1.5	179.5 ± 4.3	52.6 ± 2.0	129.5 ± 6.3	101.3 ± 7.8	23.7 ± 1.9	27.5 ± 1.9	3.5 ± 0.5	2.3 ± 0.1	33.5 ± 1.3	5.9 ± 0.3
p vs. no familial diabetes		0.05					0.003	0.0001	0.05			0.02
No familial diabetes (n = 40)	85.8 ± 1.3	10.9 ± 0.9	172.7 ± 5.2	47.9 ± 1.9	127.9 ± 6.2	100.6 ± 7.8	19.9 ± 0.7	18.5 ± 0.9	2.02 ± 0.1	2.1 ± 0.1	31.7 ± 2.0	5.0 ± 0.2

Legends: Total Chol = total cholesterol, HDL = high density lypoprotein, LDL = low density lypoprotein, AST= aspartate amino transferase, ALT= alanine amino transferase.

Table 2. Metabolic parameters of women with PCOS and overweight/obesity selected for the study (n = 84) according to baseline insulin levels ≤ 12 or > 12 µIU/mL.

	Glucose mg/dL	Insulin µUI/mL	Total Chol mg/dL	HDL mg/dL	LDL mg/dL	Trigliceride mg/dL	AST U/L	ALT U/L	HOMA Index	C-Peptide mg/dL	BMI	HIE
Familial diabetes (n = 44)												
Ins >12 µIU/mL (n = 23)	94.9 ± 4.4	21.4 ± 2.1	167.3 ± 5.5	46.7 ± 2.9	123.2 ± 10.5	112.3 ± 12.4	23.2 ± 1.3	29.2 ± 2.8	5.3 ± 0.9	2.9 ± 0.2	36.5 ± 1.8	7.2 ± 0.3
p vs. Ins ≤ 12 µIU/mL	0.03	0.000004	0.005	0.006		0.01			0.0004	0.0009	0.03	0.000004
p vs. No Fam Diab Ins > 12 µIU/mL							0.05	0.03				0.05
Ins≤ 12 µIU/mL (n = 21)	84.3 ± 1.8	7.9 ± 0.5	190.6 ± 5.9	57.7 ± 2.5	135.7 ± 7.7	89.6 ± 9.4	24.3 ± 1.5	25.8 ± 2.7	1.6 ± 0.1	1.8 ± 0.1	30.8 ± 1.8	4.7 ± 0.3
p vs. No Fam Diab Ins ≤ 12 µIU/mL				0.03			0.03	0.006				
No familial diabetes (n = 40)												
Ins >12 µIU/mL (n = 11)	90.7 ± 3.6	17.2 ± 2.0	170.0 ± 15.5	41.8 ± 3.9	115.0 ± 19.3	127.2 ± 13.9	17.8 ± 1.4	19.0 ± 1.7	3.8 ± 0.5	2.8 ± 0.2	36.1 ± 5.4	5.9 ± 0.3
p vs. Ins ≤ 12 µIU/mL		0.00000002		0.04					0.0000005	0.000004	0.04	0.001
Ins≤ 12 µIU/mL (n = 29)	84.6 ± 1.4	8.1 ± 0.5	176.8 ± 5.4	50.5 ± 2.2	129.4 ± 7.0	93.9 ± 9.0	20.4 ± 1.1	18.1 ± 1.2	1.6 ± 0.1	1.7 ± 0.1	29.6 ± 1.9	4.6 ± 0.2

Legends: BMI = Body Mass Index, HIE = Hepatic Insulin Extraction, Total Chol = total cholesterol, HDL = high density lypoprotein, LDL = low density lypoprotein, AST= aspartate amino transferase, ALT= alanine amino transferase.

The expected and observed frequencies of familial diabetes and baseline insulin levels $\geq$ 12 μIU/mL, as calculated by the Chi-square test, had significant results ($p < 0.05$). This means that the combined presence of familial diabetes and insulin $\geq$ 12 μIU/mL is not a random event and occurred more frequently in PCOS patients with familial diabetes (Table 3).

Table 3. Observed frequencies of familial diabetes and baseline insulin levels $\geq$ 12 μIU/mL.

	Baseline Insulin >12 μU/mL	Baseline Insulin ≤12 μU/mL	Totals
PCOS with familial diabetes	23 *	21	44
PCOS without familial diabetes	11	29	40
Totals	34	50	84

* p is 0.020878, significant at $p < 0.05$ (Chi-square test).

