Increased Insulin Sensitivity and Fibrinolytic Capacity After Dietary Intervention in Obese Women With Polycystic Ovary Syndrome

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In overweight women with polycystic ovary syndrome (PCOS), increased insulin resistance has been observed. Since abdominal obesity is associated with impaired fibrinolytic capacity and elevated levels of plasminogen activator inhibitor (PAI-1) and since PAI-1 seems to be related to insulin resistance, we investigated the possible effects of dietary intervention on lipids, fibrinolysis, coagulation, and insulin sensitivity in obese PCOS women. Nine women aged 22 to 39 years (median weight, 97 kg) ate a protein-rich very–low-calorie diet (VLCD) (Nutrilett, Nycomed Pharma, Oslo, Norway; 421 kcal/d) for 4 weeks (part 1). After significant reductions of body fat (13%, $P < .01$), two of nine women achieved regular menstruation and became pregnant. Six of the remaining women continued on a conventional low-calorie diet (1,000 to 1,500 kcal/d) for the next 20 weeks (part 2), during which time they were generally able to preserve the body fat loss obtained in part 1 of the study. During part 1, significant reductions of total serum cholesterol (29%, $P = .001$) and fasting triglyceride ([TG] 31%, $P < .05$) levels were observed, as well as significant reductions of fasting glucose (6%, $P < .05$) and insulin (20%, $P < .05$). Insulin sensitivity (glucose disposal rate [GDR]) was increased by 93% ($P < .05$). After finishing part 2, insulin sensitivity was still significantly increased (86%, $P < .05$) and PAI-1 activity was significantly reduced (54%, $P < .05$). Moreover, overall fibrinolytic activity was significantly improved (serum D-dimer concentration increased by 75%, $P < .05$). In conclusion, through intensive dietary intervention with adequate loss of weight it is possible to change an unfavorable atherothrombogenic risk profile in overweight (PCOS) women. Most convincingly, significantly increased insulin sensitivity and fibrinolytic capacity were observed.

STEIN AND LEVENTHAL described the association of amenorrhea, infertility, and hirsutism with enlarged cystic ovaries in 1935.[1] Since then, it has become clear that the presence of polycystic ovaries may be associated with a variety of clinical and biochemical features. With the increased use of ultrasound imaging in women with ovulatory disorders, our knowledge about the prevalence, clinical presentation, diagnosis, and management of the polycystic ovary syndrome (PCOS) has changed. Although no single entity is diagnostic of PCOS in the individual woman, obesity is encountered in 35% to 80% of cases.[2] Hyperinsulinemia and insulin resistance are found in both lean and obese PCOS women, but obese women with PCOS also have higher systolic blood pressure, serum triglyceride (TG) levels, and plasma glucose concentrations than lean women with PCOS and controls.[3]

In healthy coronary high-risk men, hypertriglyceridemia has been associated with reduced fibrinolytic capacity,[4] and increased levels of the fast-acting tissue plasminogen activator inhibitor (PAI-1) are shown to be positively correlated with serum TG levels in young survivors of myocardial infarction.[5] Furthermore, increased fibrinolytic potential was found after dietary intervention in healthy coronary high-risk individuals.[6] Since abdominal obesity is associated with impaired fibrinolytic activity and elevated PAI-1[7] and since PAI-1 seems to be related to insulin resistance,[8] we investigated the possible effects of dietary intervention on lipids, fibrinolysis, and insulin sensitivity in obese PCOS women.

SUBJECTS AND METHODS

Subject Selection

The study group contained nine obese PCOS women aged 22 to 39 years (weight, 82 to 119.0 kg). The diagnosis of PCOS was established by the presence of polycystic ovaries on vaginal ultrasound examination combined with three or more of the following criteria: oligomenorrhea/amenorrhea, hirsutism, serum luteinizing hormone to follicle-stimulating hormone ratio greater than 2, elevated serum luteinizing hormone levels, or hyperandrogenemia. Late-onset 21-hydroxylase deficiency, Cushing's syndrome, androgen-secreting tumors, and hyperprolactinemia were excluded by appropriate tests. None of the women had acanthosis nigricans. Seven women had involuntary infertility with patent fallopian tubes.

