Human Reproduction Vol.18, No.11 pp. 2289–2293, 2003 DOI: 10.1093/humrep/deg440

Difference in body weight between American and Italian women with polycystic ovary syndrome: influence of the diet

Enrico Carmina[1,3], Richard S.Legro[2], Kelly Stamets[2], Jennifer Lowell[2] and Rogerio A.Lobo[3,4]

[1]Department of Clinical Medicine, University of Palermo, Italy,[2]Department of Obstetrics and Gynecology, Pennsylvania State University, Hershey, PA and[3]Department of Obstetrics and Gynecology, Columbia University, College of Physicians and Surgeons, New York, USA

[4]To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Columbia University College of Physicians and Surgeons, 622 West 168th Street, New York, NY 10032, USA. E-mail: ral35@columbia.edu

Presented in part at the 83rd Annual Meeting of the Endocrine Society, Denver, Colorado, June 20–23, 2001

BACKGROUND: The study aim was to determine differences in body mass in two populations of women (USA and Italy) with polycystic ovary syndrome (PCOS), and to assess the effect of diet on body mass and cardiovascular risk factors. METHODS: Pools of women with PCOS from the USA (n = 343) and Italy (n = 301), seen between 1993 and 2001, were available for assessment. From these populations, 20 women who were seen consecutively in 2001 at each site had detailed analyses of diet and cardiovascular risk factors. RESULTS: In the entire group, American women had a significantly higher body mass compared with Italian women ($P < 0.01$). Also, the 20 women consecutively evaluated in the USA had a significantly higher mean (± SD) body mass index (40.3 ± 1.0 kg/m^2) than in Italy (29.7 ± 1.0 kg/m^2). US women had worse insulin resistance, lower levels of high-density lipoprotein-cholesterol (HDL-C) ($P < 0.01$) and higher levels of triglycerides ($P < 0.01$). Dietary analysis in the two groups indicated that the total daily calorific intake was similar (USA 2277 ± 109; Italy 2325 ± 68 Kcal), with no appreciable differences in dietary content of protein, carbohydrate and fat. However, the dietary saturated fat content was significantly higher in US women (31.9 ± 3 versus 18.2 ± 2 g/day, $P < 0.01$). Saturated fat intake correlated negatively with HDL-C ($P < 0.01$). CONCLUSIONS: Among women with PCOS, body mass was significantly higher in US women compared with Italian women. However, total calorie intake and dietary constituents were similar, except for a higher saturated fat in US women. It is hypothesized that diet alone does not explain differences in body mass; genetic and lifestyle factors likely contribute. An increased saturated fat intake may worsen the cardiovascular risk profile.

Key words: diet/dyslipidaemia/hyperinsulinaemia/obesity/polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder characterized by hyperandrogenism and frequently, the findings of menstrual irregularity and insulin resistance, which have significant reproductive and metabolic consequences (Carmina and Lobo, 1999; Lobo and Carmina, 2000). Insulin resistance and hyperinsulinaemia may contribute to the hyperandrogenism and anovulation, dyslipidaemia, and glucose intolerance in PCOS. Obesity is another important feature of PCOS which worsens the clinical, endocrine and metabolic features of the syndrome, mostly by increasing insulin resistance and hyperinsulinaemia (Dunaif *et al*., 1989). The prevalence of obesity in PCOS has been estimated to be around 40–50% (Goldzieher and Axelrod, 1963; Yen, 1980; Carmina *et al*., 1992; Balen *et al*., 1995). However, marked variation has been noted in this frequency, which also varies according to ethnicity and geographical location (Lobo and Carmina, 1997).

The pathogenesis of obesity in PCOS is unclear. Obesity could be the consequence of genetic factors, or alternatively be due to lifestyle factors such as diet and a sedentary existence (Bringer *et al*., 1997). More specifically, the role of diet in the genesis of obesity and lipid abnormalities in women with PCOS has not been established. In the general population and in certain ethnic groups, it is well known that diet markedly influences the prevalence of obesity and metabolic abnormalities (Marshall *et al*., 1994; Hodge *et al*., 1996). Diet has also been shown significantly to influence cardiovascular risk (Glick *et al*., 1998; Pekkarine *et al*., 1998).

