Clustering of Cardiovascular Risk Factors Associated With the Insulin Resistance Syndrome

Assessment by principal component analysis in young hyperandrogenic women

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OBJECTIVE — Hyperinsulinemia is often associated with several metabolic abnormalities and increased blood pressure, which are risk factors for cardiovascular disease. It has been hypothesized that insulin resistance may underlie all these features. However, recent data suggest that some links between insulin resistance and these alterations may be indirect. The aim of our study was to further investigate this issue in a sample of young hyperandrogenic women, who often show insulin resistance and other metabolic abnormalities typical of the insulin resistance syndrome.

RESEARCH DESIGN AND METHODS — We tested the hypothesis of a single factor underlying these features by principal component analysis, which should recognize one component if a single mechanism explains this association. The analysis was carried out in a sample of 255 young nondiabetic hyperandrogenic women. Variables selected for this analysis included the basic features of the insulin resistance syndrome and some endocrine parameters related to hyperandrogenism.

RESULTS — Principal component analysis identified four separate factors, explaining 64.5% of the total variance in the data: the first included fasting and postchallenge insulin levels, BMI, triglycerides, HDL cholesterol, and uric acid; the second, BMI, blood pressure, and serum free testosterone; the third, fasting plasma glucose, postchallenge glucose and insulin levels, serum triglycerides, and free testosterone; and the fourth, postchallenge plasma insulin, serum free testosterone, and gonadotropin-releasing hormone agonist–stimulated 17-hydroxyprogesterone.

CONCLUSIONS — These results support the hypothesis of multiple determinants in the clustering of abnormalities in the so-called insulin resistance syndrome.

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Many subjects show a clustering of metabolic abnormalities, suggesting that these alterations have shared pathogenetic mechanisms. These abnormalities include hyperglycemia, obesity, dyslipidemia, and hypertension, which are classical risk factors for cardiovascular disease (1–3). As impaired insulin action is a common finding in these subjects, it has been hypothesized that insulin resistance and the associated hyperinsulinemia may be the common link among these alterations. Clustering of these abnormalities has been called insulin resistance syndrome, metabolic syndrome, or syndrome X. According to the working definition recently proposed by the National Cholesterol Education Program Expert Panel (4), prevalence of the metabolic syndrome in the U.S. general population is as high as 20–25% (5).

The high prevalence and the serious implications of this condition indicate the need for an integrated preventive strategy. However, it remains unclear whether insulin resistance directly underlies all these features or whether some links are indirect, being mediated by other factors. A recent report by Meigs et al. (6) supported this latter hypothesis. These authors assessed, by factor analysis, clustering of cardiovascular risk variables in a large cohort of nondiabetic subjects of the Framingham Offspring Study. Their results were consistent with three different factors underlying the classical features of the insulin resistance syndrome: a central metabolic domain, comprising hyperinsulinemia, dyslipidemia, and obesity; a glucose intolerance domain, linked to the central domain through shared correlations with insulin levels; and a hypertension domain, linked to the central domain through BMI. However, it could be hypothesized that a multiple factor pattern emerges only in an advanced stage of the natural history of the syndrome and that in an earlier phase, when metabolic abnormalities are still subtle, the entire spectrum of alterations would appear directly linked to insulin resistance.

To further assess this issue, we tested the hypothesis of a single factor underlying features of syndrome X in a sample of young hyperandrogenic women. These subjects often show insulin resistance and other metabolic abnormalities typical of the insulin resistance syndrome (7–10). They thus represent a valuable model to study the relationships between metabolic abnormalities in a relatively early phase of their natural history. In many of these women, hyperandrogenism itself, especially ovarian hyperandrogenism, appears to be generated by hyperinsulinemia (7,11,12), suggesting that it is a

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Abbreviations: DHEAS, dehydroepiandrosterone sulfate; GnRH, gonadotropin-releasing hormone.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

component of the insulin resistance syndrome. Interestingly, polycystic ovary syndrome was recently included among the risk factors of the insulin resistance syndrome (13).

