Metabolic aspects of obesity and lean PCOS

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Voor Rob, Daniël en Josephine

Contents

Introduction

Chapter 1

General introduction and outlines of the studies

Introduction

A worldwide increasing prevalence of obesity has been established since the last 30 years.[1] Variation in prevalence of obesity between different cultures is observed and several environmental, genetic and behavioural factors contributing to the development of obesity have been identified.[2,3] Besides, there is evidence that obesity is related to co-morbidities.[4] Obesity is, for example, associated to an increased risk for the development of anovulation, diabetes, cancer and cardiovascular diseases and, therefore, represents a cost consuming healthcare issue.[5,6] Also in daily gynaecological practice there is an increasing number of overweight and obese women, who among others present with subfertility.

Obesity is in part associated with ovulatory subfertility. This manifests itself in lower spontaneous and lower ongoing pregnancy rates.[7] In the scope of obesity-related metabolic aspects of subfertility the starting point of this thesis is obesity related anovulatory subfertility and/or Polycystic Ovary Syndrome (PCOS).[8] Normogonadotropic normo-oestrogenic oligo-/anovulation is a common ovulation disorder presenting with a variety of clinical symptoms.[9,10] Anovulation due to PCOS is the most common endocrine disorder in women with a prevalence of up to 15% according to the Rotterdam criteria.[11] In 2003, an update consensus about the definition of PCOS was achieved in Rotterdam, which was subsequently revised in 2004. At least two of three predefined criteria had to be met:

1: PolyCystic Ovaries (PCO) on ultrasound, showing more than 12 follicles as measured in its greatest diameter,
2: hyperandrogenaemia,
3: oligomenorrhoea presenting a cycle length of more than 42 days.[12]

The aetiology of PCOS is still unknown and its presentation is heterogeneous. It has to be noted that in daily practice PCO are found on ultrasound in up to 10% of regularly menstruating women without any other clinical symptoms of PCOS. In adolescents with irregular cycles 36% show normal ovaries on ultrasound whereas in adolescents without cyclic irregularity a high prevalence of polycystic ovaries is found.[13,14] About 30-70% of PCOS patients present with overweight or obesity[15] and may suffer from hypertension, dyslipidaemia, hyperandrogenaemia, hirsutism, and peripheral insulin resistance resulting in compensatory hyperinsulinaemia.[16]

In general, these women are, therefore, advised to reduce weight in order to regain ovulation, increase ongoing pregnancy rates[17] and optimize physical condition. However,

weight reduction and sustained weight reduction in particular are difficult to achieve. In addition to common dietary and exercise programs psychological intervention like behavioural strategies are essential for the success rate of weight reduction programs.[18] Lifestyle modification programs are supposed to be efficacious to improve the metabolic syndrome and its associated risk for cardiovascular disease as well as its risk/prevalence for type 2 diabetes.[19,20,21]

The pathogenetic mechanism leading to follicular deregulation as occurs in PCO is still not fully understood. Research has focussed on the question whether there is an underlying metabolic/endocrine factor, a genetic factor, an intrinsic ovarian factor or a combination of factors that can clarify the arrest in follicular development.[22] Until recently clinical studies mainly concern obese patients with PCOS and analyse obesity-related factors that influence follicular maturation and body composition.[23,24,25]

However, PCOS is complex and a subset of women with PCOS is lean. This directly leads to another question, i.e. the specific aetiologic factors in lean PCOS patients, and the possible role of body fat distribution and its relevance regarding fertility.

Obesity related aspects in PCOS

Obesity and insulin resistance are observed in 30-70% of women with PCOS[10,26] and is clearly associated with centrally deposited fat. An imbalance between energy intake and expenditure results in weight gain but evidence also points to a genetic factor conceptualized as “thrifty gene” hypothesis.[27] This hypothesis was first described by Hales and Barker in 1992. They proposed that fetal and early postnatal malnutrition may impair pancreatic cell development resulting in an increased susceptibility to early onset type 2 diabetes[28] in association with fat storage in the visceral compartment.[29,30,31] This phenomenon can be explained by the theory that during a state of malnourishment energy homeostasis is preserved by low energy expenditure associated with low insulin requirement. In times of nutritional abundance energy conservation results in the so-called catch-up fat storage. Fat storage preferentially occurs in the visceral fat compartment.[29,30,31] Glucose is mainly used for de novo lipogenesis instead of oxidation in skeletal muscles. Insulin resistance impairs insulin signalling at the cellular level which is associated to lowering of peripheral glucose uptake by skeletal muscle cells. Reduced peripheral glucose uptake in an insulin resistant state causes increased levels of free fatty acid (FFA) as an

alternative energy source in mitochondria. Finally, ineffective burning of “FFA fuel” leads to fat accumulation in skeletal muscle cell, oxidative stress and clinically decreased fitness.[32,33]

Although obesity is a risk factor for the development of other diseases it is remarkable that obesity per se is not related with metabolic disorders in all obese individuals. A certain subset of obese individuals obviously are “healthy”. This group of individuals show higher insulin sensitivity as compared to weight matched obese individuals known with metabolic disturbances. In general, obese individuals benefit from lifestyle intervention in terms of improvement in insulin sensitivity and reduction of visceral adipose tissue.[34,35,36,37] Weight reduction affects peripheral insulin sensitivity and causes metabolic improvements.[37]

Visceral fat distribution is suggested to estimate the metabolic risk of obesity. Visceral fat accumulation in particular is related to insulin resistance.[38] Gender difference is an ideal model for studying the role of insulin sensitivity, visceral fat mass and energy expenditure/homeostasis.[39]

Obese children, who stay obese during growth have a twofold increased risk of diabetes type 2, hypertension, dyslipidaemia and atherosclerosis.[40] In PCOS also an increased risk of cardiovascular disease has been established.[15] Change of lifestyle and consistent weight reduction is supposed to reduce or even reverse the risk of development of cardiovascular disease.

Obesity, on the other hand, is suggested to affect reproductive function. It is generally accepted that obesity is associated to increased miscarriage rates and reduced success rates in artificial reproductive techniques. Besides, PCOS is especially found in women who are obese. In a systematic review including 28538 women conceiving spontaneously there was a higher rate of miscarriage in the obese population as well as a higher rate of recurrent miscarriage.[41] The necessity for prospective studies analyzing the effects of obesity on reproductive function and outcomes is gaining interest especially as fertility centres are confronted to increasing BMI of subfertile population. Regarding the treatment of anovulation in an obese population, a recent study promises benefits from total weight reduction in favour of visceral weight loss.[42]

Reviewing literature for the effects of lifestyle intervention programs on body composition and insulin resistance reveal that obese PCOS patients may benefit from weight loss in particular.[43] Meaningfully, weight reduction programs must focus at

lowering of total as well as visceral fat mass,[44,45] recovery of insulin sensitivity and improved fitness. Energy homeostasis is complex and influenced by anabolic and catabolic factors. The main regulating centre of energy homeostasis is located within the hypothalamus. The existence of a precise regulatory system implies a complex dialogue between the brain and peripheral tissues, including adipose tissues, but also other organs, such as pancreas, liver, muscles, gut and ovaries. Many communicating peptides are involved in a complex regulatory network of peptides among which some are derived from adipocytes, such as leptin, adiponectin, IL-6, other gut derived peptides, such as oxyntomodulin, GIP, and GLP-1 as well as brain derived peptides such as neuropeptide Y, dopamine, serotonine. This complex endocrine and paracrine network seems to be constructed in order to maintain the human species, especially in the past meant to increase possibility of survival during periods of famine. During starvation peripheral insulin, leptin and glucose levels are low. Oppositely, on overeating these levels become high. Although there is little evidence for a monogenetic role of genes on energy homeostasis in humans, the discovery of a single mutation in the leptin gene[46] has contributed to acknowledgement of mono-genomic deletions in obesity.

It is well established that obesity is under genetic influence, which has been initially demonstrated in laboratory animal models. Much less is known about genes involved in human obesity because the obese phenotype in humans is not only determined by a number of different genes but also by a large number of environmental factors. Genes that are involved in the regulation of catecholamine function may be of particular importance for human obesity because of the central role they have in energy expenditure both as hormones and neurotransmitters. A key factor in the energy balance is the mobilization of lipids through lipolysis in fat cells and the beta-2 adrenoceptor is a major lipolytic receptor in human fat cells. Several polymorphisms of the beta-2 adrenoceptor gene are markedly associated with human obesity.[47,48] The leptin-melanocortin system is also playing an important role in energy homeostasis. The most critical the discovery in this field is the finding of a single mutation in the leptin gene[46] which mainly contributed to the obesity and hyperphagia in well described families. Weight loss induced by leptin replacement in homozygous leptin deficient children encouraged more research in the field of the leptin-melanocortin-system.[49] Several groups have identified mutations of the MC4receptor in obese subjects.[50,51,52] The endogeneous cannabinoid system interferes with energy intake

and homeostasis by central and peripheral pathways.[53] Both animal and human studies have associated the endocannabinoid system with obesity. Blockade of the cannabinoid receptor 1 (CB1) causes weight loss and reduction in waist size. Recent studies on common variants of the CB1 receptor points towards a link between CB1 receptor gene polymorphism and body fat distribution.[54,55,56]

Another factor interfering with obesity is serotonin, a central acting peptide that reduces food intake.[57] Fluoxetine, introduced in the clinical arena as an antidepressant, acts on serotonin receptors in the arcuate nucleus and induces hypophagic effects due to stimulation of the 5HT 2c receptor. Fluoxetine in high dose is FDA approved in treatment of disorders like bulimia nervosa. Administration of fluoxetine decreases appetite and induces modest weight loss when administered over a period of 12 to 57 weeks.[58] Available data concerning weight reducing effects of fluoxetin are scarce and analysis is difficult due to high patient drop-out during studies observing the long term effects of pharmacotherapy on weight. There is some evidence that administration of antidepressants in addition to dietary treatment and/or lifestyle intervention is more effective in achieving weight reduction than diet restrictions and/or lifestyle modification alone.[59]

One of the 3 criteria for the diagnosis of PCOS is hyperandrogenaemia. There appears to be a relation between hyperinsulinaemia and high testosterone levels in PCOS. Whether this is a causal relationship remains largely unresolved[60] but there are arguments to point into the direction of direct ovarian production of androgens stimulated by the high insulin levels. Moreover, it has been established that insulin stimulates steroidogenic enzymes in theca cells[61,62] such as P450c17 alpha activity. This enzyme converts adrenal steroids such as DHEAS and androstenedione into testosterone and dihydrotestosterone. This concept has been confirmed indirectly by the study of Nestler in 1996.[63] Administration of metformin 500mg three times daily for four to eight weeks to clomiphene resistant anovulatory patients reduced circulating insulin and decreased ovarian P450c17 alpha activity resulting in lower androgen levels.

Already in 1980, dysregulation in the hypothalamic-pituitary-adrenal axis as well as increased urinary cortisol clearance was reported in obese women.[64] Late-onset adrenal hyperplasia (CAH) had been regarded as a source of excessive steroidproduction. Partial enzyme deficiencies (3ß-HSD, 21α-hydroxylase and 11ß-hydroxylase) in steroidogenesis were supposed to represent an important pathophysiologic mechanism in anovulation in at

least one third of the anovulatory population.[65,66] The study of Eldar-Geva is a key study in which a selected group of women was studied.[65] In this study a population of eastern European desent has a high prevalence of CAH (3.7%). Many of these women had ovulatory dysfunction related with the afore mentioned enzymatic defects. Besides, in obese women increased cortisol clearance was observed while morning cortisol levels were normal to low. Recently, it was demonstrated that cortisol levels were high in obese ovulatory women as well as in women with PCOS[67] which suggests enhancement of extra-adrenal steroidogenesis in obese subjects in general. Analysis of steroid precursors in lean and obese women with PCOS as well as in lean and obese ovulatory controls revealed an extra-adrenal source of steroidogenesis rather than a mild form of CAH. A plausible explanation for this could be the conversion of steroid precursor hormones into cortisol by the fat tissue.[68] Moreover, the expression of a "steroid-shuttle" in liver and fat tissue was shown to depend on body composition. Indeed, in visceral adipose tissue the expression of 11ß-HSD type 1 is increased, converting cortisone to cortisol.[69] It is questioned whether high androgens and other steroid hormone precursors are mainly produced by visceral fat to inhibit ovulation or whether it remains nothing else but an epiphenomenon in anovulatory disease, i.e. oligomenorrhoea in obese women.[70]

Insulin and obesity

Insulin resistance is positively related to visceral fat mass and has demonstrated to inhibit follicle dominance in the ovary. Still, 10 to 15% of women with PCOS has a normal body weight without the typical features of metabolic syndrome (lean PCOS). Arguments in favour of the concept that hyperinsulinaemia is universal in PCOS is the pioneer work of Dunaif.[71] Notably, these data were obtained in women with and without PCOS looking at insulin resistance and total body fat, but without assessments of body fat distribution. Apart from this lack of information, it has been shown that only half of PCOS patients were hyperinsulinaemic.[72,73] Moreover, in approximately 50% of women with PCOS unique autophosphorylation defects of the insulin receptor have been discovered but also post-receptor insulin signalling defects.[74] In mice is proposed that in an insulin resistant state the ovary preserves its sensitivity for insulin as opposed to other peripheral tissues.[75,76]

Therefore, insulin-sensitizing drugs may be effective for ovulation induction in women with PCOS, and hyperinsulinaemia regardless whether these women are lean or obese.[77,78,79]

Outlines of the studies in this thesis

This thesis addresses several questions from clinical practice that were raised during more than a decade of therapeutic innovations for treatment of infertile women with obesity and/or PCOS. In part one of this thesis we concentrated on different aspects of obesity as obesity is a main expression of PCOS. In the second part of this thesis we added data on body fat distribution and relation to PCOS in a scarce group of lean PCOS.

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Part 1

Metabolic aspects of obesity

Chapter 2

Different cycle ergometer outcomes in severely obese men and women without documented cardiopulmonary morbidities before bariatric surgery

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Chest 2005;128(1):256 – 62

Abstract

Study objectives:
The number of severely obese patients undergoing bariatric surgery is increasing. No incremental cycle ergometer data are available in this category of patients. The current study was undertaken to provide information and to compare outcomes between severely obese men and women during physical exercise.

Design:
Cross-sectional study.

Participants:
Twenty-two men and 34 women, all with a body mass index (BMI) of at least 40 kg/m^2, were selected from among persons participating in a dedicated weight management program that was carried out in the outpatient clinic of a large teaching hospital.

Measurements and results:
Body composition was estimated with bioelectrical impedance. Oxygen uptake (Vo_2) was obtained by breath-by-breath minute ventilation (ventilated hood) and was measured under resting energy expenditure (REE) conditions. Endurance was measured with an incremental cycle ergometer test. Male and female groups were balanced for mean (± SD) age (42.7 ± 7.6 vs 41.8 ± 8.9 years, respectively), BMI (43.0 ± 4.9 vs 41.3 ± 5.2 kg/m^2, respectively), and fat weight (55.5 ± 14.0 vs 56.8 ± 2.2 kg, respectively). Fat-free mass (FFM), FFM index, fasting blood glucose level, insulin level, REE, and Vo_2 at rest and during subjective maximal endurance were higher in the male group. However, maximal Vo_2 (women, 119 ± 19% predicted; men, 92 ± 16% predicted) and anaerobic threshold were higher in the female group (women, 64 ± 12% predicted; men, 48 ± 76% predicted, respectively; $p < 0.0001$).

Conclusions:
Severely obese men were more carbohydrate-intolerant and sustained less physical endurance than was predicted according to standards in comparison with obese women. The cycle ergometer data indicated that male gender was associated with less physical fitness.

Introduction

The World Health Organization considers the problem of overweight and obesity as a matter of priority.[1] Early mortality related to excess body weight was demonstrated in a previously reported meta-analysis.[2] The burden of disease and costs due to overweight have been recently reviewed,[3] and a call for urgent action has been placed for all clinicians who are dealing with obese patients.

Obesity is the principal driver for metabolic syndrome and diabetes. Thus far, substantial and sustained weight reduction with clinical improvements have been demonstrated only after bariatric surgery.[4] In terms of co-medications, long-term weight loss of at least 10% is necessary to reduce the number of prescriptions for diabetes and cardiovascular disease, whereas weight reduction of ≥ 15% is needed to prevent the issuing of new prescriptions.[5] The National Institutes of Health consensus conference[6] in 1991 established guidelines and indications for the surgical management of severe obesity that are now widely accepted. A body mass index (BMI) exceeding 40 kg/m^2 indicates that a person is severely obese and therefore has an indication for surgery. Bariatric surgery may also be a feasible treatment option for people with a BMI between 35 and 40 kg/m^2 who experience life-threatening problems like severe obstructive sleep apnoea syndrome, with or without alveolar hypoventilation syndrome, and obesity-related cardiovascular disease or diabetes. Although cardiovascular disease and diabetes are specifically related to upper body obesity, guidelines do not differentiate between male-type (upper body) vs female-type (lower body) fat distribution.

The number of people with various obesity-related complaints who have undergone bariatric surgery in the United States has more than doubled within the last decade.[7] Prior to surgery, many of these people report obesity-related dyspnoea complaints, while other patients deny any dyspnoea at all. Exercise ventilation monitoring appears to be an elegant tool to provide insight into the evaluation of dyspnoea.[8] Moreover, cardiopulmonary endurance analysis has been shown to be valuable in selecting patients who are at high risk for surgery-related complications.[9] Yet, no such data have been obtained from groups of severely obese persons. The aim of the current study was to perform cardiopulmonary exercise capacity tests in severely obese patients who are scheduled for anti-obesity surgery, using cycle ergometer tests (CETs) under standardized stress conditions. The aim

of the current study was to document CET outcomes in consecutively referred individuals, independently from complaints, and to compare the obtained results of men and women.

Materials and Methods

Patients

Patients were eligible for participation in the study if they had grade III severe obesity (BMI, ≥ 40 kg/m^2), according to the classification of the World Health Organization.[1] All patients participated in a dedicated weight management program (WMP) that was carried out in the outpatient clinic of a large teaching hospital, with the intent that they would undergo laparoscopic gastric banding. Our WMP offers a multidisciplinary approach to obese patients, aiming to reduce weight and to optimize general health before undergoing planned bariatric surgery. In a WMP, all patients receive general information about nutrition, lifestyle modification, and physical exercise. Each individual is seen by the same dietician and is advised to keep a mildly energy-restricted diet (2,092 kJ/d less than the daily requirement). Patients with a fasting blood glucose (FBG) concentration of ≥6.1 mmol/L (≥110 mg/dL) and those individuals with a BMI of ≥40 kg/m^2, irrespective of FBG concentration, received additional pharmacotherapy with metformin at a maximal dose of 1,000 mg bid.[10] Each individual was specifically asked about laxative use, purging, and whether they experienced binge eating attacks. Indeed, all patients reported frequently occurring unstoppable eating, and many experienced multiple binge eating attacks each day. Notably, gastric banding is a restrictive antiobesity procedure, which can be hazardous to anyone with extreme eating impulses. Based on these arguments, all patients were treated with fluoxetine, in a daily dose of 20 mg tid, prior to undergoing planned gastric banding. It is of note that a dosage of 60 mg of fluoxetine per day has been approved by the US Food and Drug Administration for the treatment of bulimia nervosa and obsessive-compulsive disorder.[11]

Excluded from participation were those patients with symptomatic coronary heart disease staged according the New York Heart Association class II and III, those with COPD disease of severity grade II and III (using Global Initiative for Chronic Obstructive Lung Disease criteria[12]), pregnant women, patients with general endocrine disorders, patients with known diabetes, patients who were treated with oral blood glucose-lowering agents

and/or insulin before the start of the WMP, individuals who already had been treated with anti-obesity drugs, antidepressants, or glucocorticoids, and patients who were incapable of performing an adequate CET.

At entry into the WMP, medical screening was performed in 70 consecutive patients, of whom 8 were receiving oral glucose-lowering agents, 2 were insulin users, and 2 had hypothyroidism. Written informed consent was obtained from the remaining 58 patients to participate in the study. One man and one woman were unable to perform a CET, hence, complete data were available for 56 patients, who were used in the study. The study was approved by the Medical Ethical Committee of Reinier de Graaf Groep Hospital.

