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ORIGINAL ARTICLE ***Reproductive endocrinology***

Insulin resistance in a large cohort of women with polycystic ovary syndrome: a comparison between euglycaemic-hyperinsulinaemic clamp and surrogate indexes

Flavia Tosi, Enzo Bonora, and Paolo Moghetti*

Endocrinology, Diabetes and Metabolism, University of Verona and Azienda Ospedaliera Universitaria Integrata Verona, P.le Stefani 1, I-37126 Verona, Italy

*Correspondence address. Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University of Verona, P.le Stefani 1, I-37126 Verona, Italy. Tel: +39-045-812-3110; Fax: +39-045-802-7314; E-mail: paolo.moghetti@univr.it

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STUDY QUESTION: Could surrogate indexes identify insulin resistant individuals among women with polycystic ovary syndrome (PCOS)?

SUMMARY ANSWER: Surrogate indexes may be able to rule in, but not rule out, insulin resistance in women with PCOS.

WHAT IS KNOWN ALREADY: Insulin resistance is a typical finding of women with PCOS and most clinical information on this issue is based upon surrogate indexes of insulin resistance. However, data on the performance of these indexes in PCOS women are very limited.

STUDY DESIGN SIZE, DURATION: A retrospective analysis of 406 women referred to our outpatient clinic for hyperandrogenism and/or menstrual dysfunction and submitted to hyperinsulinemic euglycaemic clamp between 1998 and 2015.

PARTICIPANTS/MATERIALS, SETTING, METHODS: In total, 375 of these women had PCOS by the Rotterdam criteria and were included in the study. Six surrogate indexes of insulin sensitivity were calculated from glucose and insulin levels, either at fasting (homeostasis model assessment (HOMA), glucose/insulin (G/I) ratio and quantitative insulin sensitivity check index (QUICKI)) or after oral glucose load (Gutt, Stumvoll$_{0-120}$ and Matsuda).

MAIN RESULTS AND THE ROLE OF CHANCE: Overall, insulin resistance, as identified by the M-clamp value, was found in 74.9% of these women. The percentage was 59.3% in normal-weight vs 77.5% in overweight and 93.9% in obese subjects. All surrogate indexes were highly correlated with the M-clamp values. However, their ability to identify insulin resistant individuals was limited, in terms of sensitivity and especially in normal-weight subjects. ROC analysis showed similar performances of these indexes (AUC values 0.782–0.817).

LIMITATIONS REASONS FOR CAUTION: Potential referral bias of PCOS patients may have caused overestimation of the prevalence of insulin resistance in these women.

WIDER IMPLICATIONS OF THE FINDINGS: By using surrogate indexes many subjects with PCOS may be erroneously diagnosed as insulin sensitive, especially among normal-weight women. These indexes can be used to rule in, but not rule out, insulin resistance in PCOS.

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Key words: euglycaemic-hyperinsulinaemic clamp / insulin resistance / HOMA-IR / PCOS / PCOS phenotypes

Introduction

Insulin resistance is a common finding in women with polycystic ovary syndrome (PCOS). However, it is not universal in these subjects. The hyperinsulinemic euglycemic clamp is unanimously considered the gold standard to evaluate insulin sensitivity *in vivo* (Ferrannini and Mari, 1988). Unfortunately, it is complex and time consuming, and requires skilled operators. Alternatively, a number of surrogate indexes, derived from plasma glucose and insulin levels at fasting or after oral glucose load, have been proposed to estimate insulin action and are widely used in clinical research.

A meta-analysis of clamp studies concluded that the impairment in insulin action is intrinsic to PCOS and independent of BMI, although obesity exacerbates insulin resistance with a disproportionately greater effect in these women than in controls (Cassar et al., 2016). It was suggested that expanding diagnostic criteria of PCOS from the original NIH phenotype (Zawadzki and Dunaif, 1992) to the more inclusive Rotterdam phenotypes (Rotterdam ESHRE/ASRM, 2004) may have a limited impact on estimates of insulin resistance. However, this conclusion was flawed by the large overlap between women diagnosed with these different criteria.

