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In women with polycystic ovary syndrome and obesity, loss of intra-abdominal fat is associated with resumption of ovulation

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BACKGROUND: It is not clear why some anovulatory women with polycystic ovary syndrome (PCOS) and obesity resume ovulation and others remain anovulatory after weight loss. The objective of this study was to compare the changes in body fat distribution and specifically intra-abdominal fat (IAF) and subcutaneous abdominal fat (SAF) between a group of anovulatory women with PCOS and obesity who resume ovulation (RO+) to those who remain anovulatory (RO−) during a lifestyle program.

METHODS: In a prospective pilot cohort study, anovulatory women with PCOS underwent a 6 month lifestyle program in a tertiary fertility clinic. Body fat distribution was assessed by anthropometrics, dual-energy X-ray absorptiometry (DEXA) and single slice abdominal CT scan at intake, after 3 months and after 6 months. Baseline-corrected changes over time were analysed using generalized estimating equations longitudinal regression analysis.

RESULTS: In 32 anovulatory women with PCOS (age, 28 ± 4 years; BMI, 37.5 ± 5.0 kg/m^2), there were no significant baseline differences in anthropometrics and biochemical assessment between 14 RO+ participants and 18 RO− participants. RO+ women lost more weight (6.3 versus 3.0%) and abdominal fat on DEXA (15.0 versus 4.3%) compared with RO− women. Resumption of ovulation was associated with early and consistent loss of IAF (12.4 versus 5.0% at 3 months and 18.5 versus 8.6% at 6 months). Loss of SAF between the RO+ women and the RO− women was similar at 3 months (6.2 versus 6.1%) but did not change any further in RO− women (6.1%) as it did in RO+ women (11.4%) at 6 months.

CONCLUSIONS: In anovulatory women with PCOS and obesity undergoing a lifestyle program, RO+ women lose more body weight and abdominal fat on DEXA than RO− women. In addition, this study shows that early and consistent loss of IAF is associated with resumption of ovulation. Future studies should address the mechanisms behind these changes and should assess interventions aimed at loss of IAF to facilitate resumption of ovulation.

Key words: obesity / infertility / anovulation / subcutaneous abdominal fat / intra-abdominal fat

Introduction

The high prevalence of overweight [body mass index (BMI): 25–29.9 kg/m²] and obesity (BMI $\geq$30 kg/m²) is significantly contributing to the overall burden of disease worldwide, and the effect on female reproduction is a growing additional concern (Haslam and James, 2005; Nelson and Fleming, 2007). Overweight and obesity in women is a main contributor to anovulation, with an exponential increase in anovulation with increasing body weight (Rich-Edwards et al., 1994; Green et al., 1988).

Increased abdominal fat accumulation (waist hip ratio >0.8 in women) contributes to reproductive dysfunction (Zaadstra et al., 1993; Wass et al., 1997; Pasquali et al., 2006) and these findings are unchanged after correcting for BMI. Abdominal fat accumulation is an indicator of higher metabolic risk profile because it is associated with insulin resistance (IR) (Despres et al., 2001). Hyperinsulinemia in women with obesity contributes to anovulation by increased ovarian androgen secretion (Pasquali et al., 2006; Bohler et al., 2010), leading to arrest of follicle growth (Willis et al., 1996).

Loss of abdominal fat, on the other hand, is associated with resumption of menstruation and ovulation in obese women with polycystic ovary syndrome (PCOS) undergoing a weight loss program (Huber-Buchholz et al., 1999; Thomson et al., 2008). It has been shown that resumption of ovulation in anovulatory women with obesity undergoing weight loss is mediated by improvement of IR and decrease in free androgen levels (Guzick et al., 1994; Holte et al., 1995; Pasquali et al., 2006). Abdominal fat consists of intra-abdominal fat (IAF) and subcutaneous abdominal fat (SAF). In study subjects with obesity who lost weight, loss of IAF had a greater beneficial effect on IR than loss of SAF (Park and Lee, 2005). The independent contribution of exercise to the improvement of IR and loss of IAF is not clear because only few studies tried to correct for diet and weight loss (Carroll and Dudfield, 2004; Christiansen et al., 2009; Hutchison et al., 2011).