Methods

At entry into the WMP, early morning fasting blood samples were taken and analyzed with commercially available assays. The following assessments were performed in men: total testosterone level (normal value, 9 to 35 nmol/L); and luteinizing hormone (LH) (normal value, 3 to 12 IU/L). Both hormones were assayed with an auto-analyzer (Immulite; Diagnostic Products Co; Los Angeles, CA). The following assessments were performed in men and women: fasting insulin level (normal value, < 20 mU/L) (Coat-a-Count radioimmunoassay; Diagnostic Products Co), and FBG concentration (normal FBG value, <6.1 mmol/L [<110 mg/dL]; carbohydrate intolerance, 6.1 to 6.9 mmol/L [110 to 125 mg/dL]; overt diabetes, ≥7.0 mmol/L [≥126 mg/dL]) (using American Diabetes Association criteria].[13] Androgen deficiency was defined as an early morning total testosterone level of ≤8 nmol/L, whereas in Leydig cell failure it was 8 to 15 nmol/L combined with an elevated LH level of > 18 IU/L (equal to 1.5 times the upper limit of the eugonadal reference range for young men).[14,15,16]

After 3 months of the WMP, body composition was measured with bioelectrical impedance analysis (BIA) Bodystat 1500; Bodystat Ltd; Isle of Man, UK] and fat-free mass (FFM), fat weight (FW), and FFM index (FFMI) [FFM × height^2] were calculated. Resting energy expenditure (REE) was determined with the ventilated hood method and was calculated with the equation of Harris and Benedict[17] Breath-by-breath oxygen uptake (Vo_2), carbon dioxide output, and minute ventilation (Ve) were measured (Oxycon α

system; Erich Jaeger, GmbH; Wuerzburg, Germany). To establish REE conditions, all participants were asked to refrain from any food, beverage, and tooth brushing for at least 8 h prior to the analysis. Measurements were performed in a special light-dimmed laboratory after 30 min in semirecumbent position, and outcomes were corrected for a respiratory exchange ratio of 0.82.

CET was performed with a bicycle ergometer (Ergoline GmbH; Bitz, Germany). Prior to CET, an indwelling catheter was inserted into the radial artery, and arterial blood samples were taken for blood gas analysis and plasma lactate determination (normal resting value, 0.9 to 2.0 mmol/L) (i-STAT; Abbott Corp; East Winsor, NJ). After a 3-min warm-up period, the net Vo_2 at 20% of the predicted maximum workload – Vo_2 measured under REE conditions (Vo_2unloaded) was calculated. This phase was followed by an interval-free slope-wise increase of workload with 10% of maximal predicted workload per subsequent minute starting at t=3 min. The anaerobic threshold (AT) was determined with the ventilatory equivalent method (ie, an increase in the ventilatory equivalent of O_2 [EqO_2 = Ve/Vo_2] without a simultaneous increase in the ventilatory equivalent of CO_2 [$EqCO_2$ = Ve/carbon dioxide output].[18] AT was expressed in absolute values (milliliters per minute) and as the percent predicted of the maximum Vo_2 (Vo_2max).[19,20] In order to achieve the Vo_2max, the CET was continued up to maximal subjective exhaustion, and otherwise was stopped in case of cardiocirculatory, ventilatory, or musculoskeletal limitations.[21,22,23] At the start of the CET and at subjective exhaustion, Borg scores for dyspnoea and leg fatigue were assessed using standard questionnaires[24] and objective measurements were also made (ie, Vo_2max, heart rate reserve, and breathing reserve).

Statistical Analysis

All results are expressed as the mean±SD. Comparisons between men and women were made with the unpaired, two-sided Student t test. Statistical significance was defined as a p-value of < 0.05.

Results

A total of 56 patients (22 men and 34 women) were included in the study. There were no group differences in terms of age, BMI, and FW (Table 1). Men had a higher FFM than women, with a mean FFMI of 25 ± 2 kg/m^2 in men vs 21 ± 1 kg/m^2 in women ($p < 0.0001$).

All men, except for one patient who had Leydig cell failure (testosterone, 10.4 nmol/L; LH, 18.2 U/L), were eugonadal. FBG concentrations and insulin levels were higher in the male group. FBG concentration was ≥6.1 mmol/L in 13 men (59%), of whom 8 men (36%) had diabetes and 5 men (23%) had carbohydrate intolerance (according to American Diabetes Association criteria[13]). FBG concentration was ≥6.1 mmol/L in 12 women (35%), of whom 4 women (12%) had diabetes and 8 women (24%) had carbohydrate intolerance.

Calculated REE in men and women matched with predicted values (Table 2). The absolute Vo_2 at 20% of maximal predicted workload and the net Vo_2unloaded were similar between men and women (men, 570 ± 183 mL/min; women, 570 ± 107 mL/min; difference was not significant).

Table 1: Demographic anthropometric data of severely obese men and women*

Variables	Men (n=22)	Women (n=34)	P Value
Demography and anthropometry			
Age, years	42.7 (7.6)	41.8 (8.9)	NS
Body weight, kg	138.9 (19.7)	115.4 (14.4)	<0.0001
Body height, m	1.795 (0.072)	1.653 (0.125)	<0.0001
BMI, kg/m^2	43.0 (4.9)	41.3 (5.2)	NS
BIA			
FW, kg	55.5 (14.0)	56.8 (12.2)	NS
FFM, kg	82.7 (8.4)	58.5 (54.9)	<0.0001
FFMI, kg/m^2	25.2 (2.0)	21.0 (1.2)	<0.0001
Laboratory test			
FBG, mmol/L	7.8 (2.1)	6.3 (1.5)	0.02
Fasting insulin, mU/L	33.1 (24.9)	16.8 (11.4)	0.02
Testosterone, nmol/L	13.6 (4.7)		

*Values given as the mean (SD), unless otherwise indicated.
NS = not significant

Dividing net Vo_2unloaded by FFM, representing the Vo_2 per kilogram of leg muscle, revealed lower levels in men compared with women (6.9±2.1 vs 9.8±1.7 mL/min/kg, respectively; p<0.0001). Furthermore, women performed better at subjective maximal endurance than predicted according to standards, while relative performance was lower in

Table 2: Metabolic rates and CET outcomes including cardiopulmonary and muscle performance during subjective maximal endurance in severely obese men and women

Variables	Men (n = 22)	Women (n = 34)	p Value
Metabolic rate			
REE			
Calculated, kcal/24 h	2,506 (509)	2,003 (371)	< 0.001
Predicted, kcal/24 h	2,576 (299)	1,895 (158)	< 0.001
% predicted†	0.99 (0.21)	1.06 (0.17)	NS
REE	0.80 (0.07)	0.81 (0.06)	NS
Cycle ergometer test			
Maximal workload			
Achieved, W/min	226 (51)	174 (38)	< 0.0001
Predicted, W/min	381 (38)	220 (81)	< 0.0001
% predicted	60 (12)	70 (33)	NS
Heart rate reserve, % predicted†	14 (12)	9 (8)	NS
O_2 pulse, % predicted†	108 (19)	113 (46)	NS
$\dot{V}_{O2}$ slope, mL/min/W	10.8 (1.8)	10.5 (2.1)	NS
Breathing reserve, % predicted†	15.8 (15.8)	12.2 (14.0)	NS
Borg score increase			
Dyspnea	2.4 (2.0)	3.1 (2.3)	NS
Leg fatigue	7.0(2.7)	6.9 (2.9)	NS
Arterial lactate increase, mmol/L	8.03 (2.34)	8.05 (2.24)	NS
$\dot{V}_{O2}$at REE, mL/min	361 (73)	296 (76)	< 0.001
$\dot{V}_{O2}$unloaded, 3 min at 20% $\dot{V}_{O2}$max predicted, mL/min	940 (192)	862 (132)	NS
Net $\dot{V}_{O2}$unloaded, mL/min	570 (183)	570 (108)	NS
$\dot{V}_{O2}$ at AT mL/min	1,534 (240)	1,303 (262)	< 0.001
$\dot{V}_{O2}$max % predicted†	48 (7)	65 (12)	< 0.0001
$\dot{V}_{O2}$max % predicted range†	37–60	43–91	
$\dot{V}_{O2}$max			
mL/min	2,934 (549)	2,328 (639)	< 0.0001
% predicted†	92 (16)	119 (19)	< 0.0001

*Values given as mean (SD), unless otherwise indicated. See Table 1 for abbreviation not used in the text.
† All predicted values are adapted from American Thoracic Society/American College of Chest Physicians statements.[38] Predicted performances are expressed as kilojoules per minute and converted to Watts per minute by multiplying by 0.2724.

men. A similar picture emerged for Vo_2 at AT (expressed as the Vo_2max percent predicted), that is, it was higher in women compared with men (Table 2).

Men had a small but significantly lower PaO_2 and a significantly higher $PaCO_2$ at rest (Table 3). This might suggest an overall lower ventilation/perfusion ratio in men compared with women. The pathophysiology behind this observation can be explained in the first place by a higher circulating blood volume in men, or by the different distribution of body fat (*ie*, male type upper-body fat vs female-type lower body fat). Differences in fat distribution may lead to diminished ventilation in the lower parts of the lung due to "abdominal compression". This may explain a relatively lower vital capacity, which results in a relatively lower level of ventilation (Table 4).

Table 3: Resting and exercise arterial bloodgas analyses in severely obese men and women*

Variables	Men		Women		p Value	
	Rest	Maximal Exercise	Rest	Maximal Exercise	Rest	Maximal Exercise
PH	7.41 (0.03)	7.33 (0.05)	7.41 (0.02)	7.33 (0.04)	NS	NS
$PaCO_2$	5.4 (0.6)	5.0 (0.5)	5.1 (0.4)	4.7 (0.4)	< 0.02	< 0.03
PaO_2	11.6 (1.5)	12.6 (1.0)	12.7 (1.3)	13.0 (1.1)	< 0.01	NS
SaO_2	96.4 (1.7)	97.2 (1.0)	97.6 (1.1)	97.0 (1.0)	< 0.01	NS
HCO_3^-	26 (2.9)	18.7 (4.0)	24.2 (1.9)	18.5 (2.2)	< 0.02	NS
BE	1.3 (2.9)	−5.1(2.8)	0.1 (1.8)	−6.4 (2.4)	NS	NS

* Values given as mean (SD), unless otherwise indicated.
SaO_2 = arterial oxygen saturation.
BE = base excess.
See Table 1 for abbreviation not used in the text.

None of the patients experienced limitations other than physiologic cardiopulmonary limitations during CET, particularly at maximal subjective exhaustion. Moreover, men and women achieved similar breathing and heart rate reserves. Maximal fatigue, expressed as Borg scores for dyspnoea and leg fatigue, as well as the increase of arterial lactate levels were similar between groups.

Table 4: Spirometry outcomes during subjective maximal endurance in severely obese men and women

Spirometry	Men (n = 22)	Women (n = 34)	p Value
Vital capacity			
L	4.75 (0.76)	3.67 (0.64)	< 0.0001
% predicted	92.4 (12.8)	104.9 (13.2)	< 0.001
FEV_1			
L	3.8 (0.6)	2.9 (0.6)	< 0.0001
% predicted	95.8 (13.7)	98.8 (15.6)	NS

Values given as mean (SD), unless otherwise indicated.
See Table 1 for abbreviation not used in the text.

Discussion

The current study demonstrated that severely obese men and women, without prior documented cardiopulmonary morbidities, sustained subjective maximal exhaustion without cardiocirculatory, ventilatory, or respiratory limitations. In men and women, leg fatigue was the main reason to stop cycling, as shown in Borg scores. The aim of this study was also to compare fitness in untrained severely obese men and women during heavy exercise. First, it appeared that men were relatively less obese but more carbohydrate-intolerant, which suggests that these men were more sensitive toward the deregulation of carbohydrate metabolism in comparison with women. Second, men and women had similar net Vo_2unloaded outcomes despite of a higher FFM in men. Third, men had a lower Vo_2max and a lower AT, in comparison with the predicted values based on existing standards.

The results of the current study appear to be in contrast with those of a previously reported study[25] that was also performed in severely obese men. These men had a similar BMI, but were able to achieve a higher peak of exercise at exhaustion and a higher AT, as predicted. The authors ascribed this phenomenon to the large muscle mass of these “big” men.[25] The subjects studied were untrained Italian employees without cardiorespiratory disorders or obesity-related complaints, whereas in the current study all patients were white Dutch persons who had the intention of undergoing gastric banding surgery, which was indicated as treatment for their severe obesity. The discrepant results between the studies may be explained by differences in patient selection and ethnicity. The finding in the current study of more deregulation of carbohydrate metabolism in men is in agreement with

that of a previously reported study looking at treadmill exercise in obese men and women. It was reported that the age-adjusted risk for having metabolic syndrome was 10.1 (95% confidence interval, 9.1 to 11.2) and was higher in men with a poor physical health compared with those who were in good shape. Differences in risk were also noted in obese women, albeit less than in men (age-adjusted risk for having metabolic syndrome, 4.9; 95% confidence interval, 3.8 to 6.3).[26] This discrepancy in risk indicates "gender interaction" with physical fitness in obese subjects. Insulin resistance may also interact with fitness. It has been shown previously that insulin resistance interacts with cardiovascular components of the metabolic syndrome by impairing capillary recruitment in muscle.[27] Furthermore, skeletal muscle tissue depends highly on lipid oxidation, particularly during exercise.[28] Obese individuals, however, usually express high intramyocellular fat levels with a decreased capacity of muscular lipid oxidation.[29,30] Such an paradoxical relationship might be of importance because an increase in intramyocellular fat is also linked with insulin resistance in obese individuals.[29,31]

A linear and inverse association between obesity and respiratory function has been previously shown in two large cohort studies.[32,33] This relationship was particularly present in men, and in both sexes with mainly male-type (upper-body) obesity.[32,33] In daily practice, however, obesity does not alter respiratory function, except in those individuals with extreme obesity (ie, weight [in kilograms]/height [in centimeters] of >1).[34] In the present study, men had significantly lower PaO_2 and a significantly higher $PaCO_2$ at rest, while PaO_2 differences disappeared during maximal physical endurance. In agreement with these observations, it was also shown that vital capacities (percent predicted) were lower in men. Nevertheless, all patients included in the study fell within the generally accepted 95% confidence limits, as observed in the general population. Whether we should recommend routine CETs or other cardiopulmonary endurance tests to analyze patients before bariatric surgery cannot yet be answered. There are no convincing data to show which severely obese patient has an unacceptably high risk to sustain bariatric surgery without major complications. However, there is a body of evidence to suggest that the determination of Vo_2max can provide crucial information about the health status of severely obese men. It has been shown previously that all-cause mortality was highest in obese men with a low Vo_2max.[35] Today, a consensus has been reached to consider all patients for bariatric who have a BMI of >35kg/m^2 with significant comorbidities, or >40kg/m^2 without

comorbidities.[36] One should realize, however, that many hospitals are unable to cope with the demand for anti-obesity surgery. Waiting times are long, and duplicate referrals to other hospitals are common. It would be a challenge to implement cardiopulmonary exercise tests to establish a clinical triage for those patients who really benefit from antiobesity surgery. More studies on this issue are certainly needed.

The current study has a few limitations. First, in contrast with dual-energy radiograph absorptiometry (DXA), BIA does not provide information on body fat distribution. An assessment of the degree of agreement between DXA and BIA in non-obese and obese subjects revealed similar accuracies for both techniques.[37] However, correlations of DXA and BIA were strongest in non-obese individuals.[37] Second, only a limited number of subjects who had decided to request surgery as a definitive obesity treatment were included in the study. Thus far, there are no studies comparing the CET outcomes of randomly selected severely obese individuals with those of individuals with obesity-related complaints requesting for bariatric surgery.

Overall, this study has demonstrated that severely obese men and women without documented cardiopulmonary morbidities were capable of sustaining maximal subjective exhaustion without cardiopulmonary limitations. We hypothesize that male gender interacts negatively with fitness in the case of abundantly deposited fat tissue. Certainly, fat deposition studies in combination with CETs are needed to clarify the biological effects of male-type obesity (upper-body) vs those of female-type obesity (lower-body) within male or female groups. This type of research, although technically complicated, is certainly required in much larger individuals. Whether gender and the amount of male-type obesity (upper-body) should be used in operational definitions for obesity severity needs further answering.

Footnotes

Abbreviations: AT = anaerobic threshold; BIA = bioelectrical impedance analysis; BMI = body mass index; CET = cycle ergometer test; DXA = dual-energy radiograph absorptiometry; FBG = fasting blood glucose; FFM = fat-free mass; FFMI = fat-free mass index; FW = fat weight; LH = luteinizing hormone; REE = resting energy expenditure; VE = minute ventilation; Vo_2 = oxygen uptake; Vo_2max = maximum oxygen consumption; Vo_2unloaded = maximum workload − oxygen uptake measured under resting energy expenditure conditions; WMP = weight management program

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Chapter 3

Daily high doses of fluoxetine for weight loss and improvement in lifestyle before bariatric surgery

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Abstract

Background:
The number of gastric restrictive bariatric operations is increasing each year, but about one-fifth of patients will become disappointed due to unsatisfactory weight reduction or annoying complications. We questioned whether weight reduction by taking high doses of fluoxetine improves lifestyle before surgery.

Methods:
84 severely obese subjects were referred by one bariatric surgeon for medical weight reduction and dietary counseling, before bariatric surgery. Subjects were voluntarily treated with fluoxetine, 60 mg per day, and followed-up at 1, 3, 6 and 12 months. Surgery was scheduled between 6 to 12 months after subjects started to take fluoxetine. Endpoints of this study were body weight reductions, the number of individuals who decided for themselves to postpone surgery for at least 6 months, and side-effects of fluoxetine therapy.

Results:
84 severely obese subjects consisting of 28 men (group 1) and 41 women (group 2) consented to take fluoxetine for its anorectic effects, whereas 12 men and 3 women who did not want to take fluoxetine served as the control group (group 3).Weight in these 3 groups at baseline was 149±26, 124±17, and 132±23 (controls) ($P<0.05$) with BMI 46±3, 44±3, and 45±2 (controls) kg/m^2 (NS). Maximum weight reduction before surgery in male and female fluoxetine users occurred at 3 and 6 months, respectively. At 6 months, men had achieved a weight reduction (kg) of -8.3 (95% CI: -9.3 to -5.9), women of -13.3 (95% CI: -16.3 to -8.8), sex difference $P<0.001$, and controls of -1.6 (95% CI: -3.8 to -2.5) kg, group difference $P<0.0001$. Only 2 men stopped fluoxetine because of annoying sexual side-effects. At 6 months, 25 fluoxetine users (29.7%) and none of the controls consented to postpone the time of surgery for at least another 6 months.

Conclusions:
Fluoxetine is effective to reduce weight in severely obese men and women who originally had requested to undergo bariatric surgery. One-third of subjects who consented to take fluoxetine as an anorectic drug agreed to delay surgery for at least 6 months later than scheduled.

Introduction

Obesity is increasing at an alarming rate and is considered today as a worldwide epidemic.[1] As the problem accounts as a risk factor for numerous medical disorders and excess mortality, it is imperative that effective treatments be developed. Longterm weight loss is crucial in high-risk populations, particularly in obese patients with co-morbidities and in those subjects with a BMI >40 kg/m^2. Substantial and sustained weight reduction with associated clinical improvements have been demonstrated only with bariatric surgery.[2,3]

In the third National Health and Nutrition Examination Survey, it was shown that many severely obese patients suffer from anxiety disorders, depression and low self-esteem.[4] Surgical weight loss resulted in improvement in physical and mental quality-of-life measures in women, younger subjects, and those with greater excess weight loss.[5] In contrast, a few studies with antidepressants have been conducted showing inconsistent anti-obesity effects. As a result of these unpredictable outcomes, approval of Selective Serotonin Reuptake Inhibitors (SSRI's) by the U.S. Food and Drug Administration (FDA) was only received for bulimia nervosa (fluoxetine) and obsessive-compulsive disorder (fluvoxamine, paroxetine, sertraline, and fluoxetine).[6]

Bariatric surgery is widely practised, in order to achieve substantial and sustained weight loss in severely obese individuals. Subjects who achieve a stable weight reduction of at least 5 to 10% will experience improvement of their physical and mental condition.[3] Moreover, in a study comparing conservative and surgical obesity treatments, it was found that a 2-year maintenance of weight loss was associated with favorable changes on practically all personality traits. These improvements occurred only after surgical treatment and were in proportion to the magnitude of the weight loss.[7] However, gastric restrictive bariatric surgery is not successful in all subjects. Long-term results remain unsatisfactory in 10-25% of subjects as a result of weight regain or unacceptable side-effects directly related to gastric restrictive surgery.[8] Optimization of patient selection before surgery is therefore mandatory.