An important knowledge gap is the limited information on the performance of surrogate indexes of insulin resistance in these women. Indeed, very few studies, all based on fasting data, performed these analyses in women with PCOS, with conflicting results (Legro *et al.*, 1998; Ducluzeau *et al.*, 2003; Diamanti-Kandarakis *et al.*, 2004; Kim *et al.*, 2006).

Therefore, the aim of this study was to evaluate the performance of several surrogate markers of insulin resistance in identifying the individual PCOS subjects with impaired insulin sensitivity, as defined by the euglycemic clamp. The secondary aim was to assess the frequency of insulin resistance in a large monocentric cohort of patients, distinguishing subjects according to BMI categories and PCOS phenotypes.

Materials and Methods

Subjects

Overall, 406 women referred between 1998 and 2015 to the outpatient clinic of our Unit—a tertiary care center of endocrinology and metabolism—for hyperandrogenism and/or menstrual dysfunction, who were submitted to a hyperinsulinemic euglycemic clamp and had a homogeneous serum insulin assay, were included. Since March 2010, the glucose clamp has been part of a systematic phenotyping of PCOS women referred to our Unit and recruited into the Verona 3 P Study (Moghetti *et al.*, 2013). Before that date, the clamp was carried out in patients participating in specific protocols.

Overall, 307 of these women had PCOS according to the original 1990 NIH criteria (classic-PCOS) (Zawadzki and Dunaif, 1992), i.e. they had clinical and/or biochemical hyperandrogenism associated with oligoanovulation, after exclusion of secondary causes. Among the remaining 99 women, 63 had hyperandrogenism and 36 oligoanovulation. Of these 99 subjects, 68 showed ultrasound evidence of micropolycystic ovarian morphology, as defined by the Rotterdam criteria (Rotterdam ESHRE/ASRM, 2004) and were classified as women with either normoandrogenic ($n = 29$) or ovulatory ($n = 39$) phenotypes of PCOS. Therefore, according to the Rotterdam criteria, 375 women, 92.4% of the whole cohort, had PCOS and were included in the analyses.

Clinical hyperandrogenism was defined by the presence of hirsutism (modified Ferriman–Gallwey score ≥8) (Hatch *et al.*, 1981) and/or acne and/or alopecia; biochemical hyperandrogenism was defined by increased levels of at least one serum androgen (total or free testosterone, androstenedione or dehydroepiandrosterone sulfate (DHEAS)). Oligoanovulation was defined as <8 menstrual cycles per year or serum progesterone levels <12 nmol/L in the luteal phase of two subsequent menses. Secondary causes of PCOS were ruled out by medical history and systematic 17-hydroxyprogesterone, prolactin, and thyroid-stimulating hormone (TSH) assays.

No patients were suffering from any other diseases or were taking medications potentially interfering with the study. In particular, no subjects had received oral contraceptives, insulin-sensitizing agents, other anti-diabetic medications, antiandrogens, or glucocorticoids in the 6 months prior to evaluation.

A sample of 41 non-hirsute, normal-weight, healthy women, with regular menses and normal ovarian morphology, recruited through advertisements at the local University, nursing school and Verona City Hospital, served to define the cut-off values of surrogate indexes of insulin resistance. These cut-offs were defined by the mean plus or minus, as appropriate, 2 SD of values in healthy subjects. The cut-off value for clamp data was determined from historical data of healthy subjects with similar characteristics, as previously described (Moghetti *et al.*, 2013).

Ethical approval

All subjects gave their written informed consent before the inclusion in the database. The study was approved by our institutional Ethical Committee.

Protocol

All subjects had undergone a complete medical examination. Blood samples for measurement of metabolic parameters (fasting glucose and insulin, total and high-density lipoprotein (HDL) cholesterol, and triglycerides) and androgens were collected at 08.00 am, after overnight fasting.

The hyperinsulinemic euglycemic clamp was performed as previously described (Moghetti *et al.*, 1996), at an insulin infusion rate of 80 mU/m^2 min. Because muscle is responsible for most insulin-induced glucose metabolism (DeFronzo, 1988), glucose disposal data were expressed per fat-free mass (mg/kg FFM min).

