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Increased thrombin generation in women with polycystic ovary syndrome
A pilot study on the effect of metformin and oral contraceptives

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ABSTRACT

Objective. Polycystic ovary syndrome (PCOS) is associated with risk factors for cardiovascular disease (CVD) which may be modified by the use of metformin and oral contraceptives (OC). Thrombin generation (TG) measures are risk markers of CVD and address the composite of multiple factors that influence blood coagulation. This prospective, randomized, intervention study evaluated the potential influence of PCOS on TG measures and the effect of OC and/or metformin on TG measures in women with PCOS.

Material and methods. Ninety patients with PCOS and 35 controls were included. Patients were randomized to 12 months of treatment with metformin, metformin + OC or OC alone. C-reactive protein (CRP), fibrinogen, total cholesterol, trunk fat mass, body mass index, estradiol, testosterone, sex hormone binding globulin (SHBG) as well as TG measures, i.e. the lag time for formation of thrombin, the endogenous thrombin potential (ETP), peak thrombin concentration (peak) and time to peak were determined at baseline and after 12 months of treatment.

Results. CRP and total testosterone were significantly higher and SHBG significantly lower in PCOS women than in controls ($P = 0.012$, $P < 0.001$ and $P = 0.008$, respectively). The TG measures ETP, peak and lag time were increased in women with PCOS compared to controls ($P < 0.01$). Significant correlations were observed between TG measures and fibrinogen, CRP, SHBG and fat trunk mass ($P>0.01$). ETP ($P = 0.006$), peak ($P = 0.003$) and lag time ($P = 0.023$) remained increased after adjustment for these potential confounders. Treatment with OC and metformin + OC further increased ETP ($P < 0.001$) and peak ($P < 0.005$) and reduced time to peak ($P < 0.04$). The increase in ETP was significantly lower in the metformin + OC group than in the OC group ($P < 0.05$). Metformin alone did not affect TG significantly.

Abbreviations: BMI, Body mass index; CVD, Cardiovascular disease; CRP, C-reactive protein; ETP, Endogenous thrombin potential; OC, Oral contraceptives; PCOS, Polycystic ovary syndrome; SHBG, Sex hormone binding globulin; TG, Thrombin generation.

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Conclusions. PCOS is associated with increase in TG measures independent of other risk factors of CVD. OC increase TG measures further and may thus add to the increased risk of CVD already present in women with PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age affecting 5%–10% of premenopausal women. The clinical identification of women with PCOS, as defined in the Rotterdam criteria, is based on anovulation, hyperandrogenism and polycystic ovaries and at least two of these three criteria must be accomplished to establish the PCOS diagnosis [1,2]. PCOS is associated with insulin resistance and enhanced risk of type 2 diabetes. Patients with PCOS have increased levels of cardiovascular risk markers [3] increased risk of venous thromboembolism [4,5] and significant subclinical atherosclerosis compared with healthy controls [6–8].

Life style intervention and treatment with metformin are applied to reduce insulin resistance and improve ovulation rates in women with PCOS [9]. Treatment with metformin may have beneficial effects on cardiovascular events [10], but no long term studies are available in patients with PCOS. The menstrual irregularities and hirsutism associated with PCOS are often treated with oral contraceptives (OC). Treatment with OC increases the risk of cardiovascular disease (CVD) in women without PCOS [11]. A comparable thrombogenic effect of OC treatment has been reported in women with PCOS [4], although recent studies have indicated that OC-treatment is without effect or may even reduce the cardiovascular risk in women with PCOS [5,7,12].

Measures of thrombin generation (TG) address the combined effect of multiple haemostatic risk markers for venous and arterial thrombotic disease [13–16]. Elevated TG measures are associated with idiopathic and pregnancy related thrombosis [13,16] and thrombin formation contributes to vascular calcification and atherosclerosis progression [17]. Age, body mass index (BMI), total cholesterol, trunk fat mass and low grade inflammation are shown to be potential confounders of measures of TG [18,19] and several studies have indicated that also sex hormones may have significant impact on TG [20–23]. It is of particular interest that OC treatment has been demonstrated to elevate measures of TG [24], whereas the function of thrombin is reduced in diabetic patients treated with metformin [25,26]. TG has rarely been studied in women with PCOS [27] and no previous studies have addressed the long term effect of OC and/or metformin on TG measures in this group of women.

In the present study we evaluated the impact of PCOS on measures of TG by comparison with control subjects of similar age and BMI after adjustment for the effect of the confounders. Furthermore we evaluated whether 12 months' treatment with metformin and/or OC affected TG measures in women with PCOS.

2. Methods

2.1. Patients

The study design has recently been published [28]. In brief, 90 white, non-diabetic women aged 18–39 years fulfilling the Rotterdam criteria for PCOS were included in the study. It should be noticed that TG as outcome measure was not defined in the original study [28], which was planned ultimo 2006. The power of the study was calculated using the area under the curve for insulin during two hours OGTT as the primary end point. No previous studies examined the effect of metformin and/or OC on TG measures and a power calculation on TG as the primary study outcome could not be performed. Thus, the present study should be regarded a pilot study with 30 patients included in each treatment arm. The development of a sensitive and convenient TG test [15] together with recent reviews identifying TG as a potent tool for studying the dynamic interactions between pro- and anticoagulant mechanisms and thereby to address the risk of CVD [29], prompted us to extend the study and focus on the relationship between PCOS, TG, metformin and OC. The patients fulfilled any two of the criteria: (1) Irregular menstrual cycles during more than a year in combination with a cycle length >35 days, (2) Total or free testosterone levels above reference interval (upper limits: total testosterone >1.8 nmol/l, free testosterone >0.035 nmol/l) and/or hirsutism, and (3) Transvaginal ultrasonography demonstrating polycystic ovaries.

The exclusion of patients with obesity (BMI $\geq$ 35 kg/m^2), pregnancy wish, contraindications for OC, endocrine diseases, diabetes, liver diseases, renal dysfunction, congestive heart disease, depression and eating disorders was previously described [28]. Our considerations regarding the cut-off for BMI are based on the ARIC study [30], a large study of cardiovascular risk in more than 15,000 subjects demonstrating that BMI is an independent risk factor for venous thromboembolism (VTE) with a hazard ratio >2 for individuals with a BMI between 30 and 35. The risk of VTE, however, increases significantly when BMI exceeds 35 kg/m^2.

Patients discontinued OC for at least three months and metformin for at least one month before evaluation. No patients were treated with medicine known to affect hormonal or metabolic parameters. Patients randomized to metformin treatment alone accepted barrier contraception during the study period or had an intrauterine device implanted.

Thirty-five healthy, white, premenopausal women comparable with the PCOS patients with respect to BMI and age were studied as controls. The controls were recruited by advertising at the local university, nursing school, and at Odense University Hospital. All controls had regular menstruation and did not have hyperandrogenemia or hirsutism. Controls discontinued OC for at least three months before evaluation and did not take any other kind of medicine. Controls underwent clinical examinations during the follicular phase of the menstrual cycle (cycle days 2–9).

2.2. Ethics

The study was approved by the local ethics committee and by the Danish Medicines Agency and all subjects gave written