Data from randomized clinical trials are sufficient to conclude that the Selective Serotonin Reuptake Inhibitor (SSRI) fluoxetine promotes weight loss for at least 6 months when given in addition to recommendations for changes in dietary habits.[9] Fluoxetine in a dose of 60 mg per day induces immediate suppressive effects on appetite.[10]

The purpose of the current study was to treat severely obese subjects with fluoxetine, aiming to suppress appetite and unstoppable eating. It was questioned whether the use of this drug may help to gain time for lifestyle changes in order to become better prepared for the effects of the surgery.

Patients and Methods

One bariatric surgeon referred 84 severely obese subjects for preoperative medical management. They were eligible for participation in the study if they had a strong wish to undergo bariatric surgery, and if they had obesity Grade III (BMI ≥40 kg/m^2) according to the classification of the World Health Organization (WHO).[11] All subjects participated in a dedicated *Weight Management Program* (WMP) carried out in the outpatient clinic, comprising extensive dietary counseling and medical treatment. In the WMP, all subjects received general information about nutrition, lifestyle modification, and physical exercise. The study was performed in a large teaching hospital (RdGG) in the western part of The Netherlands.

The WMP included 5 visits to the outpatient clinic of the hospital (0, 1, 3, 6, 12 months). Baseline fasting blood was drawn to exclude co-existing endocrine disorders and diabetes. A normal fasting blood glucose concentration (FBG) is <6.1 mmol/l (<110 mg/dl), adopted from the criteria of the American Diabetes Association (ADA).[12] Impaired fasting glucose was diagnosed when FBG was 6.1 to 6.9 mmol/l (110 to 125 mg/dl), and diabetes when FBG was ≥7.0 mmol/l (≥126 mg/dl). Those patients who were on glucose-lowering agents were considered to have diabetes.

Excluded from analysis were all referred subjects <18 and >70 years old, subjects with a BMI <40 kg/m^2, pregnant women and women with a pregnancy wish, regular users of non-steroidal inflammatory drugs (NSAIDs), anti-epileptics, glucocorticoids, subjects with abnormal thyroid function and no established treatment, and subjects who preferred treatment with registered anti-obesity drugs. Patients on antidepressants or antipsychotic drugs were included for analysis but served as the control group. None of these subjects were intentionally treated with fluoxetine as an anorectic drug because of an increased risk for toxic interactions. Patients on fluoxetine before inclusion were excluded from further analysis.

Each subject received information about appetite suppressive properties of fluoxetine, advantages and goals of intensive lifestyle modification before undergoing gastric restrictive surgery, and possible side-effects of fluoxetine. Information was also provided about the duration and meaning of the WMP, i.e. a full effort by each participating subject to change lifestyle for a period of at least 1 year. Lifestyle modification included the intention to adjust to a mildly energy-restricted diet (2092 kJ/day less than needed daily), to eat three meals each day at regular times, to stop junk food and to keep a food diary. One experienced dietician frequently saw all subjects. Finally, each subject received instructions by a physiotherapist for tailormade exercise for at least 30 minutes per day.

In principal, all surgical procedures were planned between 6 and 12 months after the start of the WMP. Subjects were allowed to delay surgery for a minimum 6 months according to their wish, at any time of the program. Each participant was free to choose either no medical treatment against obesity or fluoxetine 60 mg once each day. Information about the study was provided by the same physician, and written informed consent was obtained from all participants. In order to optimize compliance, fluoxetine in a dose of 60 mg was taken once daily at bedtime. In the final analysis, three groups were compared: men (group 1) and women (group 2) all taking fluoxetine, and controls who either refused to take fluoxetine or were excluded because of co-medication (group 3). The study had an open-label design, i.e. the control group did not receive placebo or any other new treatment for at least 6 months. Moreover, all three groups waited at least 6 months for the surgery.
At each visit, subjects were requested to answer the following questions:

1) ‘Do you think that fluoxetine helps you to control your appetite?’
2) ‘Did you take fluoxetine regularly?’
3) ‘Do you agree to continue fluoxetine until the next visit or do you want to stop it?’
4) If applicable: ‘For what reason did you stop taking fluoxetine?’
5) Your surgery is planned between 6 and 12 months of the WMP. Are you happy to go along with this time schedule? YES/NO.
   Would you prefer to delay your planned surgery for at least 6 months? YES/NO.
   In case of ‘NO’, laparoscopic adjustable gastric banding (LAGB) was performed after 6 months. In case of ‘YES’, LAGB was delayed for at least another 6 months.

The Medical Ethical Review Committee of Hospital Reinier de Graaf Groep, The Netherlands, approved the study.

Statistical Analysis

Baseline characteristics between groups were compared with Student *t*-tests and χ^2-tests if appropriate. Follow-up measurements on weight(-loss) were analyzed with a linear mixed-effect ANOVA model, with time as fixed factor and patient as random factor. Weight change was compared between men and women using the same mixed-effect model extended with gender as a fixed factor, and with the interaction between gender and time. Comparisons with control subjects were similarly performed.

Results

Of all 84 subjects, there were 6 individuals who were excluded for intentional treatment with fluoxetine, because they were using co-medication listed as exclusion-drugs according to the protocol. These drugs were antidepressants other than fluoxetine (n=5) and anti-epileptics (n=1). Another 9 subjects preferred not to take any anti-obesity medication, but accepted all other WMP conditions. Therefore, a total of 15 subjects were excluded from intentional treatment with fluoxetine and served in the current study as control group.

A total of 69 subjects started with the lifestyle modification program of the WMP including intentional fluoxetine therapy, while the remaining 15 subjects received only the lifestyle modification program. Fluoxetine users and controls were comparable for age, BMI, alcohol consumption, and degree of carbohydrate intolerance. Tobacco smoking was less prevalent in controls (Table 1).

Twentyseven out of 28 men (96.4%) consented to take fluoxetine and completed 38±7 ‘fluoxetine-weeks’, with a range from 28 to 52 weeks. Sixteen of them (57.1%) preferred to go along with fluoxetine for the entire study period of 52 weeks. Thirty-nine of all 41 women (95.1%) agreed to take fluoxetine and completed 35±6 ‘fluoxetine-weeks’, with a range from 27 to 52 weeks. Eighteen women (43.9%) preferred to go along with fluoxetine up to 52 weeks (Table 2). All male and female fluoxetine users reported appetite suppression at 1 and 3 months, while in controls this was reported by 3 subjects at 1 month (20%), and by 4 subjects at 3 months (27%) of the WMP. Seventeen male (61%) and 35

Table 1: Baseline characteristics of men and women taking fluoxetine 60 mg once a day vs controls

Individuals - No.	Men 28	Women 41	Controls 15	P-value
Fluoxetine Treatment	+	+		
Age (years)	46±13	44±13	ns	
Body weight (kg)	149±26	124±17	132±23	<0.05
Body Mass Index (kg/m^2)	46±3	44±3	45±2	ns
No. of glasses wine/ beer/ liquor per week	12±9	10±6	13±9	ns
No. of daily smokers	24 *(86%)*	30 *(73%)*	8 *(53.3%)**	<0.05
No. of IFG patients	14 *(50%)*	17 *(41%)*	6 *(40%)*	ns
No. of patients with diabetes	9 *(32%)*	12 *(29%)*	4 *(27%)*	ns

IFG = Impaired Fasting Glucose.
*The control group consisted of a significantly lower no. of daily smokers than the other groups.
Italics: Percentage of positive findings per group.

female fluoxetine users (85%) reported a suppression of their appetite at 6 months (sex difference $P<0.001$), while at that time appetite suppression never occurred in controls. Forty-four of the 69 fluoxetine users (18 men (64.2%) and 26 women (63.4%)), and all 15 controls insisted on having their surgery done as was originally scheduled. At 6 months, they all responded 'No' to the question 'Would you prefer to delay your planned surgery for at least 6 months?'

The remaining 25 subjects (10 men (35.7%) and 15 women (36.5%)) agreed to postpone surgery since they answered 'YES' to the question 'Would you prefer to delay your planned surgery for at least 6 months'? In other words, the subjects who were initially referred by the surgeon to follow the WMP for a period of 6 months had now decided to delay the surgery for another 6 months. All of them took fluoxetine during the entire 12-months follow-up.

Table 2.: Comparisons of body weight, fluoxetine use, and postponements of surgery in 84 severely obese patients

	At WMP start	At 1 month	At 3 months	At 6 months	At 12 months
Men					
Body weight (kg)	149±26	-5.9 (-7.8 to -2.9)	-9.7 (-11.8 to -6.3)	-8.3 (-9.3 to -5.9)†	-17. 2 (-24.2 to -9.8)
Fluoxetine users (no.)	n=28	n=28	n=27	n=27	n=16
No. of surgical delays	nobody	4 (14%)	9 (32%)	10 (36%)	10 (36%)
Women					
Body weight (kg)	124±17	-4.9 (-5.9 to -3.0)	-8.1 (-10.3 to -5.7)	-13.3 (-16.3 to -8.8)	-15.4 (-18.6 to -6.2)
Fluoxetine users (no.)	n=41	n=41	n=41	n=39	n=18
No. of surgical delays	nobody	12 (29%)	17(41%)	15 (37%)	15 (37%)
Controls					
Body weight (kg)	132±23	-1.9 (-2.8 to -0.4)*	-3.1 (-6.3 to -1.0)*	-1.6 (-3.8 to 2.5)*	-9.0 (-12.6 to -6. 2)*
No. of surgical delays	nobody	nobody	nobody	nobody	nobody

Body weight reductions are expressed as mean (kg) (95% Cl).
Data at 12 months are pooled from subjects who had surgery after 6 months and from subjects who delayed surgery.
WMP = Weight Management Program.
†Men achieved less weight reduction than women (P<0.01).
*Controls achieved less weight reduction than male and female fluoxetine users (P<0.001)

Table 3: Body weights in patients who consented to take fluoxetine and were divided in a group who had the surgery after 6 months and a group who delayed the surgery for another 6 months

	At WMP start	At 1 month	At 3 months	At 6 months	At 12 months
Surgeries (No.)	(n=0)	(n=0)	(n=0)	(n=0)	(n=44)
Body weight (kg)	132±16	-5.3 (-7.1 to -2.6)	-7.8. (-10.1 to -6.0)	-9.3 (-10.9. to -7.3)	-16.4 (-22.6 to -7.9)
Delays (No.)	(n=84)	(n=84)	(n=84)	(n=84)	(n=25)
Body weight (kg)	136±17	-4.8 (-6.1 to -3.2)	-8.6 (-10.9 to -5.9)	-9.1 (-11.2to-6.8)	-8.7 (-10.4 to -5.7)*

*Non-surgical subjects achieved less body weight reduction than surgical subjects at 12 months of the WMP (P<0.001).

WMP =Weight Managment Program.

Weight reductions in male and female fluoxetine users as well as in controls are shown in Table 2. Weight loss expressed as percent loss of total body weight (kg) at 3 months was -6.5% in men and women, and -2.3% in controls. At 6 months, it was -5.5% in men, -10.7% in women, whereas in controls no weight reduction was noticed. At 12 months, pooled data obtained from subjects either with or without laparoscopic gastric banding revealed 11.5% weight loss in men, 8.0% in women, and 6.8% in controls. Weight reduction in surgically and non-surgically treated men and women on fluoxetine are listed in Table 3. Weight loss expressed as percent loss of total body weight (kg) at 12 months was 12.4% in surgically treated and 6.3% in non-surgically treated subjects.

Sexual side-effects were reported as the main reason to stop fluoxetine by two men. The remaining subjects (11 men and 21 women) who decided to stop fluoxetine did so immediately after surgery. All of them reported that they quit because they felt that the drug had lost its appetite-suppressive effect. All other reported side-effects did not cause any patient to stop taking fluoxetine (Table 4).

Discussion

The current observational study was conducted among severely obese men and women, and confirmed significant rapid weight loss with fluoxetine as has been previously reported.[9,10] Perhaps the most important question of the current study was whether fluoxetine treatment could contribute towards a better time management before bariatric surgery. Indeed, it appeared that around one-third of subjects consented to delay their bariatric surgery for at least 6 months. All subjects who decided against fluoxetine treatment or had contraindications for its use, insisted to have their surgery on time.

All subjects reported frequently-occurring unstoppable eating, and many experienced multiple binge eating attacks each day. Notably, gastric banding is a restrictive bariatric procedure, which can be hazardous to anyone with extreme eating impulses.

Table 4: Reported side-effects ascribed to use of 60 mg fluoxetine each day in men and women.

Subjects	Men	Women
No.	28	41
Sleepiness	4 (14.2%)	8 (19.5%)
Increased perspiration	24 (85.7)	28 (68. 2)
Annoying ejaculatory retardation/lack of orgasms	2 (7.1%)	1 (2.4%)
Annoying loss of sexual interest	3 (10.7%)	1 (2.4%)
Annoying nausea longer than 1 week	0	4 (9.8%)
Annoying anxiety within the first week of drug taking	0	2 (4.8%)
Annoying anxiety for more than 1 week	0	1 (2.4%)
Easy bruising	0	1 (2.4%)

Our approach to treat severely obese subjects with fluoxetine preoperatively is based on reported weight reduction ascribed to this drug, and its effective suppression of binge eating and impulsiveness.[13] The first encouraging results with fluoxetine in nondepressed obese subjects have been published in the late 1980s.[14,15] In times of increasing numbers of subjects requesting bariatric surgery, it was decided to study fluoxetine efficacy in terms of weight reduction and time management before surgery. It was hypothesized that fluoxetine, with its combined anorectic and stress suppressive properties, can help subjects to maintain a diet for a longer period and to go along with lifestyle modification.

In the current study, subjects received medical appetite suppression before bariatric surgery regardless of the presence or absence of severe eating problems. Another approach is to add medical treatment after bariatric surgery. Topiramate is an anticonvulsive drug that is shown to be effective in subjects with inadequate weight loss after adjustable gastric banding because of compulsive binge eating.[16,17] There are no reports on fluoxetine after surgery in this category of patients. Whether the use of fluoxetine to all subjects before the surgery might help to select patients with hidden severe binge eating or obsessive-compulsive eating disorder remains to be answered in future studies.

With respect to all limitations of an open-label observational design, the results of the current study indicate that fluoxetine facilitates the medical management of obese subjects before surgery. Obviously, randomized clinical trials (RCTs) are needed to confirm these results and to compare the effects of fluoxetine with newer agents registered for anti-obesity treatment. The current study was not designed to study medical weight reduction before surgery in order to make the surgery easier (smaller liver, more room in peritoneal cavity)[18] or to analyze whether fluoxetine-treated subjects would tolerate their LAGB better than those who were not on fluoxetine. RCTs on quality of life assessments and follow-up CT-scans for organ size determinations are needed to find answers to these clinical questions.

The signalling network underlying central regulation of hunger and satiety includes peripheral hormonal signals from adipose and intestinal tissues, as well signals via the vagus nerve. These gathered signals are routed to the arcuate nucleus (ARC) and the nucleus tractus solitarius (NTS), which in turn activate distinct neuronal networks.[19-22] Yet, the exact mechanism of how fluoxetine suppresses appetite and controls binge eating behavior has not been completely elucidated. There is clear evidence for a strong association between serotonin-driven changes through stress or trauma and impulsive eating behavior.[23] For example, binge eating in the rat becomes stronger after pretreatment with painful stimuli such as foot shocks. Fluoxetine ameliorated binge eating in all animals although most particularly in animals who received prior foot shocks. Serotonin (5-HT) is one of the major appetite-modulating neurotransmitters and is expressed in neurons projecting from the raphe nucleus to the hypothalamus.[24] Neuropeptide Y (NPY) is a powerful feeding stimulant that is synthesized in the hypothalamic arcuate nucleus (ARC) and is found abundantly throughout the central and peripheral nervous systems.[25] 5-HT and NPY neurons share close anatomical links in the paraventricular nucleus (PVN) and ARC.[26] Fluoxetine given to genetically obese young rats led to a reduction in body weight, sustained satiety-inducing effects, and a decrease in NPY immunostaining in the PVN without changes in ARC.[27] A reduction of total as well as fat weight in obese rats on sibutramine was also observed, but this time with similar NPY immunostaining in the PVN region.[28] In other words, by using two different serotonin reuptake inhibitors, successful weight reduction was achieved by apparently different mechanisms.

The FDA approved fluoxetine therapy for bulimia nervosa, but the efficacy in obesity is still debated.[9,29,30] In a systematic review of the literature, it was demonstrated that fluoxetine, primarily given for weight reduction in patients with type 2 diabetes, led to a statistically significant weight loss, thereby improving the metabolic control.[31] In type 2 diabetes, treatment with fluoxetine resulted in a weight reduction of -3.4 kg (95% CI, -1.7 to -5.2 kg) at 8-16 weeks, -5.1 kg (95% CI, -3.3 to -6.9 kg) at 24-30 weeks, and 5.8 kg (95% CI, -0.8 to -10.8 kg) at 52 weeks of follow-up.

However, the current study included a different type of patient and was primarily focused at a rapid reduction of weight, thereby gaining time for lifestyle modification. All subjects were initially referred by general practitioners to one bariatric surgeon for LAGB. This surgeon referred all these patients to the WMP for endocrine screening, dietary counselling and lifestyle medication. Compared with controls, fluoxetine led to a significant weight reduction, while more than one-third of men and women gave up their idea to undergo surgery as quickly as possible.

In conclusion, the current study demonstrated that fluoxetine 60 mg once a day before bedtime along with a dedicated Weight Management Program (WMP) resulted in substantial weight reduction in <6 months without serious side-effects. One-third of subjects who agreed to take fluoxetine accepted to have their surgery postponed for a period of at least 6 months. It is obvious that careful implementation of a healthy diet is beneficial, particularly before restrictive bariatric surgery. However, the presented data need careful interpretation because of the openlabel design of the study and the inclusion of subjects with BMIs between 40 and 50 kg/m^2. More studies are needed to improve our knowledge into how to optimize the medical management of severely obese subjects before surgery.

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Chapter 4

Low 11-deoxycortisol to cortisol conversion reflects extra-adrenal factors in the majority of women with normo-gonadotrophic normo-oestrogenic infertility

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Abstract

Background:
Women with normogonadotrophic normo-estrogenic oligomenorrhoea often disclose a variety of clinical symptoms. Many of these individuals are obese with features of pseudo-hypercortisolism. In the current study, 11-deoxycortisol and cortisol concentrations were determined in this group and compared with ovulatory controls.

Methods and results:
Twenty-six women with clomiphene citrate-resistant infertility, 12 lean and 11 obese ovulatory controls were studied. Women with infertility had the highest 11-deoxycortisol concentrations (mean ± SD: 4.1 ± 1.5 ng/ml) compared with obese and lean controls (3.1 ± 1.4 and 2.4 ± 0.9 ng/ml) ($P < 0.01$), but similar morning cortisol concentrations (0.47 ± 0.15, 0.45 ± 0.16 and 0.47 ± 0.18 nmol/l). Baseline 11-deoxycortisol/cortisol ratios (>90th percentile of ovulatory controls) were elevated in 23/26 infertile women (88%), and in 3/26 women (12%) after adrenocorticotrophic hormone (ACTH) stimulation. Three out of six lean infertile women had elevated baseline 11-deoxycortisol/cortisol ratios, but none of these women had elevated ratios after ACTH stimulation. Stepwise regression analysis, after exclusion of testosterone, revealed significant correlations between the groups (lean controls, obese controls, infertility) and ACTH-stimulated 11-deoxycortisol/cortisol ratio ($P < 0.05$), but not with fasting glucose, insulin, cortisol, 11-deoxycortisol and baseline 11-deoxycortisol/cortisol ratios.

Conclusions:
Congenital adrenal hyperplasia was not observed in the majority of infertile women. The data indicate that extra-adrenal factors were involved in most of the infertility syndromes that were studied.