Dietary Intervention

Before the start of the study, all women were advised to eat a 3-day standardized four-meal diet containing approximately 2,000 kcal and 250 g carbohydrates. All subjects then received a 421-kcal/d diet (Nutrilett, Nycomed Pharma, Oslo, Norway) for 4 weeks. This very–low-calorie diet (VLCD) contained 51.1 g protein, 25.4 g carbohydrates, 5.0 g fat, and 4.5 g fiber per 100 g. In the next 20 weeks, the women followed a dietary regimen of 1,000 to 1,500 kcal/d that contained 22% protein, 59% carbohydrates, and 20% fat. During this period, the women were examined by the dietician twice the first month and then monthly for the remaining period. None of the women were especially active during leisure time throughout the study, but the dietician regularly advised them to take a daily walk.

Diet and Body Fat Measurements

Past and recent energy intake were determined by interviews and a 4-day record including food weighing and inventories. Waist and hip circumference were measured and the waist to hip ratio (WHR) was calculated. Weight and height were recorded for the

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calculation of body mass index ([BMI] weight in kilograms divided by square of height in meters). Percent body fat was assessed by the near–infrared-light measurement technique (Futrex 5000, NIR Medical, Oslo, Norway).[9]

Blood Sampling Procedure

At baseline and 4 and 24 weeks after intervention, each individual was tested in a fasting state between 8 and 10 AM before and after venous occlusion ([VO] blood pressure cuff at 90 mm Hg for 20 minutes). Before VO, blood was collected in Vacutainer tubes (Beckton Dickenson, Plymouth, UK) devoid of anticoagulants for determination of serum total cholesterol, high-density lipoprotein (HDL) cholesterol, TG, glucose, insulin, C-peptide, and sex hormone-binding globulin (SHBG). Citrated blood (Vacutainer tubes containing 0.129 mol/L trisodium citrate in dilution 1:10) and acidified citrated blood (Stabilyte tubes; Biopool, Umeå, Sweden) were collected for determination of hemostatic variables.[10] Platelet-poor plasma was obtained by centrifugation at 2,500 × *g* for 15 minutes at 4°C, except for analyses of coagulation factor VII (fVII) and the fVII-phospholipid complexes (fVII-PLC), where cold activation was avoided by centrifugation at room temperature. In addition, blood was collected in 5-mL glass tubes devoid of anticoagulants (Vacutainer) and left at room temperature for standardized coagulation and further measurement of serum D-dimer levels as a global fibrinolytic test.[11] After VO, blood was drawn for determination of tissue plasminogen activator (tPA) activity, and serum D-dimer and euglobulin clot lysis time (ECLT) were assessed for global fibrinolytic evaluation.[12]

Laboratory Methods

Total cholesterol, total HDL cholesterol, and TG levels were determined in serum using conventional enzymatic methods (Boehringer, Mannheim, Germany). Plasma glucose concentration was determined by the glucose dehydrogenase method (Hoffmann-La Roche, Basel, Switzerland). Serum levels of insulin, C-peptide, testosterone, and SHBG were measured by radioimmunoassay.[2] Reference values were as follows: total HDL cholesterol, 0.95 to 2.0 mmol/L; fasting TG, 0.20 to 1.30 mmol/L (women aged 20 to 34 years); fasting glucose, 3.6 to 6.0 mmol/L; fasting insulin, <20 mU/L; C-peptide, 0.23 to 0.51 nmol/L; testosterone, 0.3 to 2.8 nmol/L; and SHBG, 30 to 90 nmol/L.