It remains unexplained why some women remain anovulatory and others resume ovulation after weight loss. It can be hypothesized that specifically loss of IAF is required for improvement of IR, decease in androgen levels and resumption of ovulation. We tested this hypothesis by comparing the changes in body fat distribution, and especially IAF and SAF, in a group of obese anovulatory women with PCOS who resumed ovulation to those who remained anovulatory during a 6-month lifestyle program.

Materials and Methods

Population

In a pilot prospective cohort study, participants were recruited from women with obesity and infertility attending the Fertility Clinic of the University Medical Center Groningen (UMCG) between 2005 and 2008. All women with a BMI >29 kg/m² who met the inclusion criteria (infertility $\geq$1 year, age <38 years, partner with total motile sperm concentration /ejaculate $\geq$10 million) were asked to participate in a 6-month lifestyle program. The baseline data of this cohort of women before start of the lifestyle program were published previously as a comparison of body fat distribution characteristics between ovulatory and anovulatory infertile women (Kuchenbecker et al., 2010). In the present study, assessing changes during the lifestyle program, only anovulatory women with PCOS were included in the analysis.

Participants were diagnosed as PCOS according to the Rotterdam consensus diagnostic criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004) if two of the following criteria were identified: anovulation, hyperandrogenemia (clinically or biochemically) or polycystic ovary morphology on ultrasound and after other endocrine causes of anovulation were excluded. Hyperandrogenemia was defined by a serum testosterone level >3.5 nmol/l or a free testosterone level >62 pmol/l or when clinical hirsutism was present. No participant received hormonal medications or metformin in the 3 months preceding inclusion. All participants were Caucasian, except for one participant of Asian origin.

Women with amenorrhea (cycle interval $\geq$6 months) were considered anovulatory. Women with cycles $\leq$42 days in whom ovulation could not be confirmed by ultrasound monitoring, as well as women with menstrual cycles between 42 days and 6 months kept a basal body temperature (BBT) chart. Women with no BBT rise or a midluteal progesterone level $\leq$15 nmol/l, 1 week after a BBT rise, were considered anovulatory. In all study subjects, hyperprolactinemia, abnormal thyroid function, nonclassical 21-hydroxylase deficiency and an androgen-secreting tumor was excluded.

During the lifestyle program, all women kept a BBT chart and a midluteal progesterone level was assessed 1 week after a maintained temperature rise. Resumption of ovulation was defined as a midluteal progesterone level >15 nmol/l at least once, or by the occurrence of conception.

Participants who did not resume ovulation (RO−) and those who resumed ovulation (RO+) but did not conceive remained in the 6-month lifestyle program. Women who conceived did not continue the lifestyle program. Women who stopped the lifestyle program before the sixth month were considered to be drop-outs. All drop-outs were contacted by telephone 6 months after drop-out to inquire about self-reported weight loss and conception.

The study was approved by the Medical Ethical Committee of the UMCG and informed written consent was obtained from all subjects.

The lifestyle program of 6 months was based on the National Institute of Health evidence report of the treatment of obesity (National Heart, Lung and Blood Institute/National Institutes of Diabetes and Digestive and Kidney diseases, 1998) and consisted of a combination of dietary advice, increased physical activity and behaviour modification.

Anthropometric assessment

Body weight (kg), height (cm), BMI [=weight (kg) divided by height in meters²], waist circumference (Wc = waist measured at the narrowest part of the torso located between the lower rib and the iliac crest), a whole body DEXA scan (DEXA) and a single slice abdominal CT scan (ssCT) were measured at baseline. All measurements were carried out by the same observer. Bodyweight was recorded every 2 weeks and the Wc, DEXA and the ssCT were measured again at month 3 and month 6 of the lifestyle program.