Introduction

Irregular menstrual cycles and associated ovulation dysfunction harbour a series of heterogeneous clinical entities that are frequently associated with hypertension, dyslipidaemia, hyperandrogenism, hirsutism, obesity and insulin resistance. Many women share three features that are generally accepted for the diagnosis polycystic ovary syndrome (PCOS): (i) oligomenorrhoea, (ii) clinical or biochemical evidence of androgen excess, and (iii) the exclusion of other endocrine disturbances.[1] Moreover, obesity and associated insulin resistance are observed in >50% of these women.[2]

For overweight women, the first step in improving ovulation rates is to encourage weight reduction.[3,4] Notably, insulin-sensitizing agents can also promote ovulation, thereby reducing circulating androgen concentrations.[5,6,7,8] Both elevated androgens as well as abnormal regulation of the hypothalamic-pituitary-adrenal (HPA) axis and elevated cortisol clearance rates have previously been reported in obese women.[9,10] By contrast, women with Cushing's syndrome commonly present with irregular menstrual bleedings, and ovarian morphology similar to that of PCOS.[11]

In a previously reported study, several isolated or combined enzyme deficiencies were reported in a third of women with irregular cycles with a substantial number of subjects exhibiting a diminished 11ß-hydroxylase activity, suggestive for mild forms of congenital adrenal hyperplasia (CAH).[12] In the current study, we analysed the calculated 11-deoxycortisol/cortisol ratios, indicative for 11ß-hydroxylase activity, in women with normo-gonadotrophic, normo-oestrogenic oligomenorrhoea and clomiphene citrate-resistant infertility, compared with lean and obese ovulatory controls. Infertility is commonly observed in obese women who frequently cope with clinical features of pseudo-hypercortisolism, even though their morning serum cortisol concentrations are normal or even low, but their 24 h free urinary cortisol excretion usually is normal or even high, meaning that although cortisol production in adipose tissue is high the clearance rate is also increased.[13]

In the present study we tested the hypothesis that changes in cortisol metabolism of infertile women are more related to extra-adrenal factors than to adrenocorticotrophic hormone (ACTH)-driven steroidogenesis. Our study objective was to determine 11-deoxycortisol/cortisol ratios before and after ACTH stimulation, thereby differentiating CAH (11ß-hydroxylase deficiency) from extra-adrenal factors.

Materials and methods

Study patients and ovulatory controls

The current study included 26 normo-gonadotrophic, normo-oestrogenic, oligomenorrhoeic women, consecutively referred by the hospital IVF programme for endocrine analysis. All women had a history of oligomenorrhoea defined if cycle lengths of >42 days were reported.[14] They were healthy and displayed no endocrine disorders such as prolactinoma, hypo- or hyperthyroidism, diabetes mellitus and hypogonadotrophic hypogonadism. The possible presence of CAH was unknown at the time of inclusion. Patients were classified as being clomiphene citrate resistant if, despite taking 150 mg clomiphene citrate from day 3 to day 7 of the menstrual cycle, progesterone did not rise to >15 nmol/l, and/or sonographic signs of ovulation were lacking. Twelve lean women (body mass index (BMI) <25 kg/m^2) and 11 obese women (BMI >25 kg/m^2), with proven regular and ovulatory cycles and with children of their own, served as the control group. Serum progesterone values of >35 nmol/l in the luteal phase of their cycle (blood samples were drawn by day 5 and 8 after ovulation) were measured in these two groups. In all participants of the study, baseline blood sampling was performed either during the follicular phase of the menstrual cycle or at random, when the last menstrual bleeding had occurred >3 months prior to inclusion in the study. At least 30 min before blood samples were taken, an indwelling catheter was inserted. All blood samples were drawn between 0800 and 0900, following 12 h of fasting.

The Medical Ethical Committee of Hospital Reinier de Graaf Groep approved the study. Both patients and controls gave their informed consent for participation in the study.

Methods

Hormone assessments were determined by commercially available assays. Hormones and steroid precursors were analysed in one assay, except for 17α-hydroxypregnenolone (17OH-preg). The latter steroid was assayed in additional runs for reasons of validation. Three experienced laboratory technicians performed all assays. All precursors were assayed at baseline and at 30 min after an ACTH bolus i.v. (250 µg synthetic $\alpha_{1\text{-}24}$-ACTH (Cortrosyn®)).

Fasting insulin, reference values: <20 mIU/l (enzyme-linked immunoassay, EIA; IMX-Abbott Laboratories, USA), cortisol, reference values: <0.73 µmol/l between 0800 and 0900; total testosterone, reference values: <3.1 nmol/l before menopause. All these hormones were measured using EIA (Immulite; DPC, Los Angeles, CA, USA). The reference value for fasting glucose was <6.0 mmol/l.

According to the manufacturer's instructions, blood samples assayed for 17OH-preg (ng/ml) were prepared with an ethyl acetate/hexane extraction and column chromatography. The final analysis was done with a solid-phase ^{3}H radioimmunoassay (ICN Pharmaceuticals, Costa Mesa, CA, USA). The entire procedure had a maximal intra- and inter-assay coefficient of variation (CV) of 10.3 and 15.0% respectively. Mean ± SD values in 24 ovulatory controls before and after ACTH stimulation were 4.5 ± 4.7 and 9.7 ± 4.7 ng/ml. 17α-Hydroxyprogesterone (17OH-prog; nmol/l) was determined with a solid-phase ^{125}I radioimmunoassay (DPC) with maximal intra- and inter-assay CV of 5.6 and 5.7% respectively. Values in ovulatory controls were 6.2 ± 3.9 and 10.9 ± 3.7 nmol/l before and after ACTH stimulation respectively. 11-Deoxycortisol (ng/ml) was determined with a solid-phase ^{125}I double antibody radioimmunoassay (ICN Pharmaceuticals) with a maximal intra- and inter-assay CV of 5.9 and 13.7% respectively. Values in ovulatory controls before and after ACTH stimulation were 2.8 ± 1.2 and 4.3 ± 1.4 ng/ml.

Steroid ‘precursor-to-product ratios'

Three enzyme activities were indirectly analysed by calculating precursor-to-product ratios; these were (i) 3ß-hydroxy-Δ^5-steroid dehydrogenase (17OH-preg/17OH-prog), (ii) 21α-hydroxylase (17OH-prog/11-deoxycortisol) and (iii) 11ß-hydroxylase (11-deoxycortisol/cortisol). Normal laboratory reference ranges of these ratios before and after the ACTH bolus were obtained from the control group, which consisted of both lean and obese ovulatory women. Ratios higher than the 90th percentile values in the controls were considered indicative of abnormal steroidogenesis. ACTH-stimulated ratios higher than the 90th percentile of values in the controls were diagnostic for late onset CAH. The reference values obtained from the current study were 1.2 (basal) and 11.1 (after ACTH) for 3ß-hydroxy-Δ^5-steroid dehydrogenase, 3.5 (basal) and 7.9 (after ACTH) for 21α-hydroxylase, and 8.3 (basal) and 18.1 (after ACTH) for 11ß-hydroxylase.

Statistical analysis

Hormone and steroid levels from the lean ovulatory, obese ovulatory and infertile groups were compared using analysis of variance. Comparisons per group of basal and post-ACTH values were performed using paired Student's t-tests. Correlations between groups and physical variables such as BMI, fasting glucose and insulin, testosterone, 11-deoxycortisol, cortisol and the calculated precursor-to-product ratio were analysed using stepwise regression analysis. $P < 0.05$ was considered statistically significant.

Results

Demographic characteristics and endocrine data are shown in Table 1. Age was not different across groups. BMI was also not statistically different between women in the infertile group compared with obese controls. Total testosterone levels, however, were highest in the group of infertile women, ~2-fold compared with obese and 4-fold higher compared with lean controls. Insulin levels were also elevated in the infertile group, but only significantly higher compared with levels in lean controls. Glucose levels were lowest in the lean ovulatory controls. None of the women with infertility had abnormal 21α-hydroxylase activities (17OH-prog/11-deoxycortisol); all baseline ratios were less than the reference value of 3.4, and all ACTH ratios were <3.9. Four women (4/26) had elevated 17OH-preg/17OH-prog ratios, i.e. a baseline value >1.2. One of these women with a BMI of 34.0 kg/m^2 had CAH (3ß-hydroxy-Δ^5-steroid dehydrogenase deficiency) presenting with a basal value of 2.3 and of 12.8 after ACTH (reference value of 11.1). The ACTH-stimulated 17OH-preg/17OH-prog ratios of the three other women remained <12.8.

Twenty-three out of 26 infertile women (88%) had a low 11-deoxycortisol to cortisol conversion (11-deoxycortisol/cortisol), i.e. a baseline value >8.1 (reference value). Three of these women (BMI: 38.0, 35.0 and 44.1 kg/m^2) had CAH (11ß-hydroxylase deficiency) exhibiting elevated ACTH-stimulated 11-deoxycortisol/cortisol ratios (35.7, 32.1 and 18.3) (reference value after ACTH: >18.1). From the infertile group, six women (23%) were lean (e.g. BMI <25 kg/m^2) and 20 (77%) were considered to be obese (e.g. BMI >25 kg/m^2). Of the lean women with infertility, three had mildly increased basal 11-deoxycortisol/cortisol ratios compared with the ratios in ovulatory women, 9.8, 14.6 and 14.8 (reference value: 8.3). None of these women had higher ACTH-stimulated deoxycortisol/cortisol ratios than the reference value of 18.1. All 20 infertile obese women had increased basal 11-

deoxycortisol/cortisol ratios but only three of them had increased deoxycortisol/cortisol ratios after ACTH (18.3, 32.0 and 35.9).

As demonstrated in Table 2, baseline and ACTH-stimulated morning cortisol concentrations were similar among the three groups. Baseline 11-deoxycortisol concentrations were elevated in the infertile group, and increased significantly after ACTH stimulation (Table 1). Finally, basal 11-deoxycortisol/cortisol ratios were significantly higher in the infertile group. Although this ratio remained unchanged after ACTH stimulation, these levels were still significantly higher than those in either of the ovulatory control groups.

Interrelationships

Following stepwise regression analysis between groups (lean ovulatory, obese ovulatory and infertile women) of the variables fasting glucose and insulin, testosterone, cortisol, 11-deoxycortisol and its ratio, the only significant relationship that existed (the only step of the regression analysis) was that of the different groups and testosterone levels ($P < 0.01$). When testosterone was excluded from analysis, a relationship between groups and ACTH-stimulated 11-deoxycortisol/cortisol ratios was seen as the sole significant step ($P < 0.05$). A similar picture emerged between groups (including obese ovulatory and infertile women) and testosterone ($P < 0.01$). After exclusion of testosterone, a significant relationship was found between groups and ACTH-stimulated 11-deoxycortisol and 11-deoxycortisol/cortisol ratios ($P < 0.05$). Finally, including only the lean and obese ovulatory groups in the analysis revealed two significant relationships, for fasting glucose levels ($P < 0.01$) and baseline 11-deoxycortisol/cortisol ratios ($P < 0.05$).

Table 1: Demographics and laboratory measurements in infertile women and in obese and lean ovulatory controls

	Infertile	Obese controls	Lean controls	P value
	N=26	N=11	N=12	
Age (years)	33 ± 5 (25-44)	34 ± 5 (25-40)	33 ± 4 (27-38)	NS
BMI (kg/m2)	31 ± 7 (20-46)	36 ± 7 (27-51)	21 ± 1[a] (19-24)	< 0.0001
Testosterone (nmol/l)	3.5 ± 0.9[b] (0.9-6.8)	1.4 ± 0.8 (0.6-2.9)	0.9 ± 0.3 (0.01-1.3)	< 0.0001
Glucose (mmol/l)	5.3 ± 0.9 (4.2-7.5)	5.3 ± 0.6 (4.4-6.1)	4.1 ± 0.4[a] (3.6-4.9)	< 0.0001
Insulin (IU/l)	22 ± 10 (4-78)	15 ± 11 (5-41)	4 ± 1[a] (2-5)	< 0.001
170H-Pregnenolone (ng/ml)				
Basal	2.6 ± 1.9 (0.9-8.6)	4.4 ± 5.3 (0.4-16.7)	4.5 ± 14.5 (0.3-13.9)	NS
Stimulated	10.4 ± 6.1 (4.4-34.6)	9.9 ± 4.5 (3.5-18.6)	9.5 ± 5.0 (4.2-19.4)	NS
170H-Progesterone (nmol/)				
Basal	4.0 ± 2.0 (1.3-8.9)	6.4 ± 4.1 (1.6-13.2)	6.0 ± 3.9 (1.2-12.9)	NS
Stimulated	10.1 ± 3.8 (3.8-18.2)	11.3 ± 1.8 (7.3-13.6)	10.6 ± 4.9 (4.6-22.9)	NS
11-Deoxycortisol (ng/ml)				
Basal	4.1 ± 1.5 (1.7-8.4)	3.1 ± 1.4 (1.3-6.4)	2.4 ± 0.9[a] (1.2-4.2)	< 0.01
Stimulated	9.0 ± 5.0[b] (3.4-25.7)	5.1 ± 1.5 (3.2-8.2)	3.6 ± 0.9 (2.2-5.2)	< 0.001

Values are mean ± SD (range). Comparisons of age, body mass index (BMI), testosterone, glucose and insulin between groups.
Women were categorized as lean if their BMI was <25 kg/m^2 and obese if their BMI was >25 kg/m^2
Values are mean ± SD. [a]Significantly (P <0.05) lower and [b]significantly higher values compared with groups without symbols.
NS = not significant

Table 2: Comparisons of morning cortisol level and 11-deoxycortisol/cortisol ratios in infertile women and ovulatory controls.

	Infertile women (n = 26)	Obese controls (n = 11)	Lean controls (n = 12)	P value
Cortisol (µmol/l)				
Baseline	0.48 ± 0.17	0.45 ± 0.16	0.47 ± 0.18	NS
ACTH stimulation	0.72 ± 0.22	0.73 ± 0.1	0.77 ± 0.14	NS
11-deoxycortisol/cortisol				
Baseline	9.9 ± 4.8[a]	6.9 ± 1.6	5.2 ± 0.9	< 0.001
ACTH stimulation	12.7 ± 7.2[a]	7.0 ± 2.2	4.7 ± 1.0	< 0.001

Values are mean ± SD
[a]Significantly higher values compared to groups without symbols
ACTH= adrenocorticotrophic hormones
NS= not significant

Discussion

Lean and obese ovulatory women, and women with normogonadotrophic, normo-oestrogenic oligomenorrhoea, were compared in the current study. All infertile women were resistant to clomiphene citrate therapy and none of them disclosed general endocrine disorders. The most interesting finding was that the majority (88%) of women in the infertile group displayed elevated 11-deoxycortisol/cortisol ratios without further increase after ACTH. These findings argue against co-existent CAH and support the idea that extra-adrenal factors are involved, possibly the increase of cortisol clearance. On the contrary, infertility and 11-deoxycortisol/cortisol ratios were stronger correlated after ACTH stimulation compared with baseline values, which indicated some involvement of adrenal steroidogenesis.

Clinical features of pseudohypercortisolism even with low morning cortisol concentrations have been reported in obese individuals.[15] Based on the current observations, we questioned whether the abnormalities of cortisol metabolism that were observed should be ascribed to a mild form of CAH and/or to the concomitant state of an obesity-related metabolic disorder. Notably, it appeared that 11-deoxycortisol/cortisol ratios were highest in the infertile group.

In previous studies, it has been demonstrated that obesity is related to several changes in the HPA axis. Obese individuals sometimes have low serum cortisol concentrations despite having an increase in cortisol production.[9,10] Subtle alterations in cortisol responses were described in obese individuals after corticotrophin-releasing factor and ACTH stimulation as well as after very low dose dexamethasone suppression.[16,17,18,19,20] Finally, stress-related cortisol hyper-responders showed interactions with clinical features of the `metabolic syndrome' (central obesity, hypertension, insulin resistance, and dyslipidaemia).[20] Apart from these changes, obesity can also be related to an increase in cortisol production rate.[21,22,23,24] Moreover, there is compelling evidence that cortisol is exposed to enzymatic transformation in peripheral target tissues, mostly in liver and visceral fat.[25] One cortisol conversion system includes the peripheral 11ß-hydroxysteroid-dehydrogenases (11ß-HSD), metabolizing cortisol to the less active cortisone (Type 2 enzymes) or back to cortisol (Type 1 enzymes).[25,26] Non-obese individuals with hypertension may have subtle adrenal 11ß-hydroxylase or peripheral 11ß-HSD Type 2 deficiencies, resulting in mild mineralocorticoid excess. Obese individuals, displaying features of `the metabolic syndrome', have evidence of an altered peripheral handling of glucocorticoids through a reinforcement of peripheral 11ß-HSD, Type 1. This enzyme system enhances the local production of cortisol, particularly in visceral adipose tissues.[27,28] We speculate that the imbalance between cortisol and its precursor steroid (11-deoxycortisol), as observed in infertile women, mirrors an insufficient cortisol production in relation to its high clearance rate. Local cortisol production in peripheral tissues (11ß-HSD, Type 1 in visceral fat) may compensate this minute systemic low cortisol status.

The presence of defects in ovarian and adrenal steroidogenesis of women with infertility has been previously reported in the laboratory[29] and in clinical studies.[30,31] Notably, (patient) populations described in previous research are heterogeneous as a result of the inclusion of different clinical syndromes with infertility. In an effort to be consistent in the

selection of patients, we only included women with normo-gonadotrophic normo-oestrogenic oligomenorrhoea who were resistant against clomiphene citrate. It is widely believed that this infertile group embodies several different reproductive disorders. In daily practice, these women are commonly divided in two distinct subgroups: obese women with high fasting insulin concentrations and lean women with insulin levels which are adequately low.[32] Convincing arguments have been provided to support the concept that PCOS includes a phenotypic spectrum of metabolic disorders. Firstly, insulin resistance is also observed in lean women with PCOS.[33] Secondly, lean and fat body mass calculations have shown that in lean women with PCOS, changes in fat distribution involved primarily an increase of visceral fat mass.[34] Finally, we have observed changes in cortisol metabolism mainly due to extra-adrenal factors in lean as well as obese women with infertility.

In conclusion, abnormal baseline 11-deoxycortisol/cortisol conversions were found in 88% of women with clomiphene citrate-resistant normo-gonadotrophic normo-estrogenic oligomenorrhoea. This abnormality disappeared almost completely after ACTH administration, thus excluding overt CAH and suggesting an alternative operational pathway in non-adrenal tissues. Further studies are needed to compare the effects of interventions such as dietary programmes or insulin-sensitizing agents, to investigate cortisol metabolism, and ultimately to improve ovulation and pregnancy rates for these women.

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Chapter 5

Early effects of metformin on glucose dynamics in women with oligomenorrhoea and regular menstrual cycles who were wearing a subcutaneous glucose sensor

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Polycystic ovary syndrome (PCOS) is a heterogeneous disorder involving three generally accepted hallmarks: oligoamenorrhoea, clinical evidence of androgen excess on laboratory testing, and the absence of other endocrine disturbances.[1] Many oligoamenorrhoeic women also share the clinical features of visceral adiposity, hypertension, and dislipidaemia. Recent observations indicate that insulin resistance is often present even in nonobese women.[2,3] Moreover, at least one-third of all PCOS women show impaired glucose tolerance, while 7%–10% have overt diabetes mellitus.[4,5] The initial step to improve ovulation rates in obese women with oligoamenorrhoea are lifestyle modifications, such as a low-calorie diet and exercise. Recently, it has been shown that insulin-sensitizing agents improve ovulation and reduce the long-term complications of insulin resistance.[6,7] Interestingly, effective use of metformin has also been shown in lean adolescents with anovulatory hyperandrogenism.[8]

Insulin sensitizers are widely recommended for the treatment of diabetes mellitus. The estimated incidence of hypoglycemia directly caused by the use of insulin sensitizers in patients with noninsulin-dependent diabetes is approximately 1%.[9,10,11] Furthermore, significant lowering of endogenous insulin concentrations after metformin therapy has been reported in obese nondiabetic ovulatory women as well as in women with oligoamenorrhoea.[12]

On the basis of these considerations, we hypothesized that glucose concentrations will not decrease in nondiabetic women taking metformin. To support this, we investigated glucose dynamics in women with oligoamenorrhoea and in women with strictly regular menstrual cycles, using a continuous glucose-monitoring device (CGMS) with the capacity to generate frequent day and night readings.

Fourteen women were consecutively referred by the hospital IVF program for endocrine analysis. All women were infertile (at least 2 years) and were oligoamenorrhoeic (cycle length of more than 42 days or less than eight cycles per year to amenorrhoea).[1] Seven strictly regular and ovulatory women, as evidenced by midluteal P values of more than 35 pmol/L, served voluntarily in the control group. All 21 participants were healthy and displayed no endocrine disorders (i.e., prolactinoma, hypo- or hyperthyroidism, diabetes mellitus, or hypogonadotrophic hypogonadism). None of the participants were taking contraceptives before inclusion. Patient recruitment lasted exactly 12 months.