After an overnight fast, a baseline euglycemic hyperinsulinemic clamp test was performed as described previously.[13] The test was performed either at random in the amenorrheic women or on days 2 to 11 after a vaginal bleeding in the oligomenorrheic PCOS women. The glucose infusion rate was adjusted to maintain a stable blood glucose level at the subjects' fasting level, and the amount of glucose given during the last 20 minutes of the 2-hour test was taken as the glucose disposal rate (GDR). Average serum insulin levels during the last 20 minutes of the clamp test ranged from 79 to 320 mU/L. This variation is due to differences in endogenous insulin secretion and in the metabolic clearance rate of insulin. Accordingly, insulin sensitivity was expressed as the ratio of GDR to the prevailing plasma insulin levels (GDR/I × 100), as suggested by DeFronzo et al.[14] The following hemostatic variables were recorded: fibrinogen according to the method reported by Clauss,[15] fVII determined in a two-stage chromogenic assay (Coa-Set fVII, Chromogenics, Mølndal, Sweden), fVII-PLC estimated with percent change in Normotest after treatment of the plasma sample with phospholipase C,[16] and fibrinolytic capacity assessed by ECLT after VO[12] and by serum D-dimer test before and after VO.[11,17] Serum D-dimer concentration was measured with an enzyme-linked immunosorbent assay method (Asserachrom D-di, Stagˊo, Asniere, France). In addition, tPA activity (using acidified plasma) and PAI-1 activity were estimated amidolytically mainly as described by Wiman et al[18] and Chmilewska et al.[19] Commercially available kits (Biopool) were used for the specific assays.

Statistics

Since the variables showed a skewed distribution, we used a paired nonparametric Wilcoxon matched test to compare data before and after diet intervention. Group data are reported as the median. Statistical significance was defined as an α less than .05 (two-tailed).

RESULTS

All PCOS women completed the initial 4 weeks on a VLCD. Posttreatment values after 4 weeks on the VLCD are listed in Table 1 (part 1 of the study).

After a significant reduction of body weight (median, 9%), two of the seven infertile women became pregnant after 4 and 6 weeks of intervention, respectively. Excluding these two women and one woman who discontinued follow-up evaluation, six women continued on the conventional low-calorie diet for the next 20 weeks, during which time they were generally able to preserve the body fat loss obtained in part 1 of the study. Posttreatment values after 24 weeks are listed in Table 2 (part 2 of the study).

Because of the pregnancies and difficulties in achieving proper intravenous access for the glucose clamp in some of these overweight women, posttreatment values for insulin sensitivity and hyperandrogenemia are based on seven subjects in part 1 and five subjects in part 2 of the study.

At baseline, a strong negative correlation was found between PAI-1 activity and insulin sensitivity ($r = -.78$, $P < .05$). Strong correlations were also found between pretreatment values for fasting insulin and PAI-1 activity ($r = .70$) and between insulin and fVII ($r = .69$), but they were only of borderline significance ($P = .05$), probably due to the small sample size. As for pretreatment fasting TG levels, strong and statistically significant correlations were found with PAI-1 activity ($r = .67$) and SHBG ($r = .70$).

Part 1 (weeks 0 to 4)

In parallel with significant reductions in BMI (8%, $P < .01$) and body fat (13%, $P < .01$), serum total cholesterol and fasting TG were reduced by 29% and 31%, respectively. Fasting blood glucose and insulin decreased by 6% ($P < .05$) and 20% ($P < .05$), respectively, and insulin sensitivity increased by 93% ($P < .05$).

After 4 weeks on the VLCD, fibrinogen and fVII levels were not significantly changed. Although PAI-1 activity decreased by 48% and tPA activity before and after VO was nearly doubled (80% and 106%, respectively), the changes were not significant. However, two of eight subjects with reduced fibrinolysis at baseline had a normalized fibrinolytic capacity as demonstrated both with the serum D-dimer test before and after VO and with ECLT after VO (data not shown).