A 75 g oral glucose tolerance test was also performed in 349 of these patients: in 278 of them, glucose and insulin were measured before and after 30, 60, 90 and 120 min from glucose ingestion; in another 51 women, glucose and insulin were measured before and after 120 min only.

From these data, several surrogate markers of insulin resistance were calculated (Table I). In particular, homeostasis model assessment (HOMA), glucose/insulin (G/I) ratio and quantitative insulin sensitivity check index (QUICKI) indexes, based on fasting glucose and insulin concentrations, were available in all women; whilst Gutt, Stumvoll$_{0-120}$, and Matsuda indexes, based on values recorded during the oral glucose load, were available in 329, 329 and 278 subjects, respectively.

Diagnosis of metabolic syndrome was carried out using the 2009 joint criteria by the International Diabetes Federation (IDF) and other Societies (Alberti *et al.*, 2009). All elements required for diagnosis were available in 348 women, as HDL cholesterol was not recorded in 27 patients.

Assays

Plasma glucose was measured using a glucose analyzer (YSI-2300 StatPlus; YSI Inc, Yellow Springs, OH). Insulin was assayed by an immunoradiometric method (Biosource, Fleurus, Belgium), cross-reactivity with proinsulin being <5%.

Serum lipids were determined by automated laboratory procedures (Dimension Vista 1500, Siemens, Erlangen, Germany).

In 232 women, investigated between 2010 and 2015, total testosterone and androstenedione were measured by liquid chromatography tandem

Table I Surrogate indexes of insulin resistance assessed in the study.

Index	Reference	Calculation
HOMA	Matthews *et al.* (1985)	Fasting glucose, mmol/L × fasting insulin, mU/L/22.5
G/I ratio	Legro *et al.* (1998)	Fasting glucose, mg/dL/fasting insulin, mU/L
QUICKI	Katz *et al.* (2000)	1/Log (fasting insulin, mU/L) + log (fasting glucose, mg/dL)
Gutt index	Gutt *et al.* (2000)	{[75 000 + (fasting glucose, mg/dL − $glucose_{120'}$) × 0.19 × body weight, kg/120]/[(fasting glucose + $glucose_{120'}$)/2]}/log[(fasting insulin, mU/L, + $insulin_{120'}$)/2]
$Stumvoll_{0-120}$	Stumvoll *et al.* (2001)	0.156–0.0000459 × $insulin_{120'}$, pmol/L − 0.000321 × fasting insulin − 0.00541 × $glucose_{120'}$, mmol/L
Matsuda	Matsuda and DeFronzo (1999)	10000/[(Fasting glucose, mg/dL × fasting insulin, mU/L) × ($glucose_{30'}$ + $glucose_{60'}$ + $glucose_{90'}$ + $glucose_{120'}$)/4 × ($insulin_{30'}$ + $insulin_{60'}$ + $insulin_{90'}$ + $insulin_{120'}$)/4]$^{0.5}$

HOMA, homeostasis model assessment; G/I, glucose/insulin; QUICKI, quantitative insulin sensitivity check index.

mass spectrometry, and free testosterone fraction was assessed by equilibrium dialysis, as previously described (Tosi *et al.*, 2016). In this subgroup of women, hyperandrogenemia was defined as follows: total testosterone >41 ng/dL, androstenedione >240 ng/dL, free testosterone >0.49 ng/dL.

In the remaining 143 women, investigated between 1998 and 2009, total testosterone and androstenedione were measured by direct radioimmunoassay methods (Diagnostic Systems Laboratories, Webster, TX). In these patients cut-offs to define hyperandrogenemia were as follows: total testosterone >86.3 ng/dL, androstenedione >430 ng/dL.

DHEAS was evaluated in all subjects by an automated chemiluminescent method (Immulite 2000, Siemens, Erlangen, German), using a cut-off value >400 ug/dL.