One experienced study nurse trained all participants on the Minimed CGMS (Minimed Inc., Northridge, CA). This device measures interstitial fluid glucose expressed as $glucose_{interst}$ with an indwelling catheter that remains in place in the SC tissue of the abdomen for up to 72 hours. CGMS is a Holter-style sensor system comprising a sterile disposable SC glucose-sensing device with an external electrical connector, a small glucose monitor, a connecting cable, and a communication device enabling data stored in the monitor to be downloaded to a personal computer. Once placed in SC tissues, CGMS measures electrical current in nanoamperes that is related to the $glucose_{interst}$. The glucose sensor signal is acquired every 10 seconds with an average of the acquired signals saved in memory every 5 minutes.

The participants were asked to take at least four finger samples per day using a blood glucose meter. These readings were entered into a CGMS monitor and paired with the corresponding calibrated sensor glucose values obtained simultaneously. After insertion of the sensor, study participants returned home to wear the CGMS for up to 3 days. Upon completion, the women returned to the outpatient clinic to have the CGMS removed and the data downloaded. Participants were instructed to perform daily calibrations and to respond to alarms as needed, all outside the direct supervision of clinical personal. Each sensor was placed in the abdominal SC tissue before 3 P.M. on the first day and removed before 3 P.M. 3 days later.

The first metformin tablet of 500 mg was taken on the second study day shortly before dinner, thus after about 1–1.5 days of sensor wearing. On the next day, metformin (500 mg t.i.d.) was taken shortly before meals. All participants were asked to keep a diary to report their experiences, compliance, and eventual side effects of the metformin therapy. All women were asked not to change diet habits, and all types of sports and physical exercises were allowed. Written informed consent for study participation was obtained from all women included in the study, which allowed the anonymous use of their medical history in further research and a 3-day long SC glucose determination. The Medical Ethical Committee of the Reinier de Graaf Group approved the study.

In patients and controls, fasting hormone samples were performed once before sensor insertion between 8 and 9 A.M. in the follicular phase or at random when the last menstrual bleeding had occurred more than 3 months before inclusion in the study. Hormone assessments were determined by commercially available assays, and we used the normal

reference ranges recommended by the manufacturer: [1] fasting insulin <20 mU/L enzyme immunoassay (EIA); IMX-Abbott Laboratories, Abbott Park, IL; [2] LH and FSH <15 U/L before menopause; [3] DHEAS <12 μmol/L; [4] total T <3.1 nmol/L before menopause; all of these hormones were measured using the EIA-Immulite assay (DPC, Los Angeles, CA); and finally, [5] androstenedione by RIA (coat-a-count, DPC) <15 nmol/L. The laboratory reference range for fasting glucose was less than 6.1 mmol/L. To convert the value for glucose to milligrams per deciliter, divide by 18.

All participants were monitored for 3 consecutive days. A day was defined to begin at 3 P.M. and to end at 3 P.M., 24 hours later. A night started at 12 midnight and ended at 6 A.M. For each subject, we determined the percentages of $glucose_{interst}$ below 2.3 and 2.8 mmol/L and the mean of all $glucose_{interst}$ per individual. The average of the mean was calculated per group (patients and controls). The average mean percentage of $glucose_{interst}$ less than 2.3 mmol/L or less than 2.8 mmol/L as well as the average mean of all $glucose_{interst}$ concentrations were recorded during full days and nights. Laboratory measurements and $glucose_{interst}$ readings (day vs. day and night vs. night episodes) were compared before and after metformin therapy with two-sided paired Student *t*-tests. The $glucose_{interst}$ readings in patients and controls were compared with two-sided unpaired Student *t*-tests. P<O.05 was considered statistically significant.

Patients (n = 14) and controls (n = 7) were balanced for age mean ± SD, 31 ± 5 and 28 ± 6 years) and body mass index (29 ± 8 and 32 ± 11 kg/m^2), and no significant differences were found among glucose (5.2 ± 0.5 vs. 5.3 ± 0.5 mmol/L), insulin (10 ± 8 vs. 16 ± 12 mU/L), DHEAS (6.1 ± 3.3 vs. 6.0 ± 2.3 μmol/L), androstenedione (8.3 ± 4.2 vs. 5.6 ± 1.2 nmol/L), T (1.7 ± 0.6 vs. 1.7 ± 1.1 nmol/L), or FSH (4.7 ± 1.5 vs. 5.7 ± 3.2 U/L). LH values were significantly higher in patients (11.4 ± 4.4) compared with controls (4.2 ± 1.0 U/L; P<0.001).

A total of 17,841 $glucose_{interst}$ sensor readings were obtained from the 21 individuals included in the study. Readings on days 1 and 2 were available in all 21 subjects, and day 3 readings in all but 5 subjects because of system errors, which mainly occurred during sleep. The average of the mean percentage of $glucose_{interst}$ less than 2.3 mmol/L or less than 2.8 mmol/L as well as the average mean of all $glucose_{interst}$ concentrations were recorded during days and nights (Table 1).

At baseline, before metformin administration (day 1), the average mean percentage of low $glucose_{interst}$ (less than 2.3 mmol/L or less than 2.8 mmol/L) and the average mean $glucose_{interst}$ were similar in both groups. In patients, metformin did not elicit significant changes in low $glucose_{interst}$ percentages or in average mean of $glucose_{interst}$. In controls, a modest but significant decrease in the low $glucose_{interst}$ percentage (day 2) and a concomitant increase in the mean $glucose_{interst}$ (day 3) was observed.

All women took all tablets according to protocol. None of them skipped regular meals, that is, breakfast, lunch, and dinner. No patients complained of low glucose–related side effects or gastrointestinal discomfort during the whole study episode.

The current study was conducted to determine early metformin-induced effects on glucose dynamics in nondiabetic women with and without oligoamenorrhoea. According to available pharmacokinetic studies analyzing the gastrointestinal absorption and bioavailability of metformin, a study period of 3 days appeared to be sufficient to detect the immediate effects of this drug.[13,14] In the oligoamenorrhoeic nondiabetic group, $glucose_{interst}$ values did not change after metformin but a trend toward higher values was noted. In controls, the drug may have reduced the percentages of low $glucose_{interst}$, whereas interstitial glucose values were moderately increased. Similar observations are reported using the insulin-sensitizing drug troglitazone, suggesting that these drugs amplify the counter-regulation against hypoglycemia.[15]

CGMS was used to generate continuous $glucose_{interst}$ determinations using an SC sensor. Although the exact relationship between interstitial and blood glucose levels remains to be uncovered, clinical studies have shown that SC sensor measurements generally follow finger stick meter measurements and venous blood laboratory values.[16,17,18,19] CGMS showed median daily correlation coefficients with capillary blood glucose meter readings of 0.92. Overall, the sensor readings were 0.28 mmol/L below the meter readings (-0.30 ± 2.45 mmol), with an average percent of difference of 0.2% ($-0.3 \pm 32.4\%$).[19]

Table 1: CGMS measurements ($glucose_{interst}$) in ovulatory and oligo/amenorrhoeic women

	[a]$Glucose_{interst}$	Days	Controls	Patients
12AM–6AM	% Below 2.3 mmol/L	1	26.4 (41.4)	22.3 (36.5)
		2	8.5 (9.4)	7.88 (15.0)
		3	7.1(14.3)	6.5 (12.9)
	% Below 2.8 mmol/L	1	42.2 (36.4)	28.7 (40.0)
		2	17.0 (18.8)	15.5 (23.4)
		3	8.9 (17.9)	11.5 (18.7)
	Mean glucose levels	1	3.39 (0.94)	3.85 (1.19)
		2	4.37 (1.35)	4.45 (1.08)
		3	4.46 (0.60)	4.76 (1.10)
24 hours	% Below 2.3 mmol/L	1	11.0 (14.4)	9.5 (12.6)
		2	2.9 (3.0)	3.1 (4.4)
		3	1.7 (4.0)	5.2 (8.5)
	% Below 2.8 mmol/L	1	18.5 (12.3)	13.5 (15.7)
		2	5.7 (6.0)	7.5 (8.0)
		3	3.1 (5.2)[b]	8.1 (12.6)
	Mean glucose levels	1	4.71 (0.43)	4.78 (0.61)
		2	5.63 (0.90)[b]	5.30 (1.11)
		3	5.62 (0.71)[b]	5.01 (0.69)

Note: $Glucose_{interst}$ data are expressed as mean (SD). Comparisons were made between night (12 A.M. to 6 A.M.) and full day (24 h) episodes and also between ovulatory and amenorrheic women.
[a] To convert the value for glucose to milligrams per deciliters, divide by 18.
[b] P<.05.

In the current study, $glucose_{interst}$ cutoff levels of 2.3 and 2.8 mmol/L were arbitrarily defined as low based on reproducible symptoms occurring in healthy individuals being subjected to hypoglycemic clamps. Subtle functional changes were documented at blood glucose concentrations in the range of 3.0 –3.6 mmol/L. At concentrations below 2.8 mmol/L, cortical functions start to deteriorate, while below 2.3 mmol/L, simple motor tasks start to slow down.[20,21] Based on the strong correlations between paired CGMS and

self-monitored capillary blood glucose readings, we assumed that blood glucose and $glucose_{interst}$ are equivalent.

In conclusion, the results of the current study are in agreement with the low incidences of clinically reported hypoglycemia with metformin and support the safe use of this agent in women without diabetes mellitus. It is of note that the primary objective of this study was to describe early metformin-induced effects on glucose concentrations in interstitial tissues. The growing interest in using metformin in the clinical management of women with PCOS warrants long-term studies on drug safety and toxicity. With the use of CGMS, more new effects of insulin-sensitizing drugs in nondiabetic individuals may be discovered.

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Interface

Chapter 6

Historical considerations, definitions, and exceptions

Introduction

The definition of Polycystic Ovary Syndrome (PCOS) has been subject of controversy since years. Variable expression of several symptoms and new insights due to continuous research operate as a threshold to develop an evidence based definition of PCOS. In 1935 Stein and Leventhal[1] described for the first time a phenotypic heterogeneous group of women suffering from subfertility and amenorrhoea/oligomenorrhoea. In common, all women appeared to have enlarged Polycystic Ovaries (PCO) with or without hirsutism and/or obesity. Treatment because of subfertility aimed to restore ovulation.

The definition of the PCOS has been adjusted over the last decades, and was lately revised according to the American Society for Reproductive Medicine (ASRM) consensus.[2] The majority of women with PCOS have (visceral) obesity as well as insulin resistance suggesting involvement of adipose tissue and its metabolic activity in the pathogenesis of the PCOS.[3] Whether visceral obesity and/or insulin resistance play a primary causal role in the pathogenesis of PCOS or mainly represent the result of another causal pathophysiological pathway is still unclear. Since PCOS is frequently observed in combination with obesity and the high prevalence of decreased insulin sensitivity in the general population, the question arises whether the definition of PCOS according to the current ASRM criteria should be considered for adjustment. In this chapter of the thesis the evolution of the definition for PCOS is described emphasizing the difficulty for a unifying point of view.

Evolution of definition for PCOS

In ancient times, since Hippocrates, several references in literature and medical records mention menstrual cycle and related disorders suggesting PCOS. For example, androgen excess occurring among a masculine type of female who was not able to become pregnant.[4] In 1328 for the first time a relation between obesity and subfertility was described. A French Rabbi interprets a biblical paragraph concerning Sarah giving permission to extramarital conception as she was not able to conceive herself. This decision out of necessity in order to warrant reproduction resulted in stress, weight loss and subsequent spontaneous conception.[5] In the 17th century an Italian scientist Antonio Vallisneri described “young married peasant women, moderately obese and infertile, with two larger ovaries, bumpy, shiny and whitish, just like pigeon eggs”.[6] The description of the typical

morphology of sclerocystic ovaries in 1844 by Chereau in France contributed to the recognition of the polycystic ovarian disorder.[7,8] In 1935 Stein and Leventhal described during surgical intervention in a group of 7 amenorrhoeic women with variable expression of hirsutism and obesity, the appearance of PCOS. The ovaries were enlarged and contained multiple cysts. All 7 women underwent wedge excision of the ovary, regained ovulation and two became pregnant.[1,6] The heterogeneity of clinical findings of the pioneer report by Stein and Leventhal resulted in a long list of variable clinical and laboratory features described in the literature and warranted a more precise clinical description of the PCOS phenotype.

At the end of the 20th century, the National Institute of Health (NIH) issued the following definition: clinical or biochemical hyperandrogenism, chronic anovulation and exclusion of other endocrine disorders.[8] Based on the NIH 1990 definition it was estimated that the global prevalence of PCOS was 4-8% in the female pre-menopausal population.[9.10] Another reappraisal of definition was initiated by US and European experts in the field of PCOS with appreciation of the typical sonographic features of the ovary. This resulted in the latest global definition issues by the American Society of Reproductive Medicine (ASRM) and the European Society of Human Reproductive Endocrinology (ESHRE) in 2003 with a detailed revision in 2004. This so called 'Rotterdam Consensus Definition' of 2003/2004 used the following phenotype description: PCOS includes at least two out of three of the following features: polycystic ovaries on ultrasound, hyperandrogenaemia and/or oligo-/anovulation.[2] With this definition, global PCOS prevalence was estimated to be 4% of an unselected population. Using oligomenorrhoea and hyperandrogenism as diagnostic features the prevalence of PCOS in an unselected population increases to about 6.6%.[11] The higher prevalence is mainly the result of addition of ultrasonographic appearance of PCO into the definition. The finding of PCO with ultrasound is called PCO, while it is called PCOS in case of presence of the *clinical picture* including subfertility, hirsutism or cyclic abnormalities. PCO is more common as compared to PCOS. In an at random sampled community-based study in the United Kingdom, it was found that PCO was particularly more common among women of South Asian origin (52%) compared with Caucasians (22%).[12,13]

Another problem of the currently used definition of PCOS is that there is no consensus about aspects like body fat distribution, insulin resistance or cytokines that are involved in metabolic syndrome.

To summarize, there is no absolute definition of a number of clinical features that represent PCOS. None of the clinical and biochemical criteria of the Rotterdam consensus is associated with body composition, fat distribution, insulin resistance or cytokines involved in the metabolic syndrome. One may argue that PCOS is characterized by subfertility and/or hyperandrogenaemia in young women, while metabolic aspects become more prominent with aging.[14] This dynamic concept with age may help to characterize and understand PCOS better in a clinical way. However, age is not a criterion for PCOS.

Metabolic aspects of adipose tissue

It remains unclear in how far metabolic criteria belong to PCOS. This unresolved question has been used as an argument to omit metabolic status from the definition. Moreover, available information about body composition, fat distribution and insulin metabolism clearly indicate that a subgroup of women does not fit into the concept of derangement of insulin metabolism.

The majority of women with PCOS are overweight or obese (up to 88%)[15] suggesting a role of adipose tissue in the development of PCOS. It remains therefore confusing to note that appr. 10% of women with PCOS are lean with no signs or symptoms of the metabolic syndrome[11], and -to our personal experience- no signs of hyperandrogenism. The influence of obesity in folliculogenesis is strongly suggested since weight reduction of at least 5% is reported to restore ovarian function.[16] Adipose tissue has demonstrated to be more than only a location for energy storage. It also plays a role in insulin sensitivity, energy homeostasis and the production of paracrine factors including steroidal hormones. The physiological consequence of altered body fat composition is mainly related with body fat distribution (abdominal/visceral or peripheral/subcutaneous). Viscerally – and not peripherally – deposited fat appears to be associated with subfertility.[17,18]

The combination of viscerally distributed fat, insulin resistance and PCO-like changes is associated with metabolic syndrome and/or (non) Classic Congenital Adrenal Hyperplasia (n)CAH. Moreover, (n)CAH is prevalent in 20-30% of women with PCOS.[19] Notably, adrenal steroidogenesis usually diminishes with aging and explains a lower prevalence of

androgen excess in older women with PCOS.[20] The opposite holds true for extra-adrenal steroidogenesis. As people become older they accumulate more viscerally deposited fat enhancing extra-adrenal steroidogenesis.[21,22,23] Another important pathological mechanism is conversion from cortisone to cortisol in liver and viscerally deposited adipose tissues.[24] There is a strong association between viscerally deposited fat and cortisol secretion rate and between viscerally deposited fat, cortisol secretion rate and insulin resistance.[25,26]

Insulin resistance affects the cytochrome p450c of the ovary and adrenal enhancing the conversion of steroid precursors into androgens.[27] Besides, insulin and luteinizing hormone (LH) act synergistically in arresting terminal differentiation of granulosa cells.[28,29,30] Although lean women with PCOS are overtly insulin resistant in the study of Dunaif [31] outcomes of another euglycemic hyperinsulinaemic clamp study in lean PCOS cases only point to the direction of a strong correlation between insulin resistance and SHBG concentrations.[32] In a comparative study it was demonstrated that insulin sensitivity was lower and viscerally deposited fat was higher in lean women with PCOS as compared with controls.[33] Overall, impaired glucose tolerance and diabetes is less common among lean PCOS as compared with obese PCOS.[34]

Although the impact of obesity on insulin sensitivity is significant, it is unlikely that reduced insulin sensitivity in combination with hyperinsulinaemia is the only factor that interferes with folliculogenesis. There is an ongoing debate on the role of body fat distribution, *intrinsic* insulin resistance and steroidogenesis in lean, overweight and obese women with PCOS. The data mentioned in the literature have been obtained from heterogeneous groups in terms of age and BMI. Besides, determinations of body fat distribution differ, and variable methods for assessment of insulin sensitivity are used. Finally, full spectrum of cytokine determinations in lean cases with PCOS are lacking.

In summary, there are variable results in literature regarding insulin sensitivity in lean PCOS. In obese PCOS insulin resistance is at least due to obesity.[35] The question in lean PCOS arises whether there is an intrinsic role of visceral fat in the pathogenesis of lean PCOS. In the second part of this thesis we therefore address body fat distribution in lean PCOS as well as possible interactions between viscerally deposited fat in lean PCOS and several laboratory parameters. For proper evaluation body fat distribution and laboratory parameters were analyzed in the similar lean subgroup and results are presented in the

subsequent chapters. It was questioned whether ASRM-defined PCOS cases who appeared to be viscerally lean share similar metabolic aspects with lean fertile individuals.

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Part 2

Lean PCOS

Chapter 7

Comparison of MRI-assessed body fat content between lean women with polycystic ovary syndrome (PCOS) and matched controls: less visceral fat with PCOS

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Abstract

Background:
Polycystic ovary syndrome (PCOS) is a heterogeneous disorder. However, PCOS has a strong resemblance to the metabolic syndrome, including preponderance of visceral fat deposition. The aim of this study is to compare fat distribution between lean women with PCOS and controls matched for body composition but with regular menstrual cycles and proven fertility.

Methods:
In this prospective cross-sectional study in a fertility outpatient clinic, 10 Caucasian women with PCOS and 10 controls, all with a BMI between 19 and 25 kg/m^2, were included. Fasting glucose, insulin and C-peptide concentrations, homeostasis model assessment (HOMA), hormonal levels and bioelectrical impedance analysis (BIA) variables were assessed and fat content and ovarian volume determinations were obtained with magnetic resonance imaging (MRI). Multiple axial cross-sections were calculated.

Results:
The age of the PCOS and control groups were [mean (SD)] 28.2 years (2.6) versus 33.7 years (2.3) $P < 0.0001$, respectively, and both groups were matched for BMI: 21.6 kg/m^2 (1.1) versus 21.8 kg/m^2 (2.1) (ns), fasting glucose, insulin, C-peptide, HOMA-insulin resistance (IR) levels and BIA parameters. PCOS cases had higher ovarian volumes and less visceral fat compared with controls.

Conclusions:
Lean women with PCOS have higher MRI-determined ovarian volumes and less visceral fat content when compared with control women.