It was hypothesized that diet would be an important variable which might help explain the differences in weight and

Table I. Mean body mass index (BMI) and centiles of BMI in US and Italian populations of women with PCOS

Parameter	Women with PCOS	
	USA	Italy
No. of women	343	301
BMI (kg/m^2)*	35.05 ± 0.5	26.98 ± 0.3
Centile		
10th	23.6	20.4
25th	27.7	23.3
50th	34.6	26.5
75th	41.1	30.1
90th	47.0	37.5

Values are mean ± SEM.

cardiovascular risk profiles between women with PCOS who come from different ethnic and geographic backgrounds. Accordingly, body weight was assessed in two large and different populations of women with PCOS; from Pennsylvania in the USA and from Palermo, Italy. The initial aim was to determine if body mass was different in these two populations, and then to assess for differences or similarities in diet and metabolic profiles.

Materials and methods

Two large populations of women with PCOS were compared. The first population consisted of 343 women from the USA who were evaluated between 1993 and 2001 in the Department of Obstetrics and Gynecology of Pennsylvania State University. The second population consisted of 301 women from Sicily, Italy, who were evaluated during the same time period at the Department of Endocrinology and the Department of Clinical Medicine of the University of Palermo. These women were seeking help for various complaints of hyperandrogenism, menstrual irregularity and/or the inability to conceive. The mean age of the Italian women was 25.8 ± 1.0 years, and that of the US women 28.1 ± 1.0 years. In both populations, the diagnosis of PCOS was based on the finding of hyperandrogenism, chronic anovulation and the exclusion of Cushing's syndrome, tumours and adrenal enzymatic deficiencies (Zawadski and Dunaif, 1992). The body mass index (BMI) was determined in all women with PCOS.

In 2001, 40 women with PCOS who were seen consecutively either in the USA ($n = 20$) or in Italy ($n = 20$) were subjected to more detailed assessment. These women were not preselected, but were consecutively encountered in Italy and the USA and were seeking assistance for the complaints as noted above. The US women were living in Central Pennsylvania, were non-Hispanic Caucasians, had a mean age of 29 ± 2 years, and were seen in the Department of Obstetrics and Gynecology of Pennsylvania State University.

Twenty other Caucasian white women with a mean age of 26.6 ± 2 years were evaluated during 2001 in the Department of Clinical Medicine of the University of Palermo in Palermo, Italy.

Institutional Review Board approval was obtained at both institutions, and all subjects provided their written informed consent for this study.

In all 40 women, a fasting blood sample was obtained between 8:00 and 9:00 for measurements of LH, FSH, testosterone (T), free or unbound testosterone (uT), dehydroepiandrosterone sulphate (DHEAS), insulin, glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides. Insulin resistance was assessed by the glucose:insulin ratio (Legro *et al*., 1998a).

Dietary analyses were carried out in the two groups of women over a 3-day time period that was considered to be reflective of their normal eating habits. All women participating in the study were sedentary and had not been attempting to gain or lose weight for the previous 6 months. The consecutively evaluated patients at both sites were afforded the opportunity to participate in various ongoing trials regarding the treatment of PCOS. At the time, the US women were about to participate in either an exercise or dietary intervention study. Subjects reported all food, drink, and vitamin and mineral supplements consumed over the 3-day assessment time, estimating serving sizes using common household measures. Mean daily nutrient intake values were obtained from a computer nutrient analysis program (Nutritionist III and IV; N-squared Computing, San Bruno, CA, USA). A dietician performed the diet analysis, which also included the evaluation of saturated and unsaturated dietary fat content. The diet analyses from the USA were sent to Italy and reanalysed for consistency with the diet analyses carried out in Palermo, Italy.

Assays were carried out at the two sites. For glucose, insulin and lipid measurements, identical methods were used. For androgen and gonadotrophin determinations, different assays were used which had almost identical normal ranges (see below). Androgen assays were utilized only to confirm hyperandrogenism.