Total, trunk and abdominal fat mass was measured by DEXA, using a Hologic A Discovery Bone Densitometer (Hologic, Inc., Bedford, MA, USA). Total fat mass was determined for the whole body, and by using computer programming, two regions (trunk and abdominal slice) were specified and measured as mentioned in previous studies (Carmina et al., 2007).

The ssCT consisting of three to four subslices was performed at the level of the umbilicus for the measurement of the IAF and the SAF (Seidell et al., 1990). The technique used to measure IAF and SAF was published previously (Kuchenbecker et al., 2010). In short, the IAF and SAF volume (cm³) was calculated by multiplying the IAF and SAF area

(cm^2) of each subslice with the subslice thickness (cm), and the mean volume (cm^3) of the subslices was recorded for analysis. All measurements were performed by a single observer.

Pregnancy was excluded before each DEXA and ssCT scan by a urine pregnancy test.

Biochemical assessment

Total testosterone, sex hormone-binding globulin (SHBG) and insulin were measured after an overnight fast of 10 h at intake, after 3 months and after 6 months of the lifestyle program. The formula according to Vermeulen (Vermeulen et al., 1999) was used to calculate free testosterone. The exact measurement techniques used for the biochemical assessments were published previously (Kuchenbecker et al., 2010).

Lifestyle program

All participants received individualized dietary advice aiming for reduction in calorie intake of at least 500 kilocalories/day, but avoiding a total calorie intake <1200 kilocalories/day. An individualized exercise program was tailored to the ability and personal and social circumstances of each participant. Each participant kept a diet record and carried a pedometer SW-200 (New Lifestyles, Lee's Summit, USA) to record the amount of steps over 2-week periods during the 6 months program. Individual guidance by a nurse practitioner consisted of visits every 2 weeks, during which body weight was measured and compliance was assessed by evaluating the diet and pedometer records completed at home. In addition, the BBT chart was evaluated and a serum midluteal progesterone level assessment was scheduled if appropriate. By means of motivational counseling techniques, problems of and resistance to lifestyle changes and behavior modification were addressed and advice was given (Levensky et al., 2007).

Statistical analysis

At the end of the lifestyle program, the participants were divided into those who had resumed ovulation (RO+) and those who had not resumed ovulation (RO−) and post hoc comparison was performed between the two groups at baseline. The drop-outs were contacted by telephone 6 months after drop-out. If no further weight loss or conception after drop-out was reported, the drop-outs were analysed as part of the RO− group until the time of drop-out.

Between-group comparisons were performed using an independent samples Student's t-test for normally distributed continuous variables or a Mann–Whitney U-test when the distribution was skewed. Normal distribution was tested using the Kolmogorov–Smirnov test. Changes over time were analysed using generalized estimating equations (GEE) longitudinal regression analysis with time, ovulatory status and an interaction term of ovulatory status and time as the predictors. GEE makes maximum use of the available measurements in a longitudinal design without the limitations of complete case analysis. For interventions such as this lifestyle program, complete case analysis does not reflect a real-life scenario and poses the risk of negative selection since only those who do not conceive or resume ovulation remain in the program. Baseline correction was performed by including the measurement at T = 0 of the respective parameter as a covariate in the GEE analysis. Participants remained in the GEE analysis until conception or drop-out. For this pilot study, no sample size calculation could be performed. All analyses were performed using SPSS, versions 16 and 17 (SPSS, Inc., Chicago, IL). Differences or effects were considered statistically significant if P < 0.05.

Results

Clinical characteristics of participants

Thirty two anovulatory women with PCOS and obesity were included in the lifestyle program and their baseline data were recorded. During the first 3 months, the four women that conceived and four drop-outs left the program, while another five women who resumed ovulation continued the lifestyle program, therefore leaving 24 participants to measure and record the data at 3 months. Out of the 24 participants continuing the second 3 months of the lifestyle program, the three women who conceived and six drop-outs left the program, leaving 15 participants to measure and record the data at 6 months.