Part 2 (weeks 0 to 24)

The six women who completed part 2 of the study were all regular responders on the VLCD, as described earlier

Table 1. Effects of VLCD for 4 Weeks on Body Fat Distribution and Metabolic and Hemostatic Variables in PCOS Women

	No. of Patients	Baseline		After 4 Weeks		
		Median	Range	Median	Range	Change (%)
Body fat distribution						
Weight (kg)	9	97.0	82.4-119.0	89.0	75.2-109.3	9§
BMI (kg/m^2)	9	34.1	28.7-40.7	31.5	26.1-37.3	8†
NIR (% body fat)	9	35.0	29.4-40.0	30.6	28.7-35.4	13†
WHR	9	0.94	0.84-1.02	0.93	0.81-1.04	1
Lipids						
TC (mmol/L)	9	6.9	5.4-7.6	4.9	3.1-6.6	29*
HDL (mmol/L)	9	1.0	0.7-1.2	0.9	0.7-1.3	10
TG (mmol/L)	9	1.6	1.1-1.8	1.1	0.9-2.4	31‡
Carbohydrates/insulin						
Glucose (mmol/L)	9	5.2	4.3-6.7	4.9	3.6-5.5	6‡
Insulin (mU/L)	7	30	17-102	24	9-77	20‡
C-peptide (nmol/L)	7	1.07	0.76-1.90	0.98	0.68-2.27	8
GDR/I (mg glucose/kg/min)	7	1.74	0.44-4.59	3.36	0.95-4.54	93‡
Androgen sex hormones						
Testosterone (nmol/L)	7	3.9	2.8-6.1	3.7	1.8-4.7	5
SHBG (nmol/L)	6	14.5	11-20	20.5	12-27	41§
Coagulation						
Fibrinogen (g/L)	9	2.35	2.15-3.20	2.95	2.05-3.90	26
fVII (%)	9	133	72-188	105	72-164	21
fVII-PLC (%)	9	15	9-25	7	9-20	53§
Fibrinolysis						
PAI-1 activity (U/mL)	9	48	12-56	25	9-32	48
tPA activity (IU/mL)						
Before VO	9	0.5	0.1-1.3	0.9	0.3-1.6	80§
After VO	9	1.7	1.0-19.2	3.5	1.9-19.3	106
Serum D-dimer before VO (μg/mL)	9	0.15	0.09-0.43	0.20	0.09-1.28	33

Abbreviations: NIR, near–infrared-light measurement; TC, total cholesterol.

*P = .001.

†P < .01.

‡P < .05.

§P = .05 to .10.

(part 1). They managed to keep body fat loss at a significant level (median 9%, P < .05), but the initial reductions in serum total cholesterol and TG were halved. However, the significant reduction in insulin levels achieved after 4 weeks was further increased when succeeded by 20 weeks on the conventional low-calorie diet, whereas the increased insulin sensitivity (GDR/I) was maintained at the same level.

As for changes in coagulation and fibrinolysis, there were still no significant changes in fibrinogen and fVII, whereas PAI-1 activity was significantly reduced (54%, P < .05). Moreover, overall fibrinolytic capacity was significantly improved (serum D-dimer concentration before VO increased by 75%, P < .05). All women who completed part 2 of the study had a reduced fibrinolytic capacity (as measured with serum D-dimer test and ECLT) at baseline. Again, two of them showed normalized fibrinolysis after 24 weeks of dietary intervention (data not shown).

Hyperandrogenemia did not change significantly during dietary intervention, but there was an almost significant increase in SHBG after 4 weeks on the VLCD (41%, P = .05 to .10; Table 1).

DISCUSSION

In obese PCOS women, weight reduction seems to improve menstrual cyclicity and to restore fertility.[20,21] Actually, in the present study, two of the seven infertile women became pregnant. Both conceptions occurred during the first treatment period with the VLCD, when insulin resistance and hyperandrogenemia concomitantly decreased. Insulin may increase ovarian androgen production, although there is no clear evidence that hyperinsulinemia per se could provoke impaired follicular maturation.[22] On the other hand, insulin has a physiologic role in follicle-stimulating hormone–stimulated follicular maturation. The improved fertility during weight loss in PCOS women may therefore be related to increased sensitivity of the ovary to the beneficial action of insulin.[23] From the present study it is also evident that it is not necessary to attain ideal body weight to achieve conception, since the pregnancies occurred in women with a BMI greater than 30 kg/m^2.