Hormone assays

At the University of Palermo, serum levels of T, uT and DHEA-S were quantified using well-established radioimmunoassay methods, which were validated previously in the present authors' laboratory (Lobo *et al*., 1980; Stanczyk *et al*., 1991). At Pennsylvania State University, serum levels of T, uT and DHEAS were determined as reported previously (Dunaif *et al*., 1996; Legro *et al*., 1998b). All assays conducted at either site had intra- and inter-assay coefficients of variation (CVs) of <10%.

Metabolic measurements

Glucose and insulin assessment utilized the same method at both sites. Plasma glucose levels were determined using a glucose oxidase technique, and insulin by a double antibody method using commercially available reagents (Linco Research, Inc., St Charles, MO, USA).

Lipid and lipoprotein measurements were carried out using by the same method at both sites (Lopes-Virella *et al*., 1977). Total cholesterol was determined using the cholesterol esterase method on a Roche automated chemistry analyser. HDL-C was determined using the cholesterol esterase method following selective precipitation of apolipoprotein-B-containing lipoproteins with a polyanion solution. LDL-C levels were calculated using the Friedewald equation (Friedewald *et al*., 1972). Triglycerides were determined enzymatically as glycerol on a Roche automated chemistry analyser following hydrolysis with lipase. All lipid analyses had intra- and inter-assay CVs of <3%.

Statistical analyses

All data were expressed as mean ± SEM. Analysis of variance (ANOVA) was used for comparisons. Post-hoc testing was carried out using Student's *t*-test with log transformation. Analysis of covariance (ANCOVA) was used to evaluate the role of body weight on differences in metabolic parameters. The Pearson product moment correlation and stepwise multivariate linear regression analysis with forward selection was used to analyse correlations. A P-value < 0.05 was considered statistically significant.

Table II. Comparison between PCOS of two different subgroups of women from different ethnic populations

Parameter	Italian PCOS	American PCOS	*P*
BMI (kg/m^2)	29.7 ± 1.0	40.3 ± 1.0	<0.01
Waist:hip ratio	0.83 ± 0.04	0.85 ± 0.02	NS
Systolic blood pressure (mmHg)	126 ± 5	130 ± 3	NS
Diastolic blood pressure (mmHg)	80 ± 4	79 ± 3	NS
Insulin (μU/ml)	18.1 ± 2	29.5 ± 2	<0.01
Glucose:insulin ratio	5.3 ± 1	3.6 ± 0.5	<0.01
Total cholesterol (mg/dl)	183 ± 12	187 ± 8	NS
HDL-C (mg/dl)	48 ± 1	40 ± 2	<0.01
LDL-C (mg/dl)	109 ± 12	116 ± 7	NS
Triglycerides (mg/dl)	91 ± 8	156 ± 18	<0.01

HDL-C = high-density lipoprotein-cholesterol; LDL-C = low--density lipoprotein-cholesterol; NS = not significant.

Results

Among women with PCOS, those from the USA had a significantly ($P < 0.01$) higher BMI (35.05 ± 0.5 kg/m^2) than those from Italy (27 ± 0.3 kg/m^2). The mean BMI values and centiles of the two populations are listed in Table I. Overall, some 69% of US women had a BMI value >30 kg/m^2 compared with only 38% of Italian women.

The BMI values and metabolic and hormonal data of the two subgroups of women seen in 2001 are listed in Table II. The mean age of these women was similar to that of the entire study population. The mean BMI of the two subgroups was higher ($P < 0.01$) than that of the overall population seen between 1993 and 2001, though the US population consistently had a higher BMI. Although some women underwent oral glucose tolerance tests as part of their general evaluation, this was not the case for all women. However, all of the women evaluated had normal fasting glucose levels.