None of the 10 drop-outs reported any further weight loss or conception in the 6 months after drop-out. They were therefore analyzed as part of the RO− group. To ensure that the anthropometric and biochemical measurements of the drop-outs did not differ from the participants who completed the study, we performed a comparison of the baseline characteristics between both groups (Table I). There was no difference in age, BMI and anthropometric assessment between the drop-outs and the participants who completed the study. Except for significantly higher free testosterone and total

Table I Baseline characteristics of anovulatory women with PCOS and obesity undergoing a 6-month lifestyle program: comparison of the 10 drop-outs with the participants who completed the lifestyle program.

Parameter	Completed (n = 22)	Drop-out (n = 10)	P-value
Age (years)[a]	28.9 ± 4.1	27.5 ± 2.9	0.35
BMI (kg/m^2)[a]	37.8 ± 5.2	36.7 ± 4.3	0.56
Waist circumference (cm)[a]	114 ± 13	110 ± 9	0.36
Total fat DEXA (kg)[a]	47.6 ± 10.1	42.7 ± 9.7	0.21
Trunk fat DEXA (kg)[a]	23.8 ± 5.8	21.7 ± 4.9	0.34
Abdomen fat DEXA (kg)[a]	4.7 ± 1.3	3.9 ± 1.3	0.14
IAF ssCT (cm^3)[a]	197 ± 61	195 ± 51	0.96
SAF ssCT (cm^3)[a]	1023 ± 183	971 ± 223	0.50
Testosterone (pmol/l)[b]	3.7 (1.6–6.1)	4.7 (1.6–6.5)	0.04*
SHBG (nmol/l)[b]	22 (10–38)	20 (8–36)	0.72
Free testosterone (pmol/l)[b]	87 (32–169)	116 (32–217)	0.04*
Fasting insulin (pmol/l)[b]	170.3 (25.8–602.7)	193.0 (25.8–595.2)	0.80

Data expressed as [a]mean ± SD using an independent sample Student's t-test or as [b]median, minimum and maximum using a Mann–Whitney U-test.
*Significant at level P < 0.05.

Table II Characteristics of 32 anovulatory women with PCOS and obesity undergoing a lifestyle program divided into those who resumed ovulation (RO+) and those who remained anovulatory (RO−) at the end of 6 months.

	Baseline			Month 3		Month 6	
	RO+ (n = 14)	RO− (n = 18)	*P*-value	RO+ (n = 10)	RO− (n = 14)	RO+ (n = 7)	RO− (n = 8)
Age (years)[a]	29.6 ± 4.0	27.5 ± 3.4	0.11				
BMI (kg/m²)[a]	37.7 ± 4.5	37.3 ± 5.4	0.83	35.4 ± 4.8	37.3 ± 5.7	32.2 ± 4.5	36.1 ± 7.4
Waist circumference (cm)[a]	114 ± 13	112 ± 11	0.59	108 ± 15	112 ± 13	103 ± 8	112 ± 19
Total fat DEXA (kg)[a]	47.7 ± 8.8	44.8 ± 11.0	0.43	43.9 ± 9.9	45.9 ± 10.2	38.5 ± 7.8	44.6 ± 13.1
Trunk fat DEXA (kg)[a]	23.4 ± 5.2	22.9 ± 5.8	0.83	21.2 ± 5.3	22.8 ± 5.2	18.4 ± 3.9	22.7 ± 6.7
Abdomen fat DEXA (kg)[a]	4.6 ± 1.4	4.4 ± 1.4	0.58	4.2 ± 1.5	4.5 ± 1.4	3.6 ± 1.3	4.6 ± 1.5
IAF ssCT (cm³)[a]	191 ± 41	201 ± 69	0.63	156 ± 26	206 ± 70	133 ± 19	192 ± 94
SAF ssCT (cm³)[a]	1024 ± 187	991 ± 205	0.65	924 ± 215	971 ± 170	847 ± 235	957 ± 241
Total testosterone (pmol/l)[b]	3.6 (1.6−5.5)	4.3 (2.8−6.5)	0.27	2.9 (2.3−3.8)	3.5 (2.4−6.0)	3.6 (3.3−3.9)	3.8 (2.4−5.0)
SHBG (nmol/l)[b]	23 (10−43)	20 (8−36)	0.33	22 (14−31)	23 (12−39)	31 (19−47)	19 (15−26)
Free testosterone (pmol/l)[b]	84 (32−169)	107 (69−217)	0.05	68 (48−95)	80 (49−117)	71 (54−81)	95 (58−137)
Fasting insulin (pmol/l)[b]	171 (26−603)	183 (79−596)	0.56	155 (72−237)	183 (79−545)	117 (108−151)	159 (86−215)
Pedometer (steps/day)	7609 ± 2313	6373 ± 2873	0.22	9633 ± 2505	7489 ± 3406	10528 ± 2109	9024 ± 2417