4. Discussion

The present study supports the hypothesis that familial predisposition to diabetes is a main trigger of hyperinsulinemia impairing both peripheral insulin sensitivity and hepatic ability to degrade insulin.

Our study demonstrates that an impaired hepatic ability to degrade insulin contributes in significantly triggering hyperinsulinemia, which is observed in overweight/obese PCOS patients. In fact, our patients showed, at a higher grade, the presence of higher baseline insulin levels together with high ALT and AST levels in the presence of first-grade diabetic relatives. Any increase in transaminase is an index of putative hepatic impairment that, as a matter of time, might trigger steatosis risks, especially if combined with IR and compensatory hyperinsulinemia [9]. To strengthen the relevance of the hepatic function, in our study, the HIE index is computed in baseline conditions, resulting in an index higher in PCOS patients with familial diabetes.

The clinical relevance of HIE is due to the fact that it is an index that reflects the dynamics of both insulin and C-peptide secretion and clearance, and it is computed as insulin-to-C-peptide ratio [20]. At the pancreatic level, pro-insulin cleavage produces C-peptide and insulin that is structured through disulphide bonds that link A- and B-chains together, thus completing insulin molecule. While C-peptide hepatic clearance is negligible, the liver clears the majority of circulating insulin [19,21]. Although the production ratio between insulin and C-peptide is 1 at the pancreatic level, this ratio was computed using the circulating levels of both peptides and greatly reflects a balance between hepatic clearance kinetics and pancreatic release, which corresponding to C-peptide since its hepatic clearance is minimal [18,20].

Previous data reported a higher HIE all along the OGTT time interval in PCOSs with familial diabetes [13]. Genazzani et al. [13] demonstrated that, in these PCOS patients, the dynamics of insulin clearance were impaired at the hepatic levels, paralleling the occurrence of higher transaminase levels. Such a study demonstrated that the presence of familial diabetes predisposes individuals to reduced insulin clearance masking the physiological occurrence of bimodal insulin secretion [22]. As a result of these events, the HIE index results in the higher presence of familial predisposition to diabetes.

Although this study [13] provided relevant insights to the understanding of the physiopathology of insulin dynamics in PCOS patients, it is not handy to perform OGTT for the time interval of at least 180–240 min. On the contrary, our present data on a larger population suggest an easy-to-perform detection of HIE, calculated only on the blood sample during early mornings, after night fasting, and not on OGTT. HIE was higher in PCOS with familial diabetes than PCOS without familial diabetes, and it was the highest when baseline insulin levels of PCOS with familial diabetes are above 12 μIU/mL. It is of interest to point out that such differences occurred also between PCOS without diabetic relatives with respect to the baseline levels of insulin. The real difference between the two

groups was in the AST and ALT plasma levels: In fact, PCOS with familial diabetes had higher transaminase levels independently of insulin baseline levels, thus suggesting that the presence of diabetic first-grade relatives affects and impairs liver function. The fact that PCOSs with familial diabetes show a higher incidence of baseline levels of insulin above 12 μIU/mL than PCOS without familial diabetes clearly suggests that this might depend on the combination of two events: an impaired hepatic insulin clearance and a relatively greater pancreatic production due to peripheral IR. The latter one is the main event occurring in PCOS without familial diabetes. Previous studies clearly reported that familial diabetes together with overweight/obesity favor abnormal liver function to increase AST and ALT levels [6,8,9]. However, our data support the hypothesis that, in addition to this, there is also a reduced/impaired expression/synthesis of the insulin degrading enzyme (IDE), as previously supposed [10,23], that induces higher circulating fasting insulin levels in fasting conditions, thus maintaining hyperinsulinemia.

In conclusion, our study sustains the fact that the HIE index computation, as a ratio between insulin and C-peptide plasma levels, on a single blood sample after overnight fasting permits showing that familial diabetes predisposes an individual to IR not only for abnormal peripheral sensitivity but also for impaired liver clearances of insulin. In these PCOS patients, the HIE index and ALT and AST levels were higher than in PCOS without diabetic first-grade relatives. According to our study, the presence of anamnestic evidence of familial diabetes together with baseline levels of insulin higher than 12 μIU/mL and elevated transaminase levels should be considered a consistent clinical suspect of insulin resistance that, also by an impaired liver function in IDE expression, might lead to NAFLD and liver steatosis.

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