Calculations

Glucose disposal rate during the steady-state period of the clamp (M-clamp) was calculated with standard formula (DeFronzo *et al.*, 1979).

Surrogate indexes of insulin sensitivity were calculated as detailed in Table I.

Specificity and sensitivity of surrogate indexes and their positive (PPV) and negative (NPV) predictive values in identifying insulin resistant individuals, as defined by M-clamp values below the reference limit (11.76 mg/kg FFM min), were calculated.

Statistics

Continuous variables were described as mean ± SD, whereas categorical variables were summarized by percentages.

Comparisons of continuous variables between subgroups of patients were made by ANOVA. Multiple post-hoc comparisons were performed using the Bonferroni correction. Not normally distributed variables were log or square-root transformed before analysis. Fasting glucose and serum triglycerides could not be normalized and were analyzed by the Mann Whitney test. The Chi-square test was used for analyzing categorical variables.

The performance of surrogate indexes in identifying insulin resistant subjects was assessed by ROC analysis.

P values <0.05 were considered statistically significant. Analyses were performed using STATA version 10.1 (Stata-Corp, College Station, TX).

Results

Table II summarizes the main characteristics of women. Clinical hyperandrogenism was recorded in 74.4% and biochemical hyperandrogenism in 63.3% of subjects. Oligoanovulation was found in 84.5% of patients.

Body weight excess was observed in 54.1% of women. In particular, 18.9% of patients were overweight (BMI 25–29.9 kg/m^2), and 35.2% obese (BMI > 30 kg/m^2). Twenty-six women (6.9%) had impaired fasting glucose (IFG). Among the 349 women submitted to oral glucose tolerance test (OGTT), 34 (9.7%) had impaired glucose tolerance (IGT) and 3 had type 2 diabetes. Overall, 50 women showed

Table II Main characteristics of subjects.

	Mean ± SD	Reference interval
Age (y)	23.1 ± 5.3	–
Caucasian (%)	97.1	–
BMI (kg/m^2)	27.6 ± 7.1	18.5–24.9
Waist circumference (cm)	88.2 ± 17.4	<80
Fat mass by bioimpedance (kg)	26.7 ± 13.4	–
Fat-free mass by bioimpedance (kg)	47.3 ± 8.0	–
Ferriman–Gallwey score	9.3 ± 6.3	<8
Hirsutism (%)	60.4	–
Systolic blood pressure (mmHg)	121 ± 13	<130
Diastolic blood pressure (mmHg)	76 ± 10	<85
Fasting glucose (mg/dL)	85.1 ± 10	70–99
Fasting insulin (mU/L)	15.7 ± 11.8	<9
Glucose 2h-OGTT (mg/dL)	101 ± 31	<140
Insulin 2h-OGTT (mU/L)	112 ± 124	–
HOMA	3.4 ± 2.79	<3.0
G/I ratio	8.67 ± 6.18	≥7.0
QUICKI	0.34 ± 0.04	≥0.32
Gutt index	4.49 ± 1.63	≥3.75
$Stumvoll_{0-120}$	0.052 ± 0.069	≥0.080
Matsuda	3.43 ± 2.28	≥3.5
M-clamp (mg/KgFFM min)[a]	9.66 ± 3.23	>11.76
Total cholesterol (mg/dL)	169 ± 34	<200
HDL-cholesterol (mg/dL)[b]	51.7 ± 13.8	≥50
LDL-cholesterol (mg/dL)[b]	99.4 ± 28.9	<130
Triglycerides (mg/dL)	88.0 ± 59.7	<150

[a]M-clamp: insulin sensitivity as assessed by the hyperinsulinemic euglycemic clamp.
[b]Available in 348 women. OGTT, oral glucose tolerance test; HOMA, homeostasis model assessment; G/I, glucose/insulin ratio; QUICKI, quantitative insulin sensitivity check index.

glucose metabolism alterations (IFG and/or IGT and/or diabetes). Metabolic syndrome was found in 99 (28.4%) out of the 348 patients evaluable from this point of view.