RESEARCH DESIGN AND METHODS

Two hundred and fifty-five nondiabetic hyperandrogenic women, recruited from the outpatients referred to our division for hirsutism, acne, and/or hyperandrogenic oligoamenorrhea, were included in the study. All of them were Caucasian. In all women, androgen-secreting tumors, congenital adrenal hyperplasia, thyroid dysfunction, hyperprolactinemia, or Cushing's syndrome were ruled out. Hirsutism was defined as a score of eight or more on the Ferriman-Gallwey scale, as modified by Hatch et al. (14). Oligoamenorrhea was defined as menstrual intervals >6 weeks. Forty healthy women, with regular ovulatory cycles and normal serum androgens, served as control subjects. All women were studied in the early follicular phase (days 3–8) of the menstrual cycle or after at least 3 months of amenorrhea. No woman suffered from any other disease or was taking medications or oral contraceptives. Subjects gave their informed consent to the study, which was conducted in accordance with the Helsinki Declaration and approved by our local ethical committee.

The women had a complete physical examination and assessment of endocrine and metabolic profiles. Physical examination comprised assessment of height, weight, and blood pressure, which was measured by a mercury sphygmomanometer, with the subject in the sitting position, after at least 5 min of rest.

Baseline blood samples were collected at ~8:00 A.M., after overnight fasting, for measurements of serum free testosterone, dehydroepiandrosterone sulfate (DHEAS), gonadotropins, HDL cholesterol, triglycerides, and uric acid. Assessment included a 75-g oral glucose tolerance test, with plasma glucose and insulin measurements at 0, 30, 60, and 120 min.

In these women, a gonadotropin-releasing hormone (GnRH) agonist challenge was also carried out (15). An increased serum 17-hydroxyprogesterone response to GnRH agonist stimulation is considered a hallmark of ovarian hyperandrogenism (16,17). This test was carried out by subcutaneous injection of 0.1 mg buserelin (Suprefact; Hoechst Roussel, Milan, Italy). Twenty-four hours after drug administration, blood was drawn for assay of stimulated serum gonadotropins and 17-hydroxyprogesterone levels. All blood samples for hormonal assays were immediately centrifuged after withdrawal, and supernatant was separated and frozen at −20°C until assayed.

Table 1—*Variables collected on the 255 women of the study and 40 healthy control subjects*

	Median (interquartile range)	Median (interquartile range) for 40 control subjects
Age (years)	22 (20–26)	24 (22.5–28.5)
BMI (kg/m^2)	23.2 (20.9–27)	21.1 (20.1–26.2)
Fasting plasma glucose (mmol/l)	4.5 (4.2–4.8)	4.8 (4.5–5.1)
Postchallenge glucose (mmol/l)	7.1 (6.2–8.1)	7.0 (6.4–8.2)
Fasting plasma insulin (pmol/l)	79 (57–112)	60 (43–79)
Postchallenge insulin (pmol/l)	682 (450–1,177)	479 (359–642)
HDL cholesterol (mmol/l)	1.45 (1.28–1.74)	1.59 (1.30–2.03)
Triglycerides (mmol/l)	0.88 (0.67–1.11)	0.75 (0.64–0.95)
Uric acid (mmol/l)	0.27 (0.22–0.32)	0.23 (0.19–0.25)
Systolic blood pressure (mmHg)	120 (115–130)	120 (110–130)
Diastolic blood pressure (mmHg)	75 (70–80)	70 (65–80)
Free testosterone (pmol/l)	7.4 (5.3–11.8)	5.2 (3.5–6.2)
DHEAS (ng/ml)	6.67 (4.72–8.36)	4.09 (2.59–5.75)
Leutinizing hormone–to–follicle-stimulating hormone ratio	1.13 (0.73–2.00)	0.75 (0.59–1.17)
Stimulated 17-hydroxyprogesterone (nmol/l)	10.8 (7.5–15.4)	5.5 (4.6–6.6)

Assays

Plasma glucose was assayed with a glucose-oxidase method in an automated analyzer (Beckman Instruments, Palo Alto, CA). Plasma insulin was measured by a specific immunoradiometric method, using a kit by Medgenix Diagnostics (Fleurus, Belgium), cross-reactivity with human proinsulin being <5%. Serum uric acid was measured by a commercial enzymatic method (Uricase-PAP) in an automatic analyzer. Serum steroids, gonadotropins, and lipids were assayed as previously described (18,19). All hormone measurements were performed in duplicate.

Statistical analysis

Variables were selected according to the basic features of the insulin resistance syndrome (BMI, systolic and diastolic blood pressure, plasma glucose and insulin at fasting and after oral glucose, serum HDL cholesterol, tryglycerides, and uric acid) (1,3). In the light of the specific characteristics of the population sample, and of the recently proposed inclusion of ovarian androgen excess among features of the metabolic syndrome, some variables related to hyperandrogenism were also considered (free testosterone, DHEAS, leutinizing hormone–to–follicle-stimulating hormone ratio, and 17-hydroxyprogesterone after GnRH agonist stimulation). Associations between the considered variables were calculated by Pearson's correlation coefficients. When necessary, variables were log transformed to achieve normality of distribution.