Although different methods were used for androgen assays in the USA and Italy, the normal ranges for these values were similar. In Italy, normal ranges were: T, 15–55 ng/dl; uT, 4–14.6 pg/ml; and DHEAS, 0.8–2.8 μg/ml. In the USA, normal ranges were: T, 15–58 ng/dl; uT, 4–15.8 pg/ml; and DHEAS, 0.8–2.7 μg/ml. The two populations of women with PCOS were found to be hyperandrogenic and had comparable levels of androgens (us women: T, 76 ± 7 ng/dl; uT, 21 ± 2 pg/ml; DHEAS, 2.2 ± 0.3 μg/dl; Italian women: T, 79 ± 10 ng/dl; uT, 18 ± 3 pg/ml; DHEAS 2.3 ± 0.4 μg/ml).

While US women with PCOS had a significantly ($P < 0.01$) higher BMI, there were no differences in either waist:hip ratio or in blood pressure (Table II). The US women also had more marked insulin resistance, as reflected by the decreased glucose:insulin ratio (Table II). A significant ($P < 0.01$) positive correlation was found between the severity of obesity (BMI) and serum insulin ($r = 0.46$), and a negative correlation with the glucose:insulin ratio ($r = –0.46$; $P < 0.01$).

The two groups of women with PCOS had similar concentrations of total cholesterol and LDL-C (Table II). However, the US women had significantly lower levels of HDL-C (40 ± 2 versus 48 ± 1.5 mg/dl; $P < 0.01$) and significantly higher levels of triglycerides (156 ± 18 versus 91 ± 8 mg/dl; $P < 0.01$) (Table II). A correction for body mass reduced, but did not eliminate, the statistical differences between the two groups of women ($P < 0.05$ for insulin, glucose:insulin ratio, HDL-C and triglycerides).

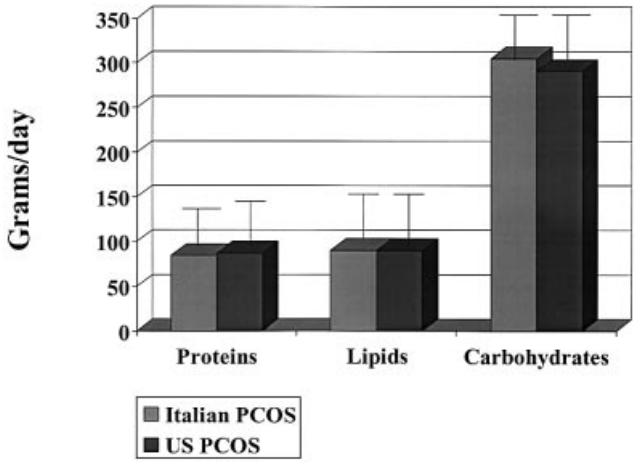

Figure 1. Mean ± SEM dietary intake of proteins, lipids and carbohydrates (g/day) in Italian and US women with PCOS.

Serum insulin correlated negatively with HDL-C ($r = –0.49$; $P < 0.01$) and positively with triglycerides ($r = 0.34$; $P < 0.05$), but not with total cholesterol or LDL-C. Each group showed separately a significant ($P < 0.05$) negative correlation between insulin and HDL-C, but the correlation with triglycerides was only shown for the pooled analysis of both groups. Similar correlations were found between BMI and HDL-C ($r = – 0.37$; $P < 0.05$) and between BMI and triglycerides ($r = 0.35$; $P < 0.05$).

An analysis of the diet showed that the total calorific intake was similar in the two groups of women with PCOS (US women 2277 ± 109 kcal; Italian women 2325 ± 68 kcal). Likewise, the proportions of the main dietary constituents were similar in the two groups (Figure 1). The amount of saturated fat, however, was significantly higher in the US women than in the Italian women (31.9 ± 3 versus 18.2 ± 2 g/day; $P < 0.01$) (Figure 2). The correlation for saturated fat, however, did not eliminate the significant ($P < 0.05$) difference in glucose:insulin ratio in the two groups.

No correlations were found between total calorific intake, carbohydrates, proteins or total fat with BMI, insulin or the glucose:insulin ratio. The total quantity of carbohydrates showed a correlation with HDL-C ($r = 0.39$; $P < 0.05$). This was also shown separately for the group from the USA ($P < 0.05$), but not separately for the Italian women. There were no other correlations between total calorific intake, total proteins, carbohydrates or fats with serum lipids or lipoproteins.