Mean M-clamp was 9.66 ± 3.23 mg/kg FFM min, significantly lower than the reference limit of our Lab (11.76 mg/kg FFM min). In particular, 281 women (74.9%) were identified as insulin resistant by the glucose clamp. The percentage of insulin resistant subjects was 59.3% in normal-weight, 77.5% in overweight, and 93.9% in obese patients.

M-clamp, adjusted for differences in BMI, was progressively lower in women with the normoandrogenic, ovulatory or classic phenotypes of PCOS (12.2 ± 3.6, 10.8 ± 3.3 and 9.3 ± 3.1 mg/kg FFM min, respectively, $P < 0.001$). The percentage of insulin resistant subjects was 37.9, 64.1 and 79.8%, respectively, in these subgroups ($P < 0.001$). Among women with classic-PCOS who had an assessment of ovarian morphology in agreement with the Rotterdam recommendations, M-clamp was slightly higher in subjects with ($n = 209$) than in those without ($n = 32$) PCO morphology (9.56 ± 3.2 vs 8.38 ± 2.8 mg/kg FFM min, respectively, $P = 0.031$).

Relationships between M-clamp and surrogate indexes

Figure 1 shows the relationships between M-clamp and each of the surrogate markers assessed in the study. All correlations were statistically highly significant (R values 0.526–0.665). However, explained variance of the clamp data was between 28 and 44%, indicating a poor performance of all markers.

The imprecision of these markers was highlighted when they were used to classify the individual subjects with PCOS, as insulin resistant or insulin sensitive. Whilst 74.9% of women showed an impaired insulin action by the glucose clamp, the percentage identified by surrogate indexes was substantially lower (HOMA 41.1%, G/I ratio 48.3%, QUICKI 46.7%, Gutt 35.6%, $Stumvoll_{0-120}$ 55.3%, Matsuda 59.4%; all $P < 0.001$ vs M-clamp).

Table III reports sensitivity and specificity, and the PPV and NPV of these markers in recognizing insulin resistant individuals. In general, specificity of surrogate markers was fair (76–94%), but sensitivity was low (45–71%). As a consequence, the PPV of surrogate indexes was good (90–96%), but the NPV was weak (36–45%). In other words, by using any of these surrogate markers, there were a few falsely positive but many falsely negative insulin resistant individuals. By combining these indexes sensitivity was only slightly improved. The best combination was QUICKI + Matsuda (sensitivity 75.5%, specificity 73.9%).

Because sensitivity and specificity, and the derived parameters, are affected by the choice of the cut-off values, we explored the effects of using different cut-offs. As expected, specificity and PPV were improved by increasing the cut-offs, but sensitivity and NPV were further worsened, without substantial improvement of results (data not shown).