We investigated the hypothesis of a single factor underlying the original variables by principal component analysis, which should recognize one component if a single mechanism explains this association. Principal component analysis was performed on the 255 hyperandrogenic women. This procedure is explained in detail in (6). Briefly, this analysis studies the correlations among several interrelated quantitative variables by grouping the variables into a few components. After grouping, the variables within each component are more highly correlated with variables in that component than with variables in other components. Variables weakly correlated to the others are not suitable for this kind of analysis.

The amounts of total variance attributable to the components are commonly known as "eigenvalues." They are the sum of the squared correlations between the original independent variables and the principal components. To avoid models

with an excessive number of factors, we excluded components with eigenvalues equal to or barely exceeding unity, i.e., we selected components with more variance of the original standardized variables. Usually, the initial component extraction is not interpretable. To produce interpretable components, the selected principal components were modified using the orthogonal varimax method. This procedure transforms the original components into other components uncorrelated with each other but highly correlated with unique subgroups of the studied variables. Factor loadings (correlations between the components and the original variables) $>\pm 0.30$ were considered. Statistical analyses were carried out by SPSS 13.0 for Windows software (SPSS, Chicago, IL).

RESULTS — Table 1 shows the main characteristics of the study sample compared with healthy control subjects. As expected, serum free testosterone and DHEAS were higher in these women. Increased serum 17-hydroxyprogesterone after GnRH agonist stimulation was consistent with the ovarian origin of androgen excess in most of these subjects. These young hyperandrogenic women had increased insulin levels and presented subtle metabolic abnormalities commonly associated with the insulin resistance syndrome. Despite BMI being normal or slightly increased in most of these subjects, 17.3% (95% CI 12.8–22.5) met the criteria for insulin resistance syndrome (13).

For an initial evaluation of risk variable clustering, the correlation matrix between variables of interest was considered (Table 2). Serum DHEAS and leutinizing hormone–to–follicle-stimulating hormone ratio, weakly correlated with the vast majority of the variables, were excluded from the following analyses. Free testosterone and postchallenge 17-hydroxyprogesterone, variables related to hyperandrogenism, were both correlated with several metabolic features.

Principal components analysis identified four dominant factors, explaining 64.5% of the total variance in the data. Factor loading patterns, after orthogonal rotation of the correlation matrix, are shown in Table 3. The first factor included fasting and postchallenge insulin levels, BMI, HDL cholesterol, triglycerides, and uric acid; the second, BMI, blood pressure, and free testosterone; the

Table 2—*Pearson's correlation coefficients among the variables of interest*

	Fasting glucose	OGTT: glucose	Fasting insulin	OGTT: insulin	BMI	HDL cholesterol	Triglycerides	Uric acid	Diastolic blood pressure	Systolic blood pressure	Free testosterone	DHEAS	Ln (stimulated 17OHP)
OGTT: glucose	0.54*	—											
Fasting insulin	0.27*	0.16†	—										
OGTT: insulin	0.25*	0.38*	0.59*	—									
BMI	0.30*	0.24*	0.46*	0.31*	—								
HDL cholesterol	−0.18‡	−0.12	−0.23*	−0.19‡	−0.23*	—							
Triglycerides	0.26†	0.29†	0.37†	0.43†	0.32†	−0.27†	—						
Uric acid	0.04	0.09	0.40*	0.34*	0.44*	−0.13†	0.24*	—					
Diastolic blood pressure	0.13†	0.19†	0.35*	0.32*	0.42*	−0.15†	0.34*	0.39*	—				
Systolic blood pressure	0.23*	0.22*	0.32*	0.29*	0.41*	−0.08	0.29*	0.20*	0.69*	—			
Free testosterone	0.20*	0.25*	0.21*	0.30*	0.27*	−0.15†	0.27*	0.16†	0.36*	0.41*	—		
DHEAS	0.04	0.06	−0.11	0.06	−0.10	−0.04	−0.05	0.06	−0.04	0.10	0.26*	—	
Ln(stimulated 17OHP)	−0.04	0.12	0.21*	0.25*	0.01	0.02	0.18‡	0.30*	0.38*	0.18‡	0.36*	0.10	—
LH/FSH ratio	0.07	0.09	0.04	0.08	−0.08	0.05	0.04	0.05	0.18‡	0.10	0.28*	−0.06	0.41*

*$P < 0.001$; †$0.01 < P < 0.05$; ‡$0.001 < P < 0.01$. Stimulated 17OHP, 17-hydroxyprogesterone after GnRH agonist test. LH/FSH, leutinizing hormone–to–follicle-stimulating hormone; OGTT, oral glucose tolerance test.