In animal studies, especially on the hormonal regulation of the rat ovary, extensive evidence has been gathered concerning the importance of the fibrinolytic system during gonadotropin-induced ovulation.[24,25] Similar results have been demonstrated in lysates of human granulosa cells and the respective follicular fluid.[26] If, in man, a corresponding influence of fibrinolysis on the ovulatory process is postulated, a significant weight reduction in PCOS women with concomitant improvement of fibrinolysis could provoke ovulation and fertility. One of the women who became

Table 2. Effects of VLCD for 4 Weeks Succeeded by a Conventional Low-Calorie Diet for 20 Weeks on Body Fat Distribution and Metabolic and Hemostatic Variables in PCOS Women

	No. of Patients	Baseline		After 24 Weeks		
		Median	Range	Median	Range	Change (%)
Body fat distribution						
Weight (kg)	6	100.3	82.4-119.0	95.5	67.0-105.6	5†
BMI (kg/m^2)	6	33.6	28.7-40.7	32.0	23.2-36.0	5†
NIR (% body fat)	6	35.4	29.1-40.0	32.2	24.7-36.3	9*
WHR	6	0.95	0.84-1.02	0.91	0.78-1.01	4
Lipid						
TC (mmol/L)	6	7.3	5.5-7.6	6.2	5.6-7.3	15
HDL (mmol/L)	6	1.0	0.7-1.2	1.1	0.7-1.4	10
TG (mmol/L)	6	1.6	1.3-1.7	1.4	0.8-2.3	13
Carbohydrates/insulin						
Glucose (mmol/L)	6	5.3	4.3-6.1	4.5	3.8-5.6	15
Insulin (mU/L)	5	42	17-102	22	14-58	48*
C-peptide (nmol/L)	5	1.07	0.76-1.90	0.94	0.57-1.12	12*
GDR/I (mg glucose/kg/min)	5	1.19	0.44-4.59	2.21	0.88-6.47	86*
Androgen sex hormones						
Testosterone (nmol/L)	5	4.0	2.8-6.1	4.0	2.1-7.1	None
SHBG (nmol/L)	5	14	11-18	15	11-17	7
Coagulation						
Fibrinogen (g/L)	6	2.35	2.15-2.90	2.75	1.90-2.95	17
fVII (%)	6	138	72-188	108	70-152	22†
fVII-PLC (%)	6	9	9-17	9	5-40	None
Fibrinolysis						
PAI-1 activity (U/mL)	6	49	12-56	22.5	8-42	54*
tPA activity (IU/mL)						
Before VO	6	0.45	0.1-0.8	0.90	0.5-2.0	100†
After VO	6	1.4	1.0-5.9	3.0	1.5-16.1	114
Serum D-dimer before VO (μg/mL)	6	0.12	0.09-0.29	0.21	0.08-2.00	75*

*$P < .05$.
†$P = .05$ to .10.

pregnant in our study had a persistingly normal fibrinolytic capacity, whereas the other one had reduced fibrinolysis that was only partially corrected after 4 weeks on the VLCD. However, measurement of fibrinolytic variables in the circulation does not necessarily disclose locally stimulated fibrinolytic activity in the ovaries. Methods other than blood sampling for analyses of changes in circulating levels of insulin sensitivity, sex hormones, and fibrinolytic capacity are obviously needed to elucidate which of these factors is the most important for the ovulatory process.