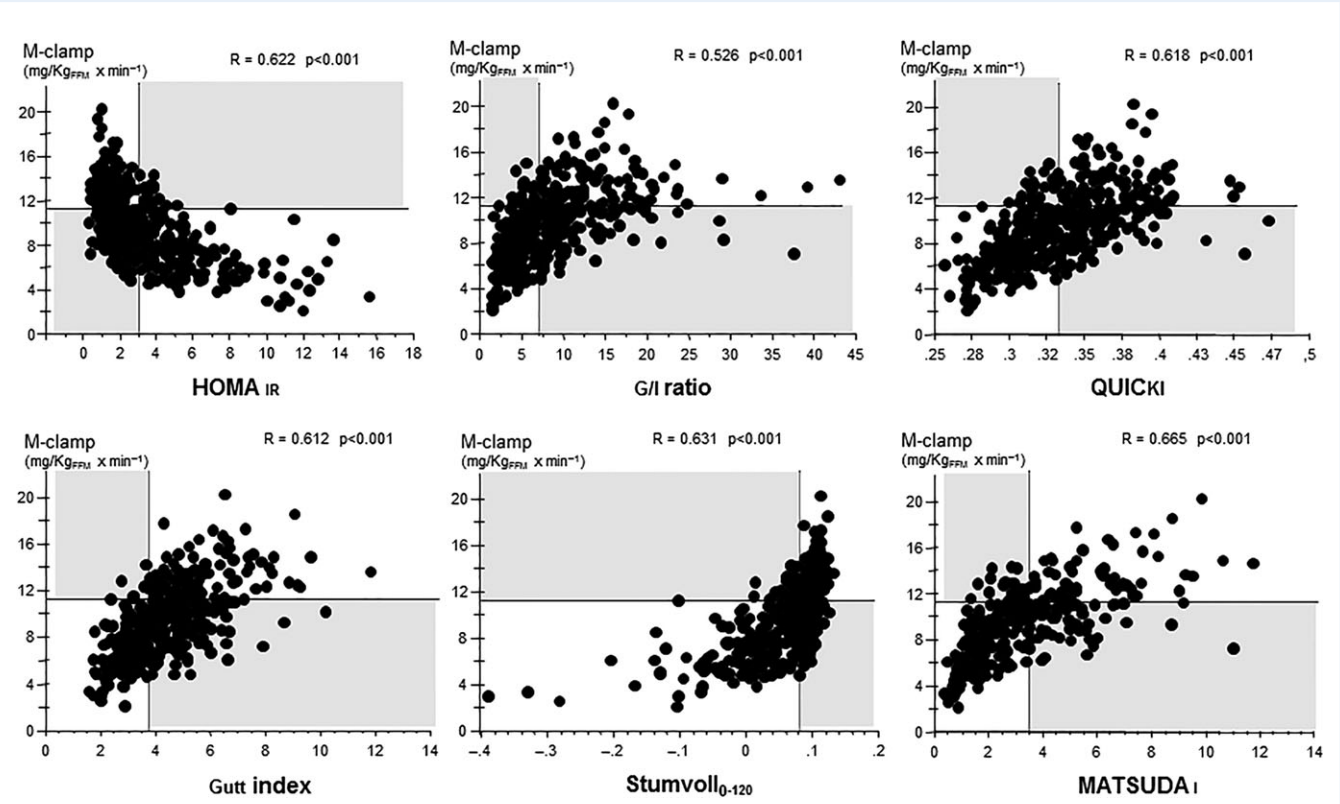

Figure 1 Correlations between M-clamp values and surrogate indexes of insulin sensitivity investigated in the study (upper panels: HOMA, G/I ratio, QUICKI; lower panels: Gutt index, $Stumvoll_{0-120}$, Matsuda index). In each panel the horizontal line indicates the cut-off for insulin resistance defined by the glucose clamp, whereas the dotted vertical line indicates the cut-off defined by the surrogate index. The shaded areas indicate the subjects inappropriately classified by surrogate indexes as insulin resistant or insulin sensitive.

Table III Sensitivity, specificity, positive and negative predictive values, and performance by ROC analysis of surrogate indexes in identifying insulin resistant subjects, as defined by the hyperinsulinemic euglycemic clamp.

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	ROC analysis	
					AUC	95% CI
HOMA	50.9	88.3	92.9	37.6	0.798	0.751–0.844
G/I ratio	59.8	86.2	92.8	41.8	0.809	0.763–0.855
QUICKI	57.7	86.2	92.6	40.5	0.798	0.752–0.845
Gutt index	45.2	93.8	95.7	35.8	0.793	0.741–0.845
$Stumvoll_{0-120}$	67.3	81.5	91.8	44.9	0.827	0.779–0.874
Matsuda	70.6	76.1	90.3	45.1	0.825	0.772–0.878

ROC analysis showed that performances of all indexes were similar (AUC values ranging between 0.793 and 0.827) (Table III). These figures were better than the performance of metabolic syndrome diagnosis, a clinical proxy for insulin resistance, which showed an AUC value of 0.653 (95% CI: 0.615–0.691).

As expected, the vast majority of subjects with metabolic syndrome were recognized as insulin resistant by the glucose clamp (94.9%). Even in this selected subgroup of subjects, surrogate indexes identified lower percentages of insulin resistant individuals (Fig. 2). However, the fraction of insulin resistant women missed by the surrogate indexes was greater among women without metabolic syndrome. Similarly, the inability of surrogate indexes to identify insulin resistant subjects was higher among normal-weight than overweigh/obese patients (Fig. 2).