Table 3—*Loadings of principal components after orthogonal rotation*

	Components			
	1: core of the metabolic syndrome	2: hypertension	3: glucose intolerance	4: hyperandrogenism
Fasting plasma glucose	0.16	0.14	**0.79**	−0.19
Postchallenge glucose	0.11	0.09	**0.83**	0.13
Fasting plasma insulin	**0.77**	0.17	0.13	0.10
Postchallenge insulin	**0.65**	0.06	**0.36**	**0.34**
BMI	**0.56**	**0.53**	0.13	−0.28
HDL cholesterol	**−0.49**	−0.02	−0.17	0.26
Triglycerides	**0.51**	0.17	**0.36**	0.15
Uric acid	**0.67**	0.24	−0.22	0.23
Diastolic blood pressure	0.28	**0.80**	0.01	0.24
Systolic blood pressure	0.11	**0.88**	0.16	0.05
Free testosterone	0.06	**0.50**	**0.33**	**0.41**
Ln(stimulated 17-hydroxyprogesterone)*	0.14	0.17	−0.02	**0.87**
Cumulative percentage of total variance	33.9	46.1	55.9	64.5

The values show the correlation of each variable with the corresponding component: variables with greater loadings characterize that specific component. Cumulative percentages of total variance are also shown. Loadings ≥0.30 are in bold type. *After GnRH agonist challenge.

third, fasting and postchallenge plasma glucose, postchallenge insulin levels, triglycerides, and free testosterone; and the fourth, postchallenge insulin, free testosterone, and stimulated 17-hydroxyprogesterone.

These results obtained in hyperandrogenic women support the hypothesis of multiple determinants in the clustering of the abnormalities in the so-called insulin resistance syndrome. In particular, our data suggest a main component including several metabolic features (insulin levels, BMI, serum HDL cholesterol, triglycerides, and uric acid). The second and the third components included variables related to other metabolic abnormalities commonly associated with insulin resistance, such as those concerning, respectively, blood pressure and glucose tolerance, with the addition in both of serum free testosterone. The fourth component was mainly formed by features of ovarian hyperandrogenism, with the noticeable presence of postchallenge plasma insulin.

CONCLUSIONS — The clustering of insulin resistance and associated hyperinsulinemia with body fat excess, hypertension, and several other metabolic abnormalities (glucose intolerance, dyslipidemia, and hyperuricemia) has been consistently reported and has been called syndrome X, metabolic syndrome, or the insulin resistance syndrome (1–3). It was hypothesized that insulin resistance might be the single underlying mechanism generating all these abnormalities (2). However, these associations could also be due to the clustering of separate components connected by shared elements. This hypothesis was recently supported by the findings of Meigs et al. (6) in the Framingham Offspring Study. Nevertheless, it cannot be excluded that a multiple factor pattern emerges only at an advanced stage of the natural history of the syndrome. In an earlier phase, all features could be still attributed to the presumed ultimate cause, i.e., insulin resistance.

Insulin resistance is also associated with hyperandrogenism, especially in women with the polycystic ovary syndrome (7,11,12,20). Interestingly, these women often show multiple metabolic abnormalities typical of syndrome X. In particular, several authors consistently reported hyperinsulinemia, obesity, altered glucose tolerance, and dyslipidemia in many of these subjects (7–10,21–23). In addition, some studies found increased blood pressure in polycystic ovary syndrome subjects (24–28). These alterations in hyperandrogenic women are usually attributed to impaired insulin action, although direct effects of androgen excess cannot be ruled out.

For these reasons, hyperandrogenic subjects may be considered as a useful model to assess the relationships among the elements included in the insulin resistance syndrome. We thus studied a sample of these women to further test the hypothesis of an underlying common mechanism generating the overall metabolic dysfunction. The principal components analysis was used to identify main clusters of variables. A single major component would have been identified if insulin resistance was the single underlying factor. Interestingly, in these young women metabolic abnormalities were mild. The prevalence of the insulin resistance syndrome was substantially lower than in other studies concerning this issue carried out in hyperandrogenic women (29,30), although it was about three times higher than in the age-matched general population (5). These characteristics of our sample allow us to model the early effects of insulin resistance, avoiding the confounding influences of overt metabolic changes. As ovarian hyperandrogenism itself could be considered a feature of the insulin resistance syndrome, the model included some specific endocrine characteristics.