Introduction

Polycystic ovary syndrome (PCOS) includes a series of heterogeneous clinical entities characterized by reproductive, hyperandrogenic and metabolic features. Obesity and fat distribution are supposed to play an important role in the aetiology of PCOS.[1,2,3] In an observational study in 2001 among 346 Dutch PCOS women, 56.1% had a BMI of <25 kg/m^2 (defined as lean), 11.3% were between 25 and 27 kg/m^2 (overweight) and 32.7% were ≥27 kg/m^2 (obese).[4] Magnetic resonance imaging (MRI) is a highly accurate way to measure fat content at different body sites. By using MRI, it has been previously demonstrated that PCOS is not as closely linked to visceral adiposity as was hypothesized. In a recent study, comparing mildly obese women with PCOS against controls, visceral fat was preponderant among PCOS cases (whole group analysis). However, there was no difference in visceral fat content between groups after subgroup analysis (matched for BMI/fat mass ratio.[5] Since all PCOS studies have included subjects who are overweight or obese it is of interest to study this issue in lean women with PCOS (BMI of 19–25 kg/m^2) excluding bias caused by external obesity-related cues, such as food, overconsumption, sedentary lifestyle or drugs associated with weight gain. These external factors might change glucose metabolism that is considered to be critical in PCOS.[6,7,8,9]

In the current MRI study, we have compared lean PCOS cases with confirmed fertile controls (women with regular cycles who have had successful pregnancies). The primary focus was to study body fat distribution in these women and secondly to include MRI-derived volume calculations of the ovaries as additional information.

Materials and Methods

Patients

Ten lean (BMI 19–25 kg/m^2) women, with PCOS according to ESHRE/ASRM criteria, who were consecutively visiting the fertility clinic of Twente Group of Hospitals, Hengelo, The Netherlands, were recruited for participation in the current study. Twelve BMI-matched controls were recruited from the outpatient clinic for gynaecology and obstetrics. All participants were Dutch Caucasians. Individuals with known drug abuse, pregnant women or patients with general or endocrine disorders were excluded from participation. Of 12 selected controls, two subjects were excluded from the study as they failed to show up for MRI analysis despite informed consent. None of the controls met any of the

diagnostic ESHRE/ASRM criteria for PCOS and all had previously completed at least one successful pregnancy. Control subjects did not use hormonal containing contraceptives and ovulatory menstrual cycles were confirmed by mid-luteal progesterone concentrations >16 nmol/l.

Inclusion of the numbers of cases was based on the original concept that visceral fat is preponderant in PCOS. Therefore, this study was powered on the findings of about 1 SD (standard deviation) difference in Mid-L4 visceral fat content (whole group analysis), as previously reported.[5] We assumed that the visceral abdominal content in women with PCOS is on average 1.25 SD different from controls, allowing the study of two groups (n = 10 per group), ensuring 80% power with a significance level of 5%.

The diagnosis of PCOS was made using the Rotterdam ESHRE/ASRM diagnostic criteria.[10,11] All included women had oligo-ovulation and/or anovulation (intermenstrual interval >42 days) and also typical polycystic ovaries shown on ultrasound; none of them had clinical and/or biochemical signs of hyperandrogenism. None of the subjects had ever received hormonal therapy for ovulation induction. Other endocrine or neoplastic causes of hyper-androgenaemia (including congenital adrenal hyperplasia, androgen-secreting tumours and Cushing's syndrome) were excluded on the basis of 8 a.m. fasting serum 17-hydroxyprogesterone (17-OH-Prog) <3 mmol/l, serum cortisol <0.54 nmol/l, dehydroepiandrosterone sulphate (DHEA-S) <12.0 µmol/l, androstenedione (Δdione) <14.4 nmol/l and absence of an ovarian tumour on intra-vaginal ultrasound. Fasting laboratory analyses and MRI assessments were performed on the same day during the follicular phase of the cycle in controls or after progesterone-induced withdrawal bleeding in all PCOS cases.

All clinical investigations were conducted in accordance with the guidelines in the Declaration of Helsinki, and the study was approved by the medical ethical review committee of the hospital. All subjects provided written informed consent.

Anthropometric and biochemical evaluation

Waist circumference (cm) was measured at the midpoint between the iliac crest and the lowest rib margin, just above the umbilicus at the end of normal expiration. Hip circumference (cm) was measured at the widest part of the buttocks. Bioelectrical impedance analysis (BIA) was performed using Quadscan equipment (version 4.04,

Bodystat Ltd, Isle of Man, UK), measuring fat mass and lean body mass. Fasting venous blood samples were drawn from all participants on the study day (on cycle days 3–7) after spontaneous menses in controls or after progesterone-induced menses in PCOS. Samples were collected in serum separator tubes, allowed to clot for at least 30 min, centrifuged for 15 min at 2000g at room temperature and aliquoted into polypropylene tubes. All biochemical parameters were immediately assayed by using commercial kits. Serum was assayed for fasting glucose with an automated analyser (COBAS 6000, Roche Diagnostics, Almere, NL). Fasting insulin, C-peptide, oestradiol (E2), 17-OH-Prog, DHEA-S, sex hormone-binding globulin (SHBG), FSH and LH were measured using chemiluminescence sandwich immunoassays (Siemens Healthcare Diagnostics, Breda, NL) and total testosterone (T) was measured with a direct (competitive) chemiluminescent assay (Siemens Healthcare Diagnostics, Breda, NL) on a fully automated analyser (IMMULITE® 2000, Siemens Healthcare Diagnostics, Breda, NL). Androstenedione assays were performed by Radioimmunoassay (RIA) after extraction and paper chromatography.[12]

Free androgen index (FAI) was calculated as total-T/SHBG multiplied by 100. Biochemical hyperandrogenism was defined as a T >3.5 nmol/l and/or FAI >8.5 as provided by the manufacturer. A model-derived estimate of insulin resistance was applied to calculate the homeostasis model assessment (HOMA) using a calculator (http://www.dtu.ox.ac.uk/homacalculator/index.php).

Magnetic resonance imaging

MRI scans were performed on the day of blood sampling. All participants were examined on a Magnetom Harmony 1.0 T MRI system (Siemens Medical Solutions USA), using the integrated Body Coil for fat measurements and CP Body Array for ovarian volume measurements. The measurements were performed on the Leonardo Stand Alone Viewing console (Siemens Medical Solutions USA). The diagnostic console has a cursor area measurement facility for all dimensional measurements.

Assessment of body fat distribution in each subject was based on measurements of cross-sectional areas of fat depots, made from axial T1-weighted Turbo Spin-Echo images taken at the following anatomically predefined levels: the mid-lumbar L4 vertebral body (Mid-L4) for measurements of visceral and subcutaneous abdominal fat depots, the upper abdominal region from diaphragm to L4 and the lower abdominal region from L4 to the

symphysis. In detail, multiple axial cross-sections were taken throughout the abdomen from the diaphragm to the pelvis and mean averages from all slices (10 mm each) in each women with PCOS were compared with the mean in the controls. In addition, measurements were performed at the greater trochanters to establish the gluteal subcutaneous fat depots and from diaphragm to symphysis to establish the whole abdomen fat depots. Slice thickness for abdominal fat measurements was 10 mm. One cross-sectional slice was taken at Mid-L4 (10 slices per group). The whole abdomen from diaphragm to symphysis included 153 slices in PCOS and 155 slices in controls.

To determine ovarian volumes, T2-weighted axial and T2-trufi axial breath-hold sequences were used providing a two-dimensional measure. The third diameter was calculated by adding the total number of axial slices of 6 mm per ovary. Ovarian volumes were calculated as $2/3\pi \times$ ovarian length $\times$ width $\times$ height.

Data for fat depot measurements disclosed a reproducibility of CV% (intra-observer): 2.5±1.4 (SD) and CV% (inter-observer): 2.6±1.5 (SD). For ovarian volumes, it was CV% (intra-observer): 3.3±2.0 (SD) and CV% (inter-observer): 6.3±7.9 (SD).

Statistical analyses

Statistical analyses were performed using Statgraphics Centurion XV software (Version 15.2.00 for MS Windows; Statpoint, Inc., VA, USA) and using Analyse-It (Version 2.21 for MS Excel; Analyse-It Software, Leeds, UK). Analyses included unpaired-sample t-tests for comparisons of demographic, laboratory and MRI data obtained from women with PCOS and controls. Shapiro–Wilks W-tests were used to check whether nominal parameters were normally distributed prior to analysis, and in case of skewness, Wilcoxon Rank tests were performed. P-values of <0.05 were considered significant.

Results

Women with PCOS were on average 5.5 years younger than controls (PCOS: 28.2±2.6 years versus controls: 33.7±2.3 years, $P < 0.0001$). However, anthropometrics and BIA parameters (fat mass and lean body mass) were similar between the groups (Table 1).

PCOS cases had significant lower mean FSH and E2 levels and the calculated FAI was higher in the PCOS group. All other laboratory results were similar between groups, including fasting glucose, insulin, C-peptide and HOMA-insulin resistance (IR) (Table 2).

MRI-determined assessments of the right and left ovarian volumes disclosed larger volumes in PCOS: 44.4% larger at the right side and 33.1% larger at the left side. None of the PCOS cases had ovarian volumes <2.3 cm^3 or >6.8 cm^3. Ovarian volumes below 2.3 cm^3 were found in 9 out of 10 control cases. Out of 10 control cases, 2 had bilateral ovarian volumes <2.3 cm^3 (Table 3).

Table 1: Demographics of lean PCOS cases and control cases

	PCOS	**Controls**	**P Value**
	n=10	n=10	< 0.0001
Age, years	28.2 ± 2.6	33.7 ± 2.3	ns
Weight, kg	64.9 ± 5.8	67.1 ± 7.2	ns
BMI, kg/m2	21.6 ± 1.1	21.8 ± 2.1	ns
Waist/hip ratio	0.83 ± 0.07	0.85 ± 0.03	ns
Mean no. of pregnancies (range)	*	2 (1–3)	ns
Months (range) between latest delivery and study entry		42 ± 11 (6–132)	ns
BIA results			
Fat mass (kg)	13.2 (4.9–19.7)	14.4 (4.0-22.5)	ns
Lean body mass (kg)	52.4 (45.2-59.4)	53.4 (44.4–59.7)	ns
Fat mass/ lean body mass	0.26 (0.09–0.35)	0.28 (0.06–0.38)	ns
BMI/ fat mass	1.63 (1.17–3.95)	1.42 (1.08–5.15)	ns

Data are shown as mean ± SD or median (range)
*1 patient had delivered 24 months before recruitment and was included

Visceral fat volumes calculated from single cross-sectional axial slices at Mid-L4 levels were similar between groups. However, comparing multiple cross-sections of the whole abdomen showed less visceral fat in PCOS cases, regardless of the region of interest: upper abdominal region: 52% less, lower abdominal region: 48% less and whole abdomen: 49% less. In PCOS, visceral fat measurements had a range of 274–854 cm^2 and in controls, the range was 644–1259 cm^2. None of the PCOS cases had visceral contents >780 cm^2, while eight control cases had visceral contents >780 cm^2. Between groups, there were no differences between peripheral fat measurements at any abdominal site or greater trochanters (Table 4).

For both groups, there were no significant correlations between any of the laboratory parameters and right- or left-sided ovarian volumes. There were also no significant correlations between fat measurements at any site and right- or left-sided ovarian volumes.

Discussion

In the current study, we compared lean PCOS cases with matched controls with the main focus on differences in fat distribution. PCOS was strictly defined according to ESHRE/ASRM diagnostic criteria, and all women had typical polycystic ovaries upon intravaginal ultrasound. Control cases were fertile having achieved at least one successful pregnancy and did not take any hormone-containing contraceptive. In contrast to expectation, the current results demonstrated that the content of visceral deposited fat was lower in PCOS cases. Since other anthropometric data except age were balanced, this finding points to higher lean body mass and lower fat mass in the PCOS cases. In addition, this discrepancy in body composition was not accompanied by differences in fasting glucose, insulin, C-peptide levels or HOMA calculations between groups. The current study is the third MRI study reporting the absence of preferential visceral fat accumulation in PCOS cases. Notably, the first MRI study included women with PCOS who were slightly obese (mean BMI: 28 kg/m^2) against controls.[5] The second study included women with PCOS (BMI: 18–47 kg/m^2) against controls (BMI: 19–41 kg/m^2), showing no differences in fat content or fat distribution, with lower insulin sensitivity in PCOS.[13]

Table 2: Fasting laboratory data for lean PCOS cases and control cases

	Normal Values	PCOS (n=10)	Controls (n=10)	P-value
Glucose (mmol/l)	4.0 – 6.4	4.7 ± 0.2	5.1 ± 0.4	ns
C-peptide (pmol/l)	200 – 1200	470 ± 140	520 ± 90	ns
17-OH-Prog (nmol/l)	1.0 – 3.0 (in follicular phase)	2.1 ± 0.4	2.2 ± 0.7	ns
DHEA-S (µmol/l)	9.0 – 12.0	6.0 ± 2.1	4.8 ± 1.4	ns
Adione (nmol/l)	1.9 – 14.4	6.4 ± 1.7	4.7 ± 2.5	ns
LH (U/l)	1 – 12	5.1 ± 2.2	6.4 ± 4.8	ns
FSH (U/l)	2.8 – 11	4.9 ± 1.4	7.9 ± 1.9	0.002
T (nmol/l)	0.35 – 4.0	1.6 (1.3 – 4.4)	1.1 (0.6 – 2.1)	ns
SHBG (nmol/l)	20 – 110	37 (22 – 71)	48 (16 – 77)	ns
FAI	0 – 8.5	4.2 (2.9 – 16.5)	2.5 (1.2 – 4.9)	0.005
E2 (pmol/l)	45 – 610	102 (62 – 569)	176 (67 – 1035)	0.04
Insulin (mU/l)	0 – 15	3.1 (2.0 – 7.2)	5.0 (2.4 – 6.6)	ns
HOMA-IR[a]	<1	0.55 ± 0.19	0.63 ± 18	ns

Data are shown as mean ± SD or median (range)
FAI = (total testosterone/SHBG) x 100
[a] http://www.dtu.ox.ac.uk/homacalculator/index.php

Table 3: Ovarian volumes for lean PCOS cases and control cases

	Ovarian volume[a] (cm^3)			
	PCOS		Controls	
	right	left	right	left
Mean ± SD	4.2 ± 1.5	4.0 ± 1.0	2.3 ± 0.9	2.7 ± 1.1
Minimum	2.3	2.7	1.5	1.6
Maximum	6.8	5.4	3.8	4.3

[a]calculated as 2/3π x ovarian length x width x height
Differences between ovarian volumes in PCOS versus controls were significant
PCOS right side ovarian volume versus controls p=0.002 and left side ovarian volume versus controls p = 0.004 (two sided unpaired-samples t-tests)

The biological influence of 'the PCO syndrome' by itself and independent from visceral fat on insulin resistance remains unclear. For example, a previous study[9] compared two PCOS groups (lean, BMI: 22.7 ± 0.5 kg/m^2, age: 28.9±1.2 years and obese, BMI: 34.5 ± 1.0 kg/m^2, age: 30.1 ± 0.9 years) and two control groups (lean, BMI: 22.9 ± 0.3 kg/m^2, age: 37.1 ± 0.8 years and obese, BMI: 31.8 ± 1.15 kg/m^2, age: 35.1 ± 1.2 years), and reported that the factor 'being obese' and not the factor 'being a PCOS patient' is predictive for insulin resistance. However, opposite results, i.e. significantly higher insulin resistance in PCOS were shown in a study[14] comparing younger women with PCOS and controls (mean age: 21.3±3.7 and 21.2±3.4 years, respectively) with normal body weights (BMI: 21.9±2.6 and 20.6±1.1 kg/m^2, respectively). Pioneer clamp studies on insulin sensitivity in lean women with PCOS compared with perfectly weight-, BMI and fat mass-matched controls showed that women with PCOS have lower insulin sensitivity indices (defined as the ratio of the glucose disposal rate to the insulin concentration at the end of the clamp).[14,15,16] However, these studies did not include MRI assessments and therefore, did not exclude for differences in visceral and subcutaneous fat volumes between lean PCOS cases and controls.

Table 4: Cross-sectional areas of subcutaneous and intra-abdominal fat content derived from axial MRI images taken at different abdominal sites

	Cross-sectional area (cm^2)		
	PCOS	**Controls**	**P-value**
	(n=10)	(n=10)	
Mid-L4			
subcutaneous	137 ± 56	134 ± 88	ns
visceral	45 ± 15	53 ± 16	ns
Upper abdomen			
subcutaneous	461 ± 155	599 ± 254	ns
visceral	241 ± 73	492 ± 234	0.005
Lower abdomen			
subcutaneous	1150 ± 450	1222 ± 622	ns
visceral	361 ± 150	696 ± 303	0.006
Total abdomen			
subcutaneous	1611 ± 431	1821 ± 743	ns
visceral	603 ± 200	1188 ± 326	0.0002
Greater trochanters	291 ± 67	250 ± 64	ns

Data are shown as mean ± SD

One of the fundamental questions in PCOS research is whether visceral fat volume changes more rapidly than subcutaneous fat volume during either ageing, use of hormone-containing formulations [such as clomiphene citrate and recombinant FSH (rFSH)], or weight change. Studies in weight-stable healthy women have shown no such preference for visceral fat accumulation through life. For example, effects on body fat distribution due to endocrine ageing (going through menopause) without concomitant weight change have been studied longitudinally with MRI imaging. Increasing subcutaneous and visceral fat content without preference for distribution was shown.[17] This coupling of subcutaneous and visceral fat content has also been shown, both in healthy premenopausal women without diabetes during cycles of weight change and during a hypo-caloric diet with or without extra exercise.[18,19] However, a different picture emerges for states of insulin resistance, for example type 2 diabetes or weight gain in anorexia nervosa. For example, in type 2 diabetes

it has been shown that subcutaneous abdominal fat content will decrease with caloric restriction, while additional exercise is needed to decrease visceral fat content resulting in less insulin resistance.[20] MRI assessment of 22% weight gain during a mean period of 10.1±2.9 weeks (range: 4–17.3 weeks) in anorexia nervosa has shown temporary accumulation of mainly visceral fat.[21] Moreover, weight recovery in anorectic women who managed to maintain body weight over a period of 1 year showed redistribution of adipose tissue deposition similarly to matched controls.[21] To date, there are no longitudinal MRI studies on body fat distribution in lean women with PCOS to test the hypothesis that preferred visceral fat deposition is primarily driven by insulin resistance. Therefore, the hypothesis that preponderant visceral fat deposition is primarily driven by insulin resistance could also be vice versa, i.e. that insulin resistance is primarily driven by preferred visceral fat deposition. PCOS cases in the current study were younger than controls but disclosed hormonal differences, less visceral fat and larger ovarian volumes. Moreover, we found a 44.4 and 33.1% larger ovarian volume of the right and left side, recording dimensions along the x-, y- and z-axes. The x-axis was the maximal longitudinal axis calculated by the number of axial 6 mm steps along the spatial orientation of the ovary, the y- and z-axis being the largest anteroposterior and transverse diameters. Calculations were performed by using a mathematical ellipsoid formula (VE = $2/3\pi$ x x y x z). Notably, volume differences of 42% on average have been previously reported with calculations based on axial, sagittal and coronal diameters (calculated as 0.5× the diameters).[22] Unfortunately, the MRI technology that we have used turned out to be inappropriate for reliable calculations of ovarian morphology (follicle number, peripheral follicle location, visible central ovarian stroma), perhaps because of the 1.0 T scanner that was used and/or the absence of coronary images. The study protocol was written in the supposition that visceral fat is preponderant in PCOS, even in lean cases. Indeed, a higher visceral fat content has been shown in PCOS women but only in mildly obese cases. However, there was no such difference after matching groups for BIA-determined total fat mass.[5] The number of included women in this study was intentionally based on reported differences in favour of visceral fat accumulation in PCOS according to calculations in the whole group. In addition, the focus of the current study was to match for fat mass as close as possible, meaning that small subjects numbers were acceptable according to power analysis. Yet, small patient numbers remain a limitation of the study leaving subtle differences of glucose/insulin metabolism

undetected. However, the strength of the current study is that it used MRI to assess body fat distribution. MRI is an objective imaging with a relatively low reliance on operator skill and with high resolution images. Moreover, serial MRI measurements were used to compare lean PCOS cases with controls. Both groups were strictly matched according to study protocol. However, there was a 5.5-years difference of age, which we believe is the consequence of how control cases were selected. Fertile women were allocated to the control group if they matched for anthropometrics, if there was no contraceptive use, if ovulatory menstrual cycles were confirmed and if they have had successful pregnancies. It remains unlikely that a small age difference among women in their late twenties and early thirties can influence current findings. This belief is supported by the demonstration of total weight gain accompanied by selective accumulation of visceral fat that commonly starts of shortly before menopause.[23]

In conclusion, this study adds new information to recent findings, i.e. mildly obese women with PCOS have no preponderant accumulation of visceral fat. The presented data demonstrated that lean PCOS cases have less visceral fat when compared with control cases. Since this is a cross-sectional study, we encourage further longitudinal studies for analysing visceral fat accumulation over time.