Discussion

In this study, we measured insulin action *in vivo* by the gold standard euglycemic clamp in a large cohort of women with PCOS, and compared these results with those of several surrogate indexes of insulin resistance, commonly used in epidemiological studies and clinical practice. We confirmed that insulin resistance is a very common feature in these women, in accordance with the findings of most of the small studies which previously investigated this issue by using this methodology (Cassar *et al.*, 2016). We also confirmed and extended our previous findings on differences in terms of insulin resistance between PCOS phenotypes (Moghetti *et al.*, 2013), showing there was a scale in metabolic risk of these subjects, independent of BMI. Insulin resistance progressively worsened from the normoandrogenic to the ovulatory and to the classic phenotype.

However, the most important finding of this study is the demonstration of the substantial pitfalls of surrogate indexes in identifying insulin resistant individuals among PCOS women. Collectively, these indexes showed a high PPV (90–96%), but a low NPV (36–45%). In other terms, many subjects with insulin resistance were not recognized by any of these surrogate markers. The limited ability of surrogate indexes to identify insulin resistant subjects was somewhat less evident in women with obesity and/or metabolic syndrome, whereas it was prominent in subjects without these alterations.

The incomplete agreement between these indexes and direct measurement of insulin action was confirmed by the ROC analysis, which is not affected by the potential bias associated with the choice of any specific cut-off. In this analysis the AUC is equal to the probability of concordance between tests. Interestingly, the AUC values of all surrogate indexes, as compared with the reference method, were similar, around 0.80.

The impairment of insulin action in PCOS subjects is of paramount clinical relevance for at least two reasons. First, it is a fundamental underlying mechanism for both type 2 diabetes and metabolic syndrome (Reusch, 2002), which are recognized risk factors for cardiovascular disease. Second, insulin resistance plays a crucial role in the pathogenesis of PCOS itself (Diamanti-Kandarakis and Dunaif, 2012). Thus, to ascertain whether a PCOS woman is insulin resistant would be important not only from a speculative perspective, but also in order to establish an appropriate monitoring of her individual metabolic risk. A (reliable) assessment of individual insulin resistance might offer key information from this point of view.

To overcome the complexity of the clamp methodology, several surrogate indexes of insulin resistance have been proposed. Although several studies reported good correlations between each of these indexes and M-clamp values in different conditions, others did not, suggesting that these indexes may be useful in large epidemiological studies but of limited worth for clinical purposes. As thoroughly discussed by Buchanam *et al.*, in an editorial on this issue, correlation coefficients are measures of association but they do not indicate whether two variables are quantitatively equivalent. A test of concordance is required to establish quantitative agreement between different measures. Moreover, surrogate indexes should be validated in each specific setting before they can be appropriately used (Buchanan *et al.*, 2010).

In contrast with this premise, very few studies have assessed the performance of surrogate indexes of insulin resistance by comparing them with the glucose clamp data in women with PCOS, and have produced conflicting results. Ducluzeau et al, in 16 obese PCOS women and 10 controls, reported a good correlation between M-clamp and the G/I ratio (Rho = 0.68) (Ducluzeau *et al.*, 2003). Kim et al, in 63 women with PCOS, reported *R* values of 0.40 between M-clamp and both HOMA and QUICKI indexes in obese patients but lower, non significant values in lean patients (Kim *et al.*, 2006). Finally, Diamanti-Kandarakis *et al.*, in 59 normoglycemic women with PCOS, did not find any statistically significant correlations between M-clamp and either HOMA or QUICKI indexes (Diamanti-Kandarakis *et al.*, 2004).

Some studies, carried out in other clinical conditions, suggested that surrogate indexes derived from the OGTT could perform better, as compared with those obtained from fasting values (Matsuda and DeFronzo, 1999; Gutt *et al.*, 2000; Stumvoll *et al.*, 2001), although the amount of additional information may indeed be limited. No data were previously available from this point of view in women with PCOS.