Finally, when the dietary constituents were assessed, the daily intake of saturated fat correlated negatively ($P < 0.01$) with HDL-C ($r = –0.52$). This correlation was found in the entire group of women with PCOS, and also separately in each of the two subpopulations (Italian women, $r = –0.53$, $P < 0.05$; US women, $r = –0.51$, $P < 0.05$).

Discussion

In the present study, the body masses of two different ethnic populations of women with PCOS were compared. The first

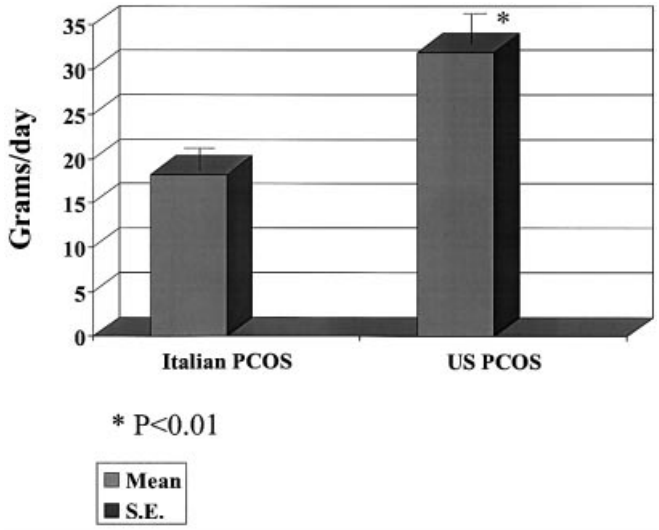

Figure 2. Mean ± SEM dietary intake of saturated fats (g/day) in Italian and US women with PCOS. *, significant difference between groups, $P < 0.01$.

population consisted of women from Central Pennsylvania, USA, who were evaluated between 1993 and 2001, while the second population included only Italian women studied at the University of Palermo during the same period. Although these patient populations were not preselected, it is clear that these comparisons of US and Italian women must be viewed as comparisons of the two specific populations, and may not reflect differences or similarities when the entire population of the countries are taken into consideration. There was a large difference in mean BMI observed between the two populations, although it cannot be determined whether this degree of difference occurs in all women with PCOS in the US compared all such women in Italy. In general, the US women with PCOS were much more obese than their Italian counterparts; indeed, some 69% of the US women were classified as obese (BMI >30 kg/m^2) compared with only 38% of the Italian women.

Previously, several populations of women with PCOS of different ethnicity were compared, including Italian, US (Latina) and Japanese. Similar biochemical characteristics were found among these groups, but ethnic differences occurred in the prevalence of obesity (Japanese women were of normal weight) (Carmina *et al*., 1992). In that study, no differences were found in the prevalence of obesity in women with PCOS coming from the USA or Italy, although the US women from Los Angeles, California and of Latina heritage. In line with the notion that the prevalence of obesity has been steadily increasing in all Western countries during the past few years, the subgroups studied during 2001 were significantly more obese than the entire population evaluated over the previous 9 years. Although obesity has been reported as being present in only 40–50% of women with PCOS (Goldzieher and Axelrod, 1963; Yen, 1980; Balen *et al*., 1995; Lobo and Carmina, 1997), only 38% of Italian PCOS women had a BMI >27 kg/m^2 (i.e. they were obese); hence, it is possible that in the USA the prevalence of obesity among women with PCOS may be higher. However, it should be noted again that the BMI of the population studied in Pennsylvania may not reflect that of all women with PCOS in the entire US population.

Obesity represents an important risk factor which can exacerbate many of the symptoms of PCOS and increase the cardiac risk profile of the syndrome. In the present study, the women with PCOS from the USA were not only more obese but also had higher insulin levels, more severe insulin resistance, and a worse lipid profile (lower serum HDL-C and higher serum triglycerides). These findings might suggest that US women with PCOS—or at least the population studied from Pennsylvania—have a higher cardiovascular risk when compared with Italian women with PCOS. The evaluated women from Pennsylvania also had more severe lipid changes than did the population studied previously, which was from California (Legro *et al*., 1999).