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Chapter 8

Normal laboratory parameters in lean women with PCOS and low visceral fat content analyzed with MRI

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Abstract

Background

A minority of women with polycystic ovary syndrome (PCOS) is lean without any symptoms of the metabolic syndrome. However, previous studies reported insulin resistance in these non-obese cases. The aim of this study is to compare metabolic and ovarian conditions in lean PCOS cases with low visceral fat content compared to controls.

Methods

In this cross-sectional study, 10 Caucasian women with PCOS and 10 controls were compared for glucose, insulin, adipokines (Adiponectin, Leptin, TNF-α, IL-6 and RBP-4), relevant steroids, gonadotrophins, Anti-Müllerian hormone (AMH) and Inhibin B. Magnetic resonance imaging (MRI) was previously used to show that viscerally fat content was lower in these PCOS cases as compared to matched controls.

Results

Glucose, insulin and adipokine levels were similar between groups. PCOS cases had significantly higher AMH levels compared to control cases (11.1±3.0 vs. 3.3±1.8 ng/ml; $P<0.01$). There was negative correlation between glucose and AMH level in PCOS (rp: -0.64; $P=0.045$) while it was positive in controls (rp: 0.79; $P=0.006$).

Conclusions

We observed neither insulin resistance nor differences in metabolic parameters in viscerally lean PCOS cases. The data presume a distinct group of lean PCOS cases with low visceral obesity and an ovarian disorder.

Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder of heterogeneous nature presenting 5–10% of women in their reproductive age. The syndrome is characterized by hyperandrogenism and chronic oligo- or anovulation.[1,2,3,4] The Rotterdam criteria of 2003 also included specific ultrasound features i.e. antral follicle counts (AFC) of more than 12 with follicles of 2-9 mm in either ovary and/or an ovarian volume of more than 5.5 cm.[3,5,6] However, this sonographic criterion is not specific as AFC's are increased and ovarian volumes are large (exceeding 10 cm^3) in a subgroup of ovulatory women.[7] The common presence of insulin resistance remains a matter of debate and is not included in the Rotterdam consensus. Pioneer scientific work in this field demonstrated that insulin-stimulated total body glucose utilization was significantly decreased in lean and obese women with PCOS.[8] PCOS is the leading cause of insulin resistance and metabolic syndrome in adolescent girls and young women, particularly those with obesity.[9,10,11]

Therapeutic interventions with metformin and thiazolidinediones (TZDs) in overweight and obese women with PCOS have shown that these drugs are effective to induce regular menstrual cycles and improve fertility.[12,13,14,15] In spite of these observations, insulin resistance can also be absent particularly in those women with small quantities of viscerally deposited fat[16,17] leaving the possibility open of an enhanced oxidant state without disruption of insulin/IGF signalling.[18] Consequently, a concept of a separate group of lean women with PCOS and no insulin resistance would argue against treatment with insulin sensitizing drugs in these patients. Three important findings should be considered in this specific group of PCOS. Selective ovary resistance to insulin signaling has been shown in granulosa cells of PCOS cases which restored after culturing these cells with the thiazolidinedione (TZD) troglitazone.[19] Pioglitazone (TZD) altered gene regulation distinct from insulin/IGF1 signaling in an obese mouse model with PCOS.[20] Obesity as a single factor altered several ovarian genes without direct involvement in insulin/IGF1 signaling.[21]

In an earlier publication we reported on the fat distribution and initial ovarian hormonal evaluation in a group of lean PCO subjects.[17] In the present study we extended the evaluation of these individuals on further metabolic parameters, with emphasis on adipokine measurements and AMH and inhibin-B levels.

Patients and Methods

For details on patient recruitment, we refer to the previous report.[17] Briefly, we included 10 lean Caucasian women with PCOS according to the ESHRE/ASRM criteria who were recruited from the fertility clinic of Twente Group of Hospitals in The Netherlands and 10 controls who were matched for BMI and Bioelectrical Impedance variables (BIA) (total Fat Mass and Lean Body Mass). The diagnosis of PCOS was made using the Rotterdam ESHRE/ASRM diagnostic criteria (Anonymous, 2004). All included women had oligo-ovulation and/or anovulation (intermenstrual interval greater than 42 days), typical polycystic ovaries shown on ultrasound and no clinical signs of hyperandrogenism. None of the subjects had ever received hormonal therapy for ovulation induction.

All clinical investigations were conducted in accordance with the guidelines in the Declaration of Helsinki, and the study was approved by the medical ethical review committee of the hospital. All subjects provided written informed consent.

Laboratory assays, MRI and BIA assessments

Fasting venous blood samples were drawn on cycle days 3–7 after spontaneous menses in controls or after progesterone-induced withdrawal bleeding in PCOS. Samples were collected in serum separator tubes, allowed to clot during at least 30 min, centrifuged for 15 min at 2000×g at room temperature and aliquoted into polypropylene tubes. Adiponectin, Leptin,TNF-α, IL-6, Retinol Binding Protein-4 (RBP-4), Inhibin B and AMH measurements were performed in samples which had been stored at -80°C and assayed with one single assay kit. Adiponectin was measured using an ELISA (Millipore EZHADP-61K; Nuclilab BV, Ede, NL) and Leptin, TNF-a and IL-6 were assayed using MILLIPLEX MAP Human Serum Adipokine Panel B (Millipore HADK2-61K-B, Nuclilab BV, Ede, NL) on a fully automated analyzer (Luminex 100/200™, Luminex BV, Oosterhout, NL). Inhibin B was assayed using an ELISA (Gen II Elisa, Beckman Coulter BV, Woerden, NL). Human RBP-4 was assayed using an extraction-free EIA Kit (EK-028-28, Phoenix Pharmaceuticals Inc, Burlingame, USA) and AMH was assayed using AMH enzyme immunoassay (A11893, Beckman Coulter BV, Woerden, NL). Laboratory reference intervals for parameters used are listed in Table 1. Adiponectin (intra-assay 1,0-7,4), Leptin (intra-assay 1,4-7,9), TNF-α (intra-assay 1,4-7,9), IL-6 (intra-assay 1,4-7,9), RBP-4 (intra-assay 1,3-9,0), Inhibin B (intra-assay 1,9-3,8), AMH (intra-assay < 12,3).

All participants were examined on a MRI scanner (Magnetom Harmony 1.0 T MRI system; Siemens Medical Solutions, USA), as reported.[17]

Table 1: Levels of metabolic parameters and ovarian parameters of the lean PCOS and control cases.

	Reference interval	PCOS (n=10)	Controls (n=10)	P-value
Metabolic parameters				
Adiponectin (pg/1)	5-16	16.4 ± 3.3	16.8 ± 3.9	ns
Leptin (µg/1)	3.7-11.1	10.0 (4.5-23.1)	9.8 (2.2-29.0)	ns
TNF-α (µg/1)	14-28	7.8 ± 3.5	7.1 ± 2.1	ns
IL-6 (µg/1)	< 3.4	2.8 (0.6-10.4)	3.8 (0.6-16.0)	ns
RBP-4 (µg/1)	19.0-57.5	30.5 (16.7-35.9)	34.0 (31.7-35.1)	ns
Ovarian parameters				
Inhibin B (µg/1)	< 350	88.7 (55.9-118.5)	127.9 (61.4-194.5)	ns
AMH pg/l	0.5-7.0	11.1 ± 3.0	3.3 ± 1.8	<0.0001

AMH reference values are derived from ovulatory women of 15-41 yrs.
Data are shown as mean±SD or median (range).

Statistical analysis

Data are shown as mean±SD or as median (range), if not normally distributed. Statistical analyses were performed using StatGraphics Centurion XV software (Version 15.2.00 for MS Windows, Statpoint Inc., VA, USA) and using Analyse-It (version 2.22 for MS Excel; Analyse-It Software, Leeds, UK). Shapiro-Wilks W-tests were performed to check whether nominal parameters were normally distributed, prior to analysis. Analyses included unpaired t-tests or Mann–Whitney–Wilcoxon rank-sum test for comparison of demographic, laboratory and MRI data obtained from women with PCOS and controls. Correlations between parameters were calculated by Pearson's linear product moment or Spearman rank correlation tests in cases and controls and pooled data.

Results

Women with PCOS were younger than controls (28.2 vs 33.7 years respectively) but both groups were balanced for body weight, Body Mass Index (BMI, 21.6 vs 21.8 respectively) and Waist/Hip ratios (0.83 and 0.85 respectively). Both groups were balanced

for Total Fat Mass and Lean Body Mass as well (13.2 vs 14.4 and 52.4 vs 53.4 respectively).

In addition to the earlier reported lower E2 and FSH levels and higher FAI calculations in PCOS cases[17], we observed significantly higher levels of AMH which were 11.1 pg/l in PCOS subjects, and 3.3 pg/l in controls (p-value <0.0001, table 1). The levels of inhibin B did not differ between both groups.

As presented in our previous report[17] blood glucose, serum insulin and HOMA–IR did not differ between lean PCOS and controls. Besides, we did not observe significant differences in the most prominent adipose-tissue derived factors, like adiponectin, leptin, TNF-α, IL-6 and RBP-4 levels when both groups were compared.

Correlations

We looked for independent correlations between the previous obtained metabolic parameters glucose and insulin versus AMH as ovarian parameter. PCOS cases showed a significant negative correlation between glucose and AMH levels (r_p: -0.64: P=0.045) in and no significant relationship between insulin and AMH levels (r_p: -0.27: NS). Control cases showed a significant positive correlation between glucose and AMH levels (r_p: 0.79: P=0.006) and no significant relationship between insulin and AMH ($r_{p:}$-0.34: NS). In PCOS cases we also looked for independent correlations between abdominal visceral fat content and glucose, insulin and AMH levels but found no significant relationships. Correlations between ovarian volumes showed a trend for AMH levels in PCOS (r_p: 0.60: P=0.06), no significance for glucose or insulin levels. There was a significant correlation between the smallest ovary per PCOS case and AMH levels (r_p: 0.84: P=0.002), no significance for glucose or insulin levels and no significance between ovarian volumes in controls cases regardless small or large versus glucose, insulin or AMH levels.

Discussion

This is one of the few metabolic studies in MRI-characterized lean subjects with PCOS. All PCOS cases were lean in terms of BMIs <25 kg/m^2 and low visceral fat contents (viscerally lean). In addition to our earlier report on finding normal fasting glucose and insulin levels[17], we also observed that there were no differences between PCO cases and controls with regards to levels of adipokines like adiponectin, TNF-α, IL-6 and RBP-4. This

underpins the absence of metabolic syndrome and insulin resistance in lean PCOS cases and criticizes the use of BMI as the sole anthropometric marker for 'lean' in PCOS cases.

The current comparison study provides additional metabolic data in lean PCOS cases but we performed no clamp studies.[17] Inspite of this, we found strong evidence for intact insulin sensitivity as metabolic symptoms were lacking, and as reported[17] lower quantities of viscerally deposited fat in the PCOS cases have been observed. Based on these findings there is no rationale to treat this category of women with insulin sensitizers in spite of some articles wherein beneficial results were reported.[23,24] It has to be noted that these observations have been reported from lean PCOS cases who were poorly characterized for insulin resistance, metabolic parameters and body fat distribution. The current finding of opposite glucose-AMH-correlations in PCOS versus controls allows speculation about glucose metabolism by the ovary.

PCOS cases in the current study had significantly lower FSH levels and larger ovarian volumes versus controls. Notice, total follicle numbers, and pre-antral follicle numbers are the main components of ovarian volume.[24] Therefore, it is likely that negative feedback between pre-antral follicle mass and FSH secretion alters as growing follicle mass expands. Two mechanisms i.e. increased sensitivity of granulosa cells to FSH or inhibitory factors of FSH secretion may explain these differences.

Ovarian reproductive biology starts with initiation of primordial dormant follicles before and throughout reproductive life (initial recruitment). Follicles develop through primordial, primary, and secondary stages before acquiring an antral cavity. At the antral stage most follicles undergo atresia but a few of them survive under optimal FSH stimulation that occurs at puberty (cyclic recruitment). Initial recruitment is a gonadotropin-responsive stage since endogenous or exogenous FSH stimulates primordial follicle growth. Cyclic recruitment is the result of the increase in circulating FSH during each reproductive cycle that rescues a cohort of antral follicles. FSH is the major survival factor to rescue pre-antral follicles from apoptosis (gonadotropin-dependent) and once the follicles reach antral and larger sizes, multiple intrafollicular factors are produced locally to ensure successful maturation and ovulation.[26]

PCOS is considered to be caused by poly-genomic changes, which all together affect signal transduction at several levels. One hypothesis about the pathogenesis of lean PCOS is that pre-antral follicles become affected by a misbalance between proliferation and

apoptosis. Granulosa cells from patients with PCOS have lower apoptotic rates associated with decreased levels of the apoptotic effector Caspase-3 and increased levels of the Anti-apoptotic survival factor Cellular inhibitor of Apoptosis proteins-2 that were coupled to higher proliferation rates.[27] The origin for this dysbalance can be ascribed to differentially expressed genes (DEGs), which differ between lean PCOS and body weight-matched controls and other DEGs which are typical for overweight/obese PCOS cases. DEGs in lean PCOS cases are characterized by down-regulated transcription factors and components of the extracellular matrix such as Wnt/β-catenin and Mitogen-activated protein kinase. DEGs in overweight/obese women with PCOS typically refer to pathways related to insulin signalling, metabolism and energy production.[28] These findings have been confirmed by other studies. For example, analyses from 7 microarray studies on PCOS tissues pointed in the direction of three overrepresented sets of genes to be involved in PCOS. These are genes involved in insulin receptor signalling, steroid biosynthesis and the regulation of gonadotropin secretion.[29] In addition, overexpression of a number of growth factors in PCOS ovaries led to a hypothesis of blocking of apoptosis and atresia leading to accumulation of pre-antral follicles.[30] Compelling data from cDNA microarray technology have demonstrated either upregulation and/or downregulation of genes involved in apoptosis and proliferation.[31] Validation of genomic expression changes, assayed at the protein level, have revealed several peptides like HSP27 and HSP10 in PCOS ovarian tissue that are considered to be critical for signal transduction in the prevention of apoptosis.[32]

The current research question focused on the question whether women with PCOS who were previously shown to be viscerally lean[17] have a different disorder compared to those individuals who are not. AMH levels were significantly higher in PCOS cases and correlated negatively with glucose levels in contrast with controls. The main physiological role of AMH in the ovary seems to be limited to the suppression of the early stages of follicular development, since both *in vivo* and *in vitro* experiments have indicated that the transition from primordial into growing follicles becomes enhanced in absence of AMH, leading to early exhaustion of the primordial follicle pool.[33,34] AMH levels become increased in conditions with increased numbers of developing follicles not only as the result of excessive accumulation of antral follicles numbers[35] but also due to increased granulosa cell secretion.[36] Moreover, AMH levels are on average 75 times higher in granulosa cells

from PCOS, compared with levels in granulosa cells from normal ovaries.[37] However, the role of AMH as a paracrine signal in early follicle development in PCOS remains unclear.[38]

In summary, lean PCOS cases were compared to control cases and showed no signs of insulin resistance or metabolic disease, but higher AMH levels and larger ovarian volumes. The inverse relationship between blood glucose and AMH levels oppositely to the positive relationship in controls suggests ovarian hypersensitivity for glucose and perhaps oxidative damage and apoptosis. The role of TZD's to protect the ovary needs to be studied in future research.

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Summary and conclusion

Chapter 9

Summary and conclusion

Patients suffering from subfertility due to obesity and/or related Polycystic Ovary Syndrome (PCOS) are frequently seen in gynaecological practices. The studies described in this thesis have been undertaken to gain new insight in the pathophysiology and treatment of such conditions. The first part of this thesis addresses aspects of obesity and related fitness based on the hypothesis that fat and fat distribution play a crucial role in the arrest of follicular development. Fat tissue (adipose tissue) has been divided into a subcutaneous and a visceral compartment. Adipose tissue represents an endocrine organ showing several metabolic aspects as described in **chapter 2-5**. These chapters focus at clinical differences of male and female pattern obesity, fat tissue as extra-adrenal source of steroidogenesis in PCOS, weight reduction caused by Selective Serotonine Reuptake Inhibition with fluoxetine prior to bariatric surgery and effects of the insulin sensitizing agent metformin on subcutaneous glucose regulation in non diabetic women.

Single metabolic aspects of adiposity and possible effects on follicular growth are widely analysed but an overall pathophysiologic hypothesis explaining the arrest of follicular growth induced by fat tissue is lacking. Moreover, PCOS is also observed in lean women. The connection to the second part of this thesis was, therefore, made by the observation that PCOS is not only diagnosed in adipose women, i.e. a subgroup of appr. 10% PCOS cases in fertility practice is not obese. Therefore, we questioned whether body fat distribution is also in favor of visceral fat deposition in lean PCOS cases **(chapter 7)**. This question is of particular interest because of recent findings that reduction of visceral adipose tissue is the major determinant of regaining ovulation.[1] Several studies point toward a reduced ovarian sensitivity for FSH resulting in follicular arrest.[2] Ovarian FSH sensitivity is subjected to endocrine, paracrine and autocrine pathways. It was questioned whether hormone and cytokine production in lean PCOS women is different as compared to proven fertile ovulatory controls matched for BMI **(chapter 8)**.

What have we learned from analysis of metabolic aspects of adiposity?

In **chapter 2** severe obese men and women with BMI above 40 kg/m2 were analysed for physical fitness prior to bariatric surgery. This study was performed in order to pre-select morbidly obese individuals with increased risk for complications immediately post surgery. The study population consisted of obese individuals who were analysed for

cardiopulmonary exercise capacity by spirometry, electrocardiography and by cycle ergometry. The study design compared females and males matched for BMI (in men typically upper body and in women typically lower body fat distribution). In total, 59% of included men and 35% of included women had glucose intolerance or overt type 2 diabetes. The men showed less physical endurance and more glucose intolerance as compared to the women. As the number of severely obese women seeking assistance in reproduction is steadily increasing, these results have considerable consequences for daily gynaecological practice. Obesity and concomitant subfertility is frequently associated with PCOS with accumulation of visceral fat and existent insulin resistance.[3] Weight loss by dietary interventions and life style programs in general improve the aspects of the metabolic syndrome and may help to regain ovulatory cycles in women. Unfortunately, the percentage of weight loss achieved by these programs is often limited to about 5-10% of initial weight and barely persistent in morbid obese individuals.[4] It is suggested that in morbid obese individuals only sustained and marked weight loss, for example by bariatric surgery, may recover adiposity-related metabolic disturbance and clinical symptoms of PCOS, and lead to successful resumption of ovulation and -ultimately- pregnancy.[5,6]

Research for innovations on the treatment of anovulation increased during the last decade. As obesity is related to increased risk of cardiovascular disease and diabetes, many weight reduction programs have been developed. Great effort has been made to develop lifestyle programs for obese individuals in order to lower visceral fat thereby creating an advantageous metabolic context. Obese PCOS patients may benefit from weight reduction as insulin sensitivity is reduced and hyperandrogenism improves.[7] In practice it has been demonstrated that weight reduction of 10% is achievable but maintenance of this reduction remains usually cumbersome. Weight reduction programs are considered to be the cornerstone in the approach of obesity treatment. Although pharmacological interventions are considered to be second line treatment, they may be supportive to achieve and/or maintain weight reduction. The Selective Serotonin Reuptake Inhibitor (SSRI) fluoxetine has shown to suppress appetite and rapid weight loss.[8,9]

In an observational study it was attempted to support morbidly obese patients participating in a structured weight management program with fluoxetine treatment in order to optimize their clinical condition prior to bariatric surgery. For this, fluoxetine in a dose of 60 mg t.i.d. was used, and we showed clear weight reducing effects during a intervention

period of 6 months (chapter 3). In long term studies among obese type 2 diabetes patients supportive psycho-pharmacotherapy with fluoxetine is effective in modest weight reduction.[10] Fluoxetine reduces appetite and is frequently used in individuals with aberrant eating pattern but is not licensed as an anti-obesity drug.[11] For patients with mild to moderate obesity suffering from type 2 diabetes supportive fluoxetine therapy is, however, effective in addition to diet or lifestyle modification programs. Besides, fluoxetine has beneficial effects on metabolic disturbances associated with diabetes.[12]

As the clinical presentation of PCOS shows similarity to other endocrine diseases such as the metabolic syndrome and atherosclerosis, it also mimics hypercortisolism and hyperandrogenaemia. Therefore, we have assessed possible disturbances of steroidogenesis in women with PCOS, as described in chapter 4. In previous studies, it were mainly adrenal enzyme deficiencies that were reported in these individuals particularly in subjects with hirsutism, together referred to as adrenal hyperplasia syndromes. However, obese women with PCOS usually present with normal fasting cortisol levels and mildly increased urinary cortisol excretion suggesting extra-adrenal cortisol production. We hypothesized that in PCOS disruption of steroidogenesis originates from extra-adrenal sources. Enzyme activities of 3β-Hydroxy-Δ^5-steroid dehydrogenase, 21α-hydroxylase and 11β-hydroxylase were calculated by determining respectively precursor to product ratio's of 17OH-pregnenolone/17OH-progesterone, 17OH-progesterone/11-deoxycortisol and 11-deoxycortisol/cortisol before and after ACTH stimulation in a group of women with PCOS. Our study confirmed the absence of adult onset Congenital Adrenal Hyperplasia (CAH) to contribute in the development and clinical features of PCOS. Quite possibly, PCOS ovarian steroidogenic cells produce androgens in excess, while the extra cortisol production comes from fat cells. Visceral deposited fat is supposed to contain sufficient 11 β-HSD type 1 activity to convert 11-deoxycortisol to cortisol explaining pseudo hypercortisolism in obese women with or without PCOS.