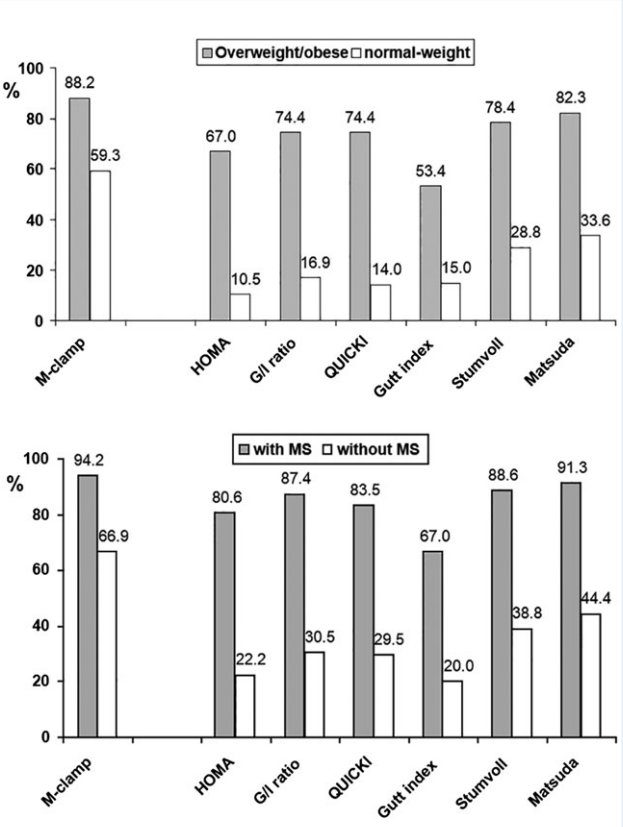

Figure 2 Percentages of insulin resistant PCOS subjects identified by the hyperinsulinemic euglycemic clamp and by each surrogate index of insulin resistance (HOMA, G/I ratio, QUICKI; Gutt index, $Stumvoll_{0-120}$ and Matsuda): upper panel, in overweight/obese (grey bars) or normal-weight (white bars) women; lower panel, in women with (grey bars) or without (white bars) metabolic syndrome (MS) (for all comparisons, *P* values <0.001 between results of glucose clamp and surrogate indexes).

Which take home messages can be drawn from these findings? First, surrogate indexes cannot be considered reliable alternatives to the glucose clamp in assessing individual insulin sensitivity, and underestimate the prevalence of insulin resistance among PCOS women, especially in subjects who are non-obese and who do not have overt metabolic syndrome. Second, whilst glucose clamp, due to its complexity, cannot be proposed in clinical practice, it is required in studies in which a clear distinction between insulin resistant and insulin sensitive subjects is a critical issue.

The main strengths of this study are the large cohort of women investigated, the use of gold standard methodology to assess *in vivo* insulin sensitivity, the evaluation of the performance of many different surrogate indexes, and the presence of a well characterized control group to define the normal limits of M-clamp and surrogate indexes. A limitation of the study, as regards the prevalence of insulin resistance in these women, is the potential referral bias of patients investigated, which may have caused overestimation of this figure (Ezeh *et al.*, 2013). Another limitation regards differences in methods used for androgen assay, although gold standard methodology was used in most subjects. Finally, this was a monocentric study, which is both a strength and a limitation, as it ensures homogeneity in the assessment of insulin sensitivity, but at the same time suggests a need for confirmation in other cohorts.

In conclusion, this study, carried out in a large cohort of women, demonstrates that insulin resistance, as assessed by the glucose clamp technique, is a very common feature in women with PCOS, even in normal-weight subjects. Surrogate indexes of insulin action show a low sensitivity in identifying insulin resistant subjects, which causes many subjects to be erroneously diagnosed as insulin sensitive.

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Authors' roles

F.T. and P.M. analyzed and interpreted data, and wrote the article, E.B. contributed to the discussion and reviewed/edited the article.

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Conflict of interest

The authors have nothing to disclose.

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