In analysing the diets of the US and Italian women with PCOS, a validated diet analysis program was used and cross-referenced in the two populations. Surprisingly, the diets were comparable, despite significant geographical differences in calorific consumption and diet composition. Nevertheless, it should be noted that these data were drawn from a subset of the entire population of PCOS women evaluated at each centre in the two countries, and therefore may not be reflective of entire populations. The two groups evaluated herein had a similar total calorific intake and on a daily basis ate similar proportions of protein, carbohydrates and total fat. A recent diet history may not reflect a lifetime of adverse dietary habits, but the women were asked to reflect on their normal eating habits and selected on the basis of not participating in any dietary or exercise modification programme.

One important difference in the diets of the two groups however was in the quantity of saturated fat consumed by American women, which was almost double that in Italian women (31.9 ± 4 versus 18.2 ± 2 g/day). The increased daily consumption of saturated fat correlated significantly, but negatively, with serum HDL-C ($r = –0.52$; $P < 0.01$), and this may at least partially explain the more abnormal lipid profile of the US women with PCOS. However, it may not have influenced the degree of insulin resistance in that, even after adjusting for dietary saturated fat intake, there was a significant difference between the two groups in their glucose:insulin ratios. Saturated fat, nevertheless, is known to be important for reducing levels of sex hormone-binding globulin and is highly correlated with the development of obesity.

As noted above, whilst dietary saturated fat intake is an extremely important variable, it is unlikely that the increased saturated fat content alone is sufficient to explain the more severe obesity and insulin resistance observed among US women with PCOS. It is also possible that a more sedentary lifestyle of women in the US may have contributed to an energy surplus and greater obesity (Friedewald *et al*., 1972), though no specific information is available to support this supposition, and further study is merited.

The total amount of calories consumed by the two groups of women was considered to be normal. It has been reported recently that women of normal weight with PCOS consume fewer calories than normal-weight women without PCOS (Taylor *et al*., 2002). This may suggest that obesity (or

overweight status) is part of the disorder in PCOS, which is related to genetic metabolic factors, and that diet and lifestyle may modify the phenotype.

In conclusion, the present results suggest that in the USA (central Pennsylvania), women with PCOS are more obese and have more significant metabolic and cardiovascular risk factors than women with PCOS who live in Italy (Sicily). The difference in obesity status between the two populations may be partly related to dietary factors (a higher consumption of saturated fat), although their total calorific consumption was the same. Hence, it is likely that genetic and other lifestyle factors play a major role.

Acknowledgements

These studies were supported by PHS grants K24 HD01476 (to R.S.L.), MO1 RR10732 (to Pennsylvania State University College of Medicine General Clinical Research Center) from the National Institutes of Health.

References

Balen, A.H., Conway, G.S., Kaltsas, G. *et al*. (1995) Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. *Hum. Reprod.*, **10**, 2107–2111.

Bringer, J., Lefebrve, P. and Renard, E.M. (1997) The confounding role of body habitus in androgen excess. In Azziz, R., Nestler, J.E. and Dewailly, D. (eds), *Androgen Excess Disorders in Women*. Lippincott Raven Publishers, Philadelphia, pp. 463–471.

Carmina, E. and Lobo, R.A. (1999) Polycystic Ovary Syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. *J. Clin. Endocrinol. Metab.*, **84**, 1897–1899.

Carmina, E., Koyama, T., Chang, L., Stanczyk, F.Z. and Lobo, R.A. (1992) Does ethnicity influence the prevalence of adrenal hyperandrogenism and insulin resistance in polycystic ovary syndrome? *Am. J. Obstet. Gynecol.*, **167**, 1807–1812.

Dunaif, A., Segal, K.R., Futterweit, W. and Dobrjansky, A. (1989) Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes*, **38**, 1165–1174.

Dunaif, A., Scott, D., Finegood, D., Quintana, B. and Whitcomb, R. (1996) The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.*, **81**, 3299–3306.

Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clin. Chem.*, **18**, 499–502.

