Lifestyle intervention in obese infertile women

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Effectiveness of lifestyle intervention in subgroups of obese infertile women: a subgroup analysis of a RCT

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Abstract

Study question: Do age, ovulatory status, severity of obesity and body fat distribution affect the effectiveness of lifestyle intervention in obese infertile women?

Summary answer: We did not identify a subgroup in which lifestyle intervention increased the healthy live birth rate however it did increase the natural conception rate in anovulatory obese infertile women.

What is known already: Obese women are at increased risk of infertility and are less likely to conceive after infertility treatment. We previously demonstrated that a 6-month lifestyle intervention preceding infertility treatment did not increase the rate of healthy live births (vaginal live birth of a healthy singleton at term) within 24 months of follow-up as compared to prompt infertility treatment in obese infertile women. Natural conceptions occurred more frequently in women who received a 6-month lifestyle intervention preceding infertility treatment.

Study design, size, duration: This is a secondary analysis of a multicenter RCT (randomized controlled trial), the LIFEstyle study. Between 2009 and 2012, 577 obese infertile women were randomly assigned to a 6-month lifestyle intervention followed by infertility treatment (intervention group) or to prompt infertility treatment (control group). Subgroups were predefined in the study protocol, based on frequently used cut-off values in the literature: age (≥ 36 or < 36 years), ovulatory status (anovulatory or ovulatory), BMI (≥ 35 or < 35 kg/m^2) and waist-hip (WH) ratio (≥ 0.8 or < 0.8).

Participants/materials, setting, methods: Data of 564 (98%) randomized women who completed follow-up were analyzed. We studied the effect of the intervention program in various subgroups on healthy live birth rate within 24 months, as well as the rate of overall live births (live births independent of gestational age, mode of delivery and health) and natural conceptions conceived within 24 months. Live birth rates included pregnancies resulting from both treatment dependent and natural conceptions. Logistic regression models with randomization group, subgroup and the interaction between randomization group and subgroup were used. Significant interaction was defined as a P-value <0.1.

Main results and the role of chance: Neither maternal age, ovulatory status nor BMI had an impact on the healthy live birth rate within 24 months, nor did they influence the overall live birth rate within 24 months after randomization. WH ratio showed a significant interaction with the effect of lifestyle intervention on healthy live birth rate ($P = 0.05$), resulting in a lower

healthy live birth rate in women with a WH ratio <0.8. WH ratio had no interaction regarding overall live birth rate ($P = 0.27$) or on natural conception rate ($P = 0.38$). In anovulatory women, the effect of lifestyle intervention resulted in more natural conceptions compared to ovulatory women (P - value for interaction = 0.02). There was no interaction between other subgroups and the effect of the intervention on the rate of natural conception.

Limitations, reasons for caution: Since this was a subgroup analysis of a RCT and sample size determination of the trial was based on the primary outcome of the study, the study was not powered for analyses of all subgroups.

Wider implications of the findings: Our finding that lifestyle intervention leads to increased natural conception in anovulatory obese women could be used in the counseling of these women, but requires further research using an appropriately powered study in order to confirm this result.

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Introduction

The prevalence of obesity, defined as a BMI ≥ 30 kg/m^2, in women of reproductive age ranges between 4% and 21% in European countries[1] and is 32% in the United States.[2] Obesity impairs reproductive health in both men and women[3], partly due to an increased incidence of anovulation and polycystic ovarian syndrome (PCOS) in obese women.[4] Also, in obese women with an ovulatory cycle, the time to conceive is longer than in ovulatory women of normal weight.[5]

Obesity poses additional challenges to women seeking infertility care and to their healthcare providers. Obese women may require higher doses of clomiphene citrate or FSH to induce ovulation or to hyperstimulate the ovaries.[6,7] In addition, obese women show lower birth rates after infertility treatment and increased miscarriage rates compared to lean women. [8,9] The impact of a woman's BMI on the costs of infertility treatment is still a matter of debate. [10,11] Pregnant obese women are at an increased risk of hypertensive pregnancy disorders, gestational diabetes, preterm birth and increased perinatal mortality and morbidity.[12-14]

The lower efficacy of infertility treatment and an increased complication rate during pregnancy in obese women has sparked a global discussion on whether infertility treatment should be restricted to women under a certain BMI.[15,16] Weight loss prior to infertility treatment in obese women is currently advised in the British and American guidelines of

reproductive medicine,[17,18] however evidence of the effectiveness of weight loss prior to infertility treatment is still limited.

We recently showed in a RCT (randomized controlled trial),[19] that a 6-month lifestyle intervention in obese infertile women preceding infertility treatment neither increased the rates of vaginal birth of healthy singletons at term nor the rates of live births compared to women who received prompt infertility treatment. Maternal and neonatal complication rates did not differ between groups. However, there were significantly more naturally conceived ongoing pregnancies in women who received lifestyle intervention preceding infertility treatment.

The LIFEstyle study included a heterogeneous population of obese infertile women. We hypothesize that the effect of lifestyle intervention might be different in subgroups of women included in the LIFEstyle study. In this manuscript, we investigate whether age, ovulatory status, severity of obesity and body fat distribution affect the effectiveness of lifestyle intervention in obese infertile women.

Materials and methods

This study consists of analyses of several predefined subgroups of patient and outcome data from a multicenter RCT, the LIFEstyle study. The study protocol and main results have been reported previously.[19,20]

The LIFEstyle study

In short, obese infertile women between 18 and 39 years of age were randomized between a 6-month lifestyle intervention preceding infertility treatment (intervention group) or prompt infertility treatment (control group). A total of 577 infertile women with a BMI ≥ 29 kg/m^2 were randomized (290 women were allocated to the intervention group and 287 to the control group). A couple was considered infertile if the woman had chronic anovulation or when the woman had ovulatory cycles and the couple had tried to conceive for at least 12 months. Chronic anovulation was classified as either normogonadotrophic anovulation (World Health Organization, WHO class II) or hypogonadotrophic hypogonadism (WHO class I).[21] Within the WHO II anovulatory women, PCOS was diagnosed using the Rotterdam criteria.[22] Women with anovulation due to hypergonadotrophic hypogonadism (WHO class III) were excluded from the study.

The lifestyle intervention consisted of a