Previous studies are in support of the concept that visceral body fat distribution affects the clinical presentation in obese individual and focus at obese women with PCOS. Indeed many of these individuals present with the combination of obesity and features of the metabolic syndrome, and 7-10% even with overt type 2 diabetes. As the drug metformin is frequently used to induce normalisation of menstrual periods and resumption of ovulation, we questioned to what degree this agent would affect glucose concentrations in women

with PCOS but without overt type 2 diabetes (chapter 5). This study on the effects of metformin on glucose dynamics was performed in a period of growing interest for this drug as treatment for ovulation induction, and the new development of glucose sensors which were capable of measuring subcutaneous glucose concentrations for a period of 3 days. Interstitial tissue glucose dynamics were measured with these sensors in a group of non-diabetic women with PCOS receiving metformin as well as in a control group. Metformin administration did not induce a change in the percentage of interstitial glucose levels below 2.3 mmol/L, whereas mean interstitial glucose level maintained similar in the PCOS group. In the control group percentage of low interstitial glucose levels decreased and mean interstitial glucose level slightly increased. The finding of similar interstitial glucose levels during metformin treatment underpins safe administration of drug in PCOS cases without diabetes.

Although the majority of PCOS patients are obese or severely obese, we have realized during the recent years that PCOS is not a single disease entity, but a complex disorder, resembling a similar complex spectrum of metabolic abnormalities as found in type 2 diabetes. In clinical practice, a significant number of patients are not obese, and we questioned whether an intrinsic metabolic role of visceral fat mass can be observed in this lean group of patients. This is of clinical significance, since there are variable results in literature regarding insulin sensitivity in lean PCOS and many lean PCOS patients had no hirsutism or other signs of increased androgen production. In chapter 7 and 8 we described an extensive evaluation of lean PCOS patients. At first, we assessed whether this subgroup presents with a different body composition compared with a BMI-matched fertile control group. Surprisingly, PCOS cases studied revealed to have less visceral body fat content and an increased ovarian volume. In addition, our metabolic and hormonal evaluation showed no indication of clinically significant insulin resistance or changes in adipokine levels, but did reveal higher AMH levels in lean PCOS cases with an inverse relationship between glucose and AMH levels. Oppositely, glucose and AMH levels were positively related in matched controls.

Conclusion and future perspective

Our observations suggest that women with PCOS do not share similar phenotypes and point into a direction of different pathologies for overweight/obese cases versus that of lean

cases. The hypothesis arises that follicular arrest in viscerally lean PCOS may be due to an intrinsic ovarian mechanism. Obese PCOS comprises a complex of symptoms with variable expression mainly due to the amount of visceral adiposity resulting in a metabolic condition to protect the individual against famine. In viscerally lean PCOS this beneficial metabolic condition is lacking. Currently, the definition of PCOS deals with the description of selected symptoms. The intrinsic causal organ responsible for different phenotypes of PCOS is left out of consideration. A more accurate definition should also include the assessment of visceral fat mass. PCOS seems to reflect a complex of symptoms in viscerally obese individuals with polycystic ovaries but not in viscerally lean individuals who also have polycystic ovaries. The latter group doesn't share any metabolic indication of a conservation mechanisms for calories like insulin resistance or visceral fat related adipokine production. It seems therefore to be more adequate to categorize viscerally lean individuals with subfertility and polycystic ovaries as polycystic ovarian disease (PCOD). This hypothetic concept of heterogeneity of normogonadotropic normoestrogenic oligo-anovulation imply different interventions for PCOS and PCOD cases based on quantified visceral fat content.

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Samenvatting en conclusie

Chapter 10

Samenvatting en conclusie

Onvruchtbaarheid op basis van obesitas is, al dan niet gerelateerd aan het Polycysteus Ovarium Syndroom (PCOS), een regelmatig voorkomend probleem in de dagelijkse praktijk van de gynaecoloog. De studies in dit proefschrift dragen bij aan nieuwe inzichten in de pathofysiologie en behandeling van PCOS. Het eerste deel van dit proefschrift richt zich op obesitas en daaraan gerelateerde metabole aspecten. Uitgangssituatie is de hypothese dat vet en de verdeling van het lichaamsvet ten gunste van het viscerale compartiment een belangrijke functie zouden vervullen bij het stagneren van de follikelgroei. Er dient onderscheid te worden gemaakt tussen onderhuids (subcutaan) vetweefsel tegenover vetweefsel dat zich in de buikholte (visceraal) bevindt. Vetweefsel is een orgaan dat de stofwisseling kan beinvloeden. De **hoofdstukken 2-5** geven een overzicht van een aantal metabole aspecten van het vetweefsel. Klinisch hebben mannen en vrouwen een verschillende verhouding tussen subcutaan en visceraal vet. Het verschil in fysieke conditie tussen man en vrouw wordt in dit proefschrift geanalyseerd. Vetweefsel als een bron voor extra adrenale steroidproduktie komt aan de orde. Het effect van de Selectieve Serotonine Heropname Remmer fluoxetine op de pre-operatieve gewichtsafname bij een ernstig obese groep wordt besproken. Tot slot is het effect van de toediening van metformine op de onderhuids gemeten glucose concentraties bestudeerd bij vrouwen zonder diabetes al dan niet met PCOS.

Er is op dit moment geen uniforme pathofysiologische verklaring voor het stagneren van de follikelgroei bij vrouwen met PCOS en de rol van vet is nog onduidelijk. Bovendien komt PCOS ook voor bij slanke vrouwen. Het feit dat PCOS zowel bij zwaarlijvige als bij slanke vrouwen voorkomt vormt de basis voor het tweede deel van dit proefschrift. Een deel van de vrouwen met onvruchtbaarheid op basis van PCOS is slank. Deze klinische waarneming noopt tot nader onderzoek naar de vetverdeling bij de slanke vrouwen met PCOS. Er zijn steeds meer aanwijzingen dat niet uitsluitend de hoeveelheid vetweefsel en de verdeling ervan een oorzakelijke rol spelen bij de stagnatie van de follikelgroei. Afvallen resulterend in de afname van de hoeveelheid visceraal vet is weliswaar een bepalende factor voor het herstel van de ovulatie. Er zijn echter ook een aantal onderzoeken waarin een verminderde gevoeligheid voor FSH gedreven follikelgroei in het polycysteuze ovarium wordt aangetoond. Dit wijst op een aandoening van het ovarium. De gevoeligheid van het ovarium voor FSH is onderhevig aan endocriene, paracriene en autocriene faktoren. Om die reden hebben wij onderzocht of de slanke vrouw met PCOS een ander cytokine- en

hormoonprofiel vertoont vergeleken met dat van de even slanke vruchtbare vrouw. **(hoofdstuk 8).**

Wat hebben wij geleerd van de analyse bij obesitas?

In **hoofdstuk 2** ondergingen ernstig obese vrouwen en mannen onderzoek naar hun lichamelijke conditie, voorafgaande aan bariatrische chirurgie. Ernstig obese patiënten hebben een verhoogd risico op postoperatieve complicaties. Het onderzoek had als doel om het operatierisico van chirurgisch handelen in kaart te brengen en te vergelijken. De studiegroep bestond uit ernstig obese mannen die een typisch androgyne vetverdeling representeren terwijl vrouwen veel meer subcutaan vet hebben. De fysieke conditie werd bepaald aan de hand van spirometrie, electrocardiografie en fiets-ergometrie. 59% Van de mannen en 35% van de vrouwen bleken glucose intolerant te zijn of hadden diabetes. Opvallend in de uitkomst van het onderzoek was dat de mannen een slechtere fysieke conditie hadden dan de vrouwen. Aangezien overgewicht en obesitas steeds vaker onderdeel vormt van de klinische problemen bij onvruchtbare vrouwen wordt behandeling hiervan steeds belangrijker in de dagelijkse praktijk. PCOS gaat vaak gepaard met obesitas ten gunste van het viscerale compartiment en dientengevolge met insulineresistentie. Afvallen dmv dieet of verandering van ‚life-style' doet het klinisch beeld van PCOS verbeteren en ovulatie veelal herstellen. Helaas is afvallen vaak beperkt tot 5 a 10% van het oorspronkelijke gewicht en is het handhaven van het gewicht op termijn een probleem. Het plaatsen van een maagband is een methode om het gewicht duurzaam te verlagen, het metabolisme te optimaliseren en de vruchtbaarheid te bevorderen.

In de afgelopen jaren is veel onderzoek verricht naar nieuwe interventiestrategieën bij vrouwen met PCOS waarbij eveneens aandacht werd gegeven aan het ontwikkelen van "life style" programma's. Dit concept is gebaseerd op de aanpak van het toegenomen risico op hart- en vaatziekten, diabetes en de anekdotische ervaring dat patiënten met PCOS makkelijker zwanger raken. Het biologisch effect lijkt te berusten op een vermindering van het viscerale vet waardoor het metabole milieu gunstiger wordt. Minder visceraal vet hangt direct samen met een toename van de insuline gevoeligheid en een afname van de hoeveelheid vrij circulerend androgeen. 10% Gewichtsverlies is al voldoende om genoemde effecten te bewerkstelligen.

Steeds weer blijkt dat leefstijlprogramma's de voorkeur hebben hoewel ondersteuning met farmaca kan helpen om succesvol af te vallen. Fluoxetine is een zogenaamde Selectieve Serotonine Heropname Remmer met een angst en depressie onderdrukkende werking maar ook een remming van de eetlust. In ons observerend onderzoek onder ernstig obese patiënten – mannen en vrouwen – die op de wachtlijst staan voor bariatrische chirurgie bleek fluoxetine toediening in een dosering van 60 mg effectief te zijn om gewicht kwijt te raken met als doel om de gezondheid voor de operatie te bevorderen **(hoofdstuk 3)**. Ook een aantal langere termijn studies onder diabetes patiënten bevestigen dit gewichtsreducerend effect.

De klinische presentatie van PCOS vertoont gelijkenis met het metabool syndroom, atherosclerose en hypercortisolisme. In **hoofdstuk 4** werden mogelijke afwijkingen in de steroidgenese bij vrouwen met PCOS geanalyseerd. Vrouwen met PCOS hebben vaak een overschot aan circulerend androgeen dat klinisch tot hirsutisme, acne en seborrhoea kan leiden. Deze afwijkingen in androgenen werden in de literatuur aan een volwassen vorm van bijnierschorshypertrofie toegeschreven, hoewel de serum cortisol concentraties normaal waren. Subtiele cortisol afwijkingen waren al lang bekend, zo blijkt dat de cortisol uitscheiding de urine licht te zijn verhoogd. Dit suggereert een andere bron dan de bijnier voor de cortisolproduktie. De hypothese is dan ook dat de oorzaak van een afwijkende steroidproduktie inclusief die van het cortisol buiten de bijnier moet zijn gelegen. De activiteit van 3β-Hydroxy-Δ^5-steroid dehydrogenase, 21α-hydroxylase and 11β-hydroxylase zijn berekend door respectievelijk de precursor-to-product ratios van 17OH-pregnenolone/17OH-progesterone, 17OH-progesterone/11-deoxycortisol and 11-deoxycortisol/cortisol voor en na ACTH stimulatie te berekenen. De studie in dit proefschrift laat zien dat er van bijnierschorshypertrofie bij PCOS geen sprake is en dat de verhoogde excretie van cortisol waarschijnlijk afkomstig is uit het vetweefsel. Het viscerale vet heeft namelijk het vermogen om met 11 β-HSD type 1 enzym activiteit 11-deoxycortisol in cortisol te converteren. Daarmee kan het pseudo-hypercortisolisme bij obese PCOS worden verklaard.

Verreweg de meeste studies betreffen vrouwen met PCOS en overgewicht/obesitas. De uitkomsten hiervan ondersteunen het concept dat het toegenomen viscerale vet betrokken moet zijn bij de klinische uitingsvormen van het syndroom. Gebleken is dat de overgrote meerderheid van deze patiënten aan insuline resistentie lijden en 7-10% aan type 2 diabetes.

Omdat metformine frequent wordt gebruikt om een ovulatoire cyclus te herstellen met als uiteindelijke doel om een zwangerschap te bewerkstelligen, beoogden wij met **hoofdstuk 5** om het effect van metformine op subcutane glucoseconcentraties te boordelen. Dit werd m.b.v. een continue glucosesensor gemeten tijdens metformine gebruik en vergeleken tussen vrouwen met PCOS en gezonde vrouwen. Wij maakten gebruik van een eerste generatie sensor die gedurende drie dagen kan registreren. Wij vonden dat het percentage lage glucose concentraties afnam, terwijl het totaal gemiddelde juist iets toenam. Gedurende de meettijd veranderde het aantal lage glucose concentraties niet en bleef de gemiddelde concentratie gelijk bij de vrouwen met PCOS.

De meeste vrouwen met PCOS hebben overgewicht of zijn te dik. Deze patiënten hebben veelal de symptomen van een metabool syndroom. PCOS is zoals de naam ook al aangeeft een typisch syndromale aandoening met een hoge diabetes prevalentie. Toch is niet elke vrouw die aan de definitie PCOS voldoet te zwaar - 'gewicht' is ook geen opgenomen criterium in de vigerende 'Rotterdam Consensus Definition'. Naar schatting is 10 – 15% van de patiënten slank. Slanke vrouwen met PCOS representeren zich fenotypisch anders dan obese vrouwen met PCOS. Dus rijst de vraag ***of*** vetmassa bij deze slanke patiënten metabool een rol speelt. En als dit het geval zou zijn, betreft dit dan het visceraal opgeslagen vet? In **hoofdstuk 7 en 8** wordt hierop in gegaan. In **hoofdstuk 7** onderzochten wij slanke vrouwen met PCOS middels magnetische resonantie beeldvorming (MRI). Zowel het viscerale als het perifere buikvet kan m.b.v. deze techniek nauwkeurig gekwantificeerd worden. Wij vonden dat slanke vrouwen met PCOS minder visceraal obese waren dan een vergelijkbare fertiele controlegroep. Het grote verschil tussen de groepen was niet zozeer het viscerale vetvolume maar het eierstok volume. De slanke patient met PCOS heeft dus behalve een laag visceraal vetgehalte ook vergrootte eierstokvolumina. In **hoofdstuk 8** onderzochten wij dezelfde twee groepen op verschillen in metabole en eierstokgerelateerde factoren. Op grond van bovenstaande vaststellingen gingen wij ervan uit dat de slanke patiënten – i.t.t. wat de literatuur aangeeft over PCOS patiënten met overgewicht of obesitas – geen afwijkende metabole doch wel afwijkende eierstokgerelateerde factoren hebben. Onze hypothese bleek de werkelijkheid, althans in het door ons bestudeerde materiaal. Viscerale slanke PCOS patiënten bleken normaal insuline gevoelig en hadden geen aanwijzingen voor metabole ziekte. De slanke patiënten hadden echter significant hoge concentraties Anti Müller Hormoon (AMH) en deze concentraties

correleerden omgekeerd met de glucose concentraties in bloed. Gezonde vergelijkbare controlepersonen hadden een significant lagere AMH concentratie en deze concentraties correleerden positief met de glucose concentraties in bloed.

Conclusie en toekomstperspectief

Dit proefschrift bestrijkt een aantal aspecten over de samenhang tussen obesitas en de rol van vetweefsel met die van de eierstok (ovarium). Deze samenhang werd bestudeerd onder mensen al dan obese en al dan niet met vruchtbaarheidsstoornissen. Het bevestigt eerdere wetenschappelijke uitkomsten dat vetweefsel inderdaad is betrokken bij de biologie van de eisprong (ovulatie). Het verminderen van visceraal opgeslagen vet bevordert het ovulatieproces en dit effect wordt in de patiëntenzorg toegepast. Leefstijlverandering al dan gecombineerd met medicijnen gericht op de verbetering van de insulinegevoeligheid bevorderen de kans op ovulatie en daarmee de kans op zwangerschap. Dier en humaan onderzoek leveren het overtuigende bewijs dat ondermeer metformine effectief is bij patiënten met PCOS. Betekent dit dat leefstijlprogramma's al dan niet ondersteund door metformine zinvol zijn voor elke patient met PCOS? De literatuur geeft hiervoor alleen steun indien vrouwen met PCOS ook overgewicht hebben.

Op basis van de uitkomsten van dit proefschrift is het waarschijnlijk dat PCOS vastgesteld o.b.v. de huidige definitie ten minste twee pathosfysiologische mechanismen bestrijkt. Het lijkt beter op zijn plaats om te spreken over een polycysteuze ovariële ziekte bij visceraal slanke individuen , bijvoorbeeld polycystic ovarian disease of PCOD en over polycysteus ovarium syndroom (PCOS) bij visceraal obese individuen. De hypothese is dat PCOD een primair intrinsieke ovariële aandoening is. PCOS omvat daarentegen een complexiteit van symptomen met ondermeer ovulatiestoornissen. Behandelingstrategieën tegen PCOD dienen primair op het ovarium te zijn gericht. Bij PCOS echter dient men de therapie te richten op het hypermetabolisme waaronder leefstijlinterventie en medicatie ter verbetering van insulinegevoeligheid.

Kortom, bij de behandeling en toekomstig onderzoek van normagonadotrope, normo-oestrogene anovulatie/oligomenorrhoe dient de behandelaar rekening te houden met het onderliggend pathosfysiologisch mechanisme. De behandelaar dient zich af te vragen of er sprake is van PCOS of PCOD.

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Curriculum Vitae

Jacqueline Dolfing werd geboren op 10 april 1969 te Amersfoort als jongste in een gezin met twee kinderen. Haar vader werd beroepshalve in 1974 overgeplaatst naar Duitsland toen Jacqueline nog kleuter was. In Bramsche behaalde zij in 1988 haar Duitse Abitur aan het Greselius Gymnasium.

Ze startte datzelfde jaar met de studie geneeskunde aan de Rijksuniversiteit te Groningen. Na haar co-schappen in Enschede verhuisde zij in augustus 1995 naar Den Haag alwaar zij een baan kreeg als fertiliteitsarts in het Diaconessenhuis Voorburg (dr. C.A.M. Jansen). Daar werd de basis voor dit proefschrift gelegd samen met dr. D.H. Schweitzer, endocrinoloog.

Op 1 december 1999 startte zij met de opleiding gynaecologie en verloskunde en werkte achtereenvolgens in het Martiniziekenhuis te Groningen (opleider, dr. W.F.A Mensink), het Universitair Medisch Centrum te Groningen (opleider Prof. dr. J.G. Aalders en later Prof. dr. M.J. Heineman) en het Deventer Ziekenhuis (opleider dr. P.J.Q. van der Linden). Tijdens de academische periode onderbrak zij op eigen initiatief de opleiding om gedurende een aantal maanden out of the box te kijken bij de pre-implantatie geneeskunde en fertiliteitsafdeling van de Vrije Universiteit te Brussel (Prof. dr. I. Liebaers, Prof. dr. A. Van Steirteghem en Prof. dr. P. Devroey).

Sedert 1 januari 2006 is zij full-time werkzaam als algemeen gynaecoloog in de Ziekenhuisgroep Twente te Hengelo en Almelo met als aandachtsgebied fertiliteit.

Zij is sinds 1999 getrouwd met Rob Neve. Samen hebben zij twee kinderen: Daniël (2005) en Josephine (2009).