Glick, M., Michel, A.C., Dorn, J., Horwitz, M., Rosenthal, T. and Trevisan, M. (1998) Dietary cardiovascular risk factors and serum cholesterol in an old order mennonite community. *Am. J. Public Health*, **88**, 1202–1205.

Goldzieher, J.W. and Axelrod, L.R. (1963) Clinical and biochemical features of polycystic ovarian disease. *Fertil. Steril.*, **14**, 631–653.

Hodge, A.M., Dowse, G.K., Gareeboo, H., Tuomilehto, J., Alberti, K.G. and Zimmet, P.Z. (1996) Incidence, increasing prevalence, and predictors of change in obesity and fat distribution over 5 years in the rapidly developing population of Mauritius. *Int. J. Obesity Rel. Metab. Disord.*, **20**, 137–146.

Legro, R.S., Finegood, D. and Dunaif, A. (1998a) A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.*, **83**, 2964–2968.

Legro, R.S., Driscoll, D., Strauss, J.F., Fox, J. and Dunaif, A. (1998b) Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. *Proc. Natl Acad. Sci. USA*, **95**, 14956–14960.

Legro, R.S., Blanche, P., Krauss, R.M. and Lobo, R.A. (1999) Alterations in low-density lipoprotein and high-density lipoprotein subclasses among Hispanic women with polycystic ovary syndrome: influence of insulin and genetic factors. *Fertil. Steril.*, **72**, 990–995.

Lobo, R.A. and Carmina, E. (1997) Polycystic Ovary Syndrome. In Lobo, R.A., Mishell, D.R., Jr, Paulson, R.J. and Shoupe, D. (eds), *Mishell's Textbook of Infertility*, *Contraception and Reproductive Endocrinology*, 4th edition. Blackwell Science Publishers, Oxford, pp. 363–383.

Lobo, R.A. and Carmina, E. (2000) The importance of diagnosing the Polycystic Ovary Syndrome. *Ann. Intern. Med.*, **132**, 989–993.

Lobo, R.A., Kletzky, O.A., Kaptein, E.M. and Goebelsmann, U. (1980) Prolactin modulation of dehydroepiandrosterone sulfate secretion. *Am. J. Obstet. Gynecol.*, **138**, 632–636.

Lopes-Virella, M.S., Stone, P., Ellis, S. and Colwell, J.A. (1977) Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin. Chem.*, **23**, 882–884.

Marshall, J.A., Hoag, S., Shetterly, S. and Hamman, R.F. (1994) Dietary fat predicts conversion from impaired glucose tolerance to NIDDM. The San Luis Valley diabetes study. *Diabetes Care*, **17**, 50–56.

Pekkarine, T., Takala, I. and Mustajoki, P. (1998) Weight loss with very-low-calorie diet and cardiovascular risk factors in moderately obese women: one-year follow-up study including ambulatory blood pressure monitoring. *Int. J. Obesity Rel. Metab. Disord.*, **22**, 661–666.

Stanczyk, F.Z., Chang, L., Carmina, E., Putz, Z. and Lobo, R.A. (1991) Is 11β-hydroxyandrostenedione a better marker of adrenal androgen excess than dehydroepiandrosterone sulfate? *Am. J. Obstet. Gynecol.*, **166**, 1837–1842.

Taylor, A.E., Hubbard, J.L., Anderson, E.J. and Hall, J.E. (2002). Role of dietary composition in the pathophysiology of Polycystic Ovary Syndrome: diet composition in normal and PCOS women. Presented at the 84th Annual Meeting of The Endocrine Society, San Francisco, CA, June 19–22, abstract P2-633.

Yen, S.S. (1980) The polycystic ovary syndrome. *Clin. Endocrinol.*,**12**, 177–207.

Zawadski, J.K. and Dunaif, A. (1992) Diagnostic criteria for polycystic ovary syndrome; towards a rational approach. In Dunaif, A., Givens, J.R., Haseltine, F. and Merriam, G.R. (eds), *Polycystic Ovary Syndrome*. Blackwell Scientific, Boston, pp. 377–384.

Submitted on April 22, 2003; accepted on July 10, 2003