Comparison of baseline characteristics and characteristics of RO+ and RO− women at month 3 and month 6 are given.
Data expressed as [a]mean ± SD were analysed using the independent samples Student's *t*-test; data expressed as [b]median, minimum and maximum were analysed using the Mann–Whitney *U*-test.

testosterone level in the drop-outs (*P* = 0.04), there were no baseline differences in the biochemical assessments between the drop-outs and the participants who completed the study.

The baseline data of the RO+ (*n* = 14) and RO− (*n* = 18) women are shown in Table II. There was no difference in age, BMI, anthropometric- and biochemical assessment at baseline between the RO+ and RO− women.

Changes during the lifestyle program

At the end of the lifestyle program, the participants were divided into those who had resumed ovulation (RO+) and those who had not resumed ovulation (RO−) and GEE analysis was performed on the two groups. The unadjusted results of the measurements of the two groups at baseline, month 3 and month 6 are presented in Table II.

The results of the GEE analyses of changes over time in body weight and abdominal fat on DEXA are presented in Fig. 1. Since GEE was performed with correction for baseline the values differ from those in Table II. The total group of RO+ women lost more weight (6.3 versus 3.0% at 6 months, *P* = 0.018, Fig. 1a) and abdominal fat on DEXA (15.0 versus 4.3% at 6 months, *P* = 0.025, Fig. 1b) compared with the total group of RO− women. Figure 2 shows that the RO+ women lost more IAF on ssCT between baseline and month 3 (12.4 versus 5.0%, *P* = 0.002, Fig. 2a) and baseline and month 6 (18.5 versus 8.6%, *P* = 0.005, Fig. 2a) compared with the RO− women. For SAF on ssCT, on the other hand, changes for RO+ women and RO− women were similar between baseline and month three (6.2 versus 6.1%, *P* = 0.946, Fig. 2b). Due to continued loss of SAF after 3 months in RO+ women, there was a difference in loss of SAF

between baseline and 6 months (11.4 versus 6.1%, *P* = 0.031, Fig. 2b) compared with RO− women.

GEE analysis showed a non-significant decrease of fasting insulin (*P* = 0.194) and free testosterone (*P* = 0.086) in the RO+ women compared with the RO− women. The increase in pedometers steps in RO+ women compared with RO− women did not reach statistical significance.

Discussion

This study shows that in anovulatory women with PCOS and obesity participating in a lifestyle program, resumption of ovulation was associated with more weight loss and loss of abdominal fat on DEXA. Resumption of ovulation was associated with early and consistent loss of IAF.