Four components were identified by the analysis. The central component comprised insulin levels together with BMI, HDL cholesterol, triglycerides, and uric acid. This suggests that, among metabolic abnormalities, obesity, dyslipidemia, and hyperuricemia were directly associated with insulin resistance.

Blood pressure was indirectly linked to the central component through BMI. The indirect relationship between hypertension and insulin resistance is supported by the discordant findings of previous studies. While some authors reported that hypertension was associated with hyperinsulinemia (31–34), others

did not (35–38). Interestingly, this association seems to be more common in obese subjects.

In this population of hyperandrogenic women, BMI and blood pressure were also linked to free testosterone levels. In this regard, inclusion of these features in the same component prompts a number of considerations. Obesity is a common finding in women with ovarian hyperandrogenism (39), although the mechanisms underlying this relationship remain largely undetermined. Fat excess may influence sex hormone metabolism both directly (40) and indirectly by impairing insulin action (41). On the other hand, either androgens or glucocorticoid hormones, which could be both oversecreted in many hyperandrogenic subjects, might favor fat accumulation, particularly in central body sites (42). The few studies assessing blood pressure in hyperandrogenic subjects have yielded different results (24–28,43,44). These discrepancies are not easily explained. Interestingly, some studies reported increased blood pressure only in obese polycystic ovary syndrome women (26–28).

The composition of the third component, including fasting and postload plasma glucose, postload plasma insulin, serum triglycerides, and free testosterone, suggests that glucose tolerance is also distinct from the central component, being linked to it by post–oral glucose tolerance test insulin levels and serum triglycerides. This is consistent on the one hand with the evidence that both insulin resistance and impaired β-cell function contribute to glucose intolerance (45) and on the other hand with the association between hypertriglyceridemia and hyperglycemia (42).

These findings are consistent with those reported by Meigs et al. (6) in the Framingham Offspring Study. These authors performed principal components analysis on main features of the insulin resistance syndrome in a large sample of nondiabetic subjects. In this study, three components were identified: a central component (fasting and postchallenge insulin, BMI, triglycerides, HDL cholesterol, and waist-to-hip ratio), including all the variables of our first component shared by the two studies; an impaired glucose tolerance component (fasting and postchallenge insulin and glucose); and a hypertension component (BMI and blood pressure). These latter components as well substantially matched our results.

It should be noticed that there are remarkable differences between the two samples, as our study included only young hyperandrogenic women, while Meigs's study was carried out on the middle-aged general population of both sexes. Mean BMI and serum lipids were also somewhat different between the two studies. Furthermore, environmental and dietary habits were likely different in Framingham, MA, and Verona, Italy. The very similar results obtained in the two studies, in spite of these differences, strongly support the hypothesis of distinct components in the insulin resistance syndrome. Evidence that young subjects with mild alterations also show separate components in the metabolic syndrome indicates that a multiple factor pattern is already present at an early stage in the natural history of the syndrome, suggesting that this pattern is primitive.

In addition, our study identified a hyperandrogenic component. This component comprised serum free testosterone and GnRH agonist–stimulated 17-hydroxyprogesterone, a hallmark of ovarian hyperandrogenism, linked to the central component by post–oral glucose tolerance test insulin levels. This finding is attributable to the inclusion of additional variables in our sample and to its unique characteristics. The fact that insulin and ovarian androgens gather together is not surprising, as there is evidence of a bidirectional link between hyperinsulinemia and free androgen levels: hyperinsulinemia is thought to stimulate androgen production (7,11,46–48) and also increases testosterone bioavailability by reducing sex hormone–binding globulin synthesis in the liver (49,50). On the other hand, androgen excess in turn seems to impair insulin action (18,51–53). Interestingly, an association between serum free androgens and the metabolic syndrome was recently found also in postmenopausal women (54).

In conclusion, in a sample of hyperandrogenic women, principal components analysis of cardiovascular risk variables associated with insulin resistance identified multiple components. These data are consistent with previous results in the general population, supporting the hypothesis that insulin resistance alone does not underlie the whole expression of the metabolic syndrome. In addition, these findings support the hypothesis that in women free androgen excess is a feature of the metabolic syndrome.

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