Before the start of dietary intervention, we found a strong negative correlation between insulin sensitivity and PAI-1 ($r = -.78$). Furthermore, strong positive correlations were noted between fasting insulin and PAI-1 activity ($r = .70$) and between insulin and fVII ($r = .69$). Vague et al[27] found that unstimulated euglobulin fibrinolytic activity correlated negatively and PAI-1 activity positively with BMI and plasma insulin level. Moreover, in the same study, they found decreased levels of plasma glucose, insulin, and PAI-1 and increased euglobulin fibrinolytic activity in obese subjects after a 24-hour fast. Although their results were already achieved after 24 hours on a "noncalorie" diet, they were similar to ours after 4 weeks on a VLCD.

It has been shown that a low-fat diet decreases fVII coagulant activity, whereas diet seems to have little or no effect on fibrinogen level.[28] In the present study, fVII coagulant activity was decreased to the same extent (median changes, 21% and 22%, respectively) in part 1 and part 2, whereas fibrinogen level showed a corresponding increase (median changes, 26% and 17%, respectively). None of these results reached statistical significance, probably due to the wide ranges and the small number of participants. Smoking has been associated with plasma fibrinogen level,[29] but none of the three smokers participating in this study had a fibrinogen level above the median baseline value (2.35 g/L) and their smoking attitudes were not changed throughout the trial.

After 4 weeks on the VLCD, there was a nonsignificant reduction of fVII-PLC, which was not observed after the following 20 weeks on the conventional low-calorie diet. As observed, the effect of dietary intervention on fasting TG was more pronounced after 4 weeks on the VLCD than after 24 weeks of dietary intervention. Since diet-induced changes in fVII-PLC are strongly correlated with corresponding changes in fasting TG levels, these findings could be expected.[30] Furthermore, the value of PLC-sensitive fVII activity as a predictor of cardiovascular disease seems questionable and may be of no greater significance than the measurement of changes in plasma TG concentration itself.[31]

Hypertriglyceridemia is associated with reduced fibrinolysis, and in our earlier experience, three of four coronary

high-risk men with TG levels greater than 3.5 mmol/L had reduced fibrinolytic capacity after VO.[4] In the present study, there was a strong correlation between fasting TG and PAI-1 at baseline ($r = .67$), although the median pretreatment TG value (1.60 mmol/L) was only slightly above the upper reference limit for the corresponding age (1.30 mmol/L). All six participants who completed part 2 of the study had reduced fibrinolytic capacity before intervention. Moreover, hyperinsulinemia and insulin resistance were more pronounced than hyperlipidemia in these individuals at baseline. After 24 weeks of dietary intervention, the profitable changes in insulin resistance and fibrinolytic activity attained after 4 weeks on the VLCD were virtually not diminished despite the concomitant lesser reductions in lipids. These results support the hypothesis of a stronger association between changes in PAI-1 and insulin resistance than between changes in PAI-1 and TG.

Abdominal obesity, ie, male-type obesity, is a risk factor for the development of cardiovascular disease and overall mortality in men and women.[32,33] Localization, as well as degree, of obesity may influence insulin resistance. Upper-body fat predominance, ie, android obesity, increases insulin resistance[34] and has also been found to be associated with a relative increase in free testosterone, ie, hyperandrogenicity, in premenopausal women.[35] PCOS premenopausal women with android obesity (increased WHR) obviously possess the same atherothrombogenic risk profile as healthy middle-aged coronary high-risk men with a low ratio of testosterone to estradiol.[36] Intervention with a protein-rich VLCD for 4 weeks preferably changed this atherothrombogenic risk profile toward a normalization of hyperlipidemia, insulin resistance, and reduced fibrinolysis.

In conclusion, we found that intensive dietary intervention in obese (PCOS) women with concomitant reductions in weight and body fat was accompanied by a significant normalization of glucose metabolism and fibrinolytic capacity. These changes were maintained after 24 weeks, despite only moderate changes in lipids. Therefore, premenopausal women with central obesity should be examined for the same metabolic cardiovascular or atherothrombogenic risk factors as overweight middle-aged coronary high-risk men, and diet intervention should be equally thoroughly conducted.

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