In anovulatory women, >5% loss of body weight is required for resumption of ovulation (Kiddy *et al.*, 1992; Guzick *et al.*, 1994; Clark *et al.*, 1995; Holte *et al.*, 1995; Huber-Buchholz *et al.*, 1999). Previous studies using DEXA to quantify the changes in abdominal fat in women with PCOS undergoing a weight loss program indicated that loss of abdominal fat is associated with resumption of ovulation (Huber-Buchholz *et al.*, 1999; Thomson *et al.*, 2008). The present study confirms these findings, since the RO+ women lost 6.3% of their body weight and 15.0% abdominal fat on DEXA during the lifestyle program, as compared with 3.0% loss of body weight and 4.3% loss of abdominal fat on DEXA in RO− women. Compared with RO− women, RO+ women lost on average 4.0 kg more body weight and 0.5 kg more abdominal fat on DEXA.
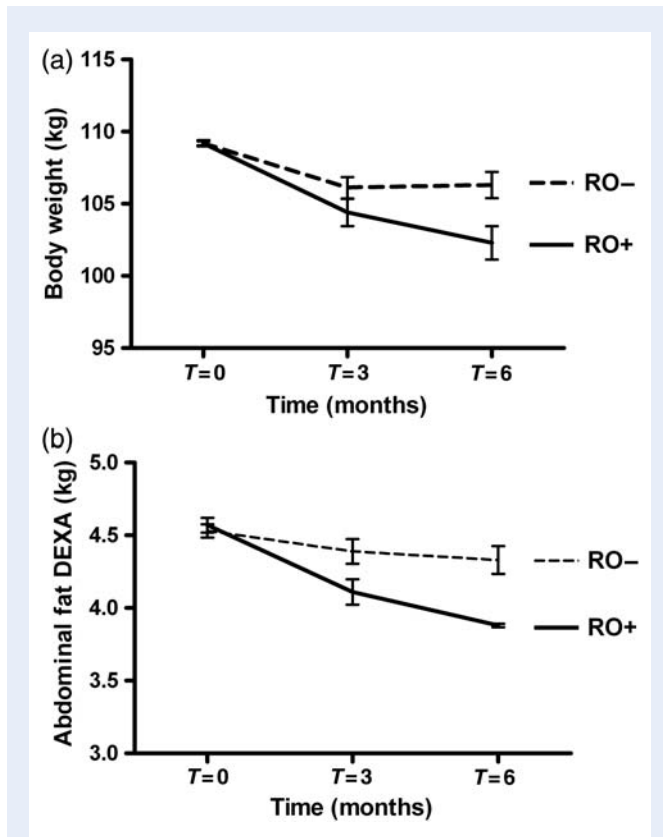
Figure 1 Baseline corrected GEE analysis of 32 anovulatory women with PCOS and obesity undergoing a lifestyle program with *post hoc* allocation of participants into those who resumed ovulation (RO+) and those who remained anovulatory (RO−) at the end of 6 months shows a difference in loss of body weight ($P = 0.018$) (a) and abdominal fat on DEXA ($P = 0.025$) and (b) between RO+ (solid line) and RO− (dashed line) participants. Error bars indicate standard errors of GEE estimates.
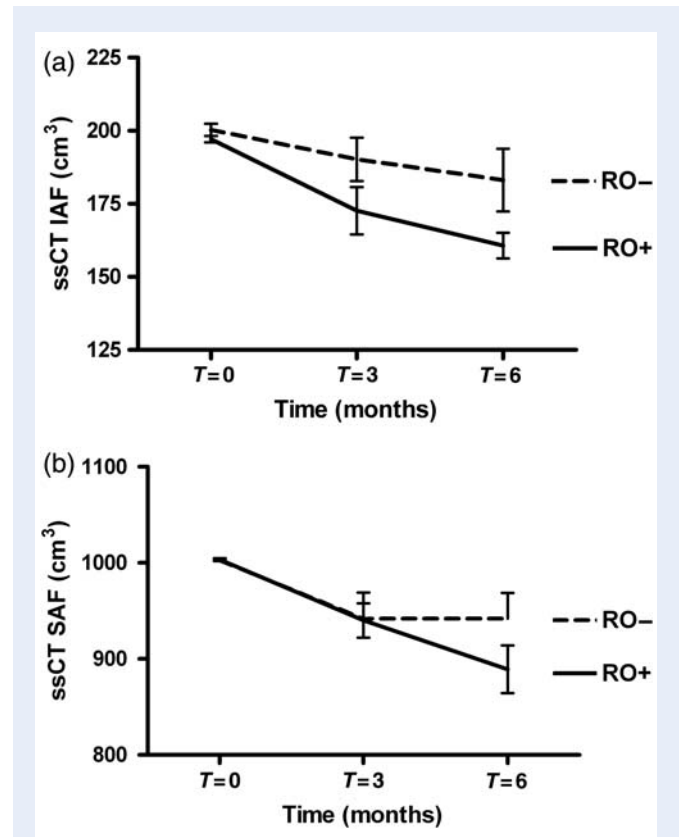

Figure 2 Baseline-corrected GEE analysis of 32 anovulatory women with PCOS and obesity undergoing a lifestyle program with *post hoc* allocation of participants into those who resumed ovulation (RO+) and those who remained anovulatory (RO−) at the end of 6 months show a difference in loss of IAF from month 0 to month 3 ($P = 0.002$) (a) and from month 0 to month 6 ($P = 0.005$) (a), and difference in loss of SAF from month 0 to month 3 ($P = 0.946$) (b) and from month 0 to month 6 ($P = 0.031$) and (b) between RO+ (solid line) and RO− (dashed line) participants. Error bars indicate standard errors of GEE estimates.

Moreover, our study shows that early and consistent loss of IAF over a 6-month period is associated with resumption of ovulation. Only after more than 3 months of lifestyle intervention, RO+ women started losing more SAF than RO− women. Previous studies in different patient populations have shown that during the initial period of calorie restriction, preferential loss of IAF occurs which correlates significantly with improvement in IR (Goodpaster et al., 1999; Park and Lee, 2005). Loss of IAF and not SAF during initial calorie restriction, can be explained by increased lipolysis of IAF due to its higher metabolic activity (Smith and Zachwieja, 1999). After surgical removal of IAF, IR has been shown to improve (Thorne et al., 2002), while removal of SAF by liposuction does not significantly alter IR (Klein et al., 2004). Based on the above-mentioned mechanism, the early and consistent loss of IAF in RO+ women might have contributed to improvement in IR and resumption of ovulation. We could not confirm the expected difference in fasting insulin levels between RO+ and RO− women in our study, which may be due to the high average BMI in our population. Moderate weight loss in women with BMI >35 kg/m^2 does not always lead to the same decrease in fasting insulin levels as in women with lower BMI,

as shown in a previous study in women with PCOS and obesity (Tang et al., 2006).

We have previously shown that in women with obesity and infertility, anovulatory women have significantly more SAF and higher fasting insulin levels and the same amount of IAF compared with ovulatory women with the same BMI (Kuchenbecker et al., 2010). A possible explanation for an increased volume of SAF in anovulatory women with obesity could be provided by the concept of a critical IAF threshold. This concept suggests that during constant high calorie food consumption, storage of fat in IAF reaches a point of saturation, after which fat is shunted to the subcutaneous fat compartments (Freedland, 2004). With increased accumulation of SAF, inflammatory changes and increase in adipocyte size occur, limiting the SAF storage capacity and contributing to the antilipolytical effects of insulin. Fat is then shunted from SAF to the liver and skeletal muscle which contributes to increased IR (Weiss, 2007; Koska et al., 2008). During a period of reduced calorie intake, fat is rapidly mobilized from IAF, liver fat and skeletal muscle fat due to the higher lipolytical activity of these fat

compartments. This mobilization of fat from IAF, liver fat and skeletal muscle fat leads to improved IR (Goodpaster *et al.*, 1999; Freedland, 2004). This mechanism could also explain the findings of the present study that early and consistent loss of IAF and not SAF was associated with resumption of ovulation.

Previous studies suggest that exercise contributes to loss of IAF (Kay and Fiatarone Singh, 2006; Ohkawara *et al.*, 2007) while another study could not show an independent effect of exercise on loss of IAF (Christiansen *et al.*, 2009). In anovulatory women with PCOS, a structured exercise program showed higher ovulation rates and improvement in IR compared with a diet program alone (Palomba *et al.*, 2008). A more recent study showed that exercise was associated with loss of IAF and improvement in IR in PCOS women, despite weight maintenance. Using the euglycemic hyperinsulinemic clamp technique, no significant correlation was found, however, between improvement in IR and loss of IAF (Hutchison *et al.*, 2011). Exercise might also improve IR by increasing muscle mass and enhancing glucose disposal in skeletal muscle (Carroll and Dudfield, 2004; Thomson *et al.*, 2008). In the present study, we cannot exclude that the non-significant increase in pedometer steps might have contributed to increased loss of IAF and resumption of ovulation in the RO+ women compared with RO− women.

As shown in previous studies, resumption of ovulation during weight loss is associated with a decrease in fasting insulin and free testosterone levels (Guzick *et al.*, 1994; Pasquali *et al.*, 2006). In addition, weight loss with improvement in IR, and therefore lower insulin levels, leads to less androgen production by the ovarian theca cells and more SHBG production in the liver (Poretsky, 1991). Lower free androgen levels in the long term, limits the amount of abdominal fat accumulation (Pasquali *et al.*, 2006; Bohler *et al.*, 2010). In our study, we found a decrease in free testosterone in the RO+ women that was not statistically significant compared with RO− women. This might be due to small numbers in our study.

This study shows that in anovulatory women with PCOS and obesity participating in a lifestyle program, resumption of ovulation is associated with early and consistent loss of IAF. The most likely mechanism of resumption of ovulation after loss of IAF during a lifestyle program is improvement of IR and lower free androgen levels but other mechanisms should also be considered. Women with anovulatory PCOS have altered adipocytokine secretion (Carmina *et al.*, 2009) and changes in the adipocytokine secretion due to loss of IAF can also be considered as a mechanism of resumption of ovulation, as these substances also have direct effects on the ovary (Mitchell *et al.*, 2005). Future studies should try to evaluate interventions aimed at loss of IAF and should try to explore the mechanism involved in resumption of ovulation due to loss of IAF.

The present study was a pilot to investigate the feasibility of a lifestyle program in a tertiary fertility center. In spite of personal guidance by a nurse practitioner using motivational interviewing techniques, there was a high drop-out rate of 31.3%. Our drop-out rate is in agreement with the rates of 27−35% reported in previous studies on lifestyle intervention in comparable patient populations (Clark *et al.*, 1995; Hoeger *et al.*, 2004; Palomba *et al.*, 2008). Based on follow-up data until 6 months after drop-out, we decided to allocate the drop-outs to the RO− group until the moment of drop-out. This decision was based on the fact that resumption of ovulation is highly unlikely in anovulatory women with obesity who do not lose weight (Clark *et al.*, 1995). We can however not exclude the possibility that women in the drop-out group had occasional ovulation in spite of no further weight loss. The high drop-out rate deserves further evaluation in order to improve the effectiveness of lifestyle programs. If predictive factors for drop-out in this patient population could be indentified (Teixeira *et al.*, 2005), specific personal guidance and intervention should be developed to improve motivation and adherence to diet—and exercise programs to achieve weight loss.

In conclusion, this study indicates that anovulatory women with PCOS and obesity who resume ovulation during a lifestyle program lose 4 kg (3.3%) more weight and 0.5 kg (10.7%) more abdominal fat on DEXA than the women who remain anovulatory. Moreover, early and consistent loss of IAF is associated with resumption of ovulation.

Future larger studies on lifestyle interventions in anovulatory women with PCOS should aim to confirm our findings and to evaluate the mechanisms of resumption of ovulation. Trials should be designed to assess the combination of diet and structured exercise programs aimed at loss of IAF and improvement of IR for resumption of ovulation, thereby decreasing the number of infertile women requiring ovulation induction.

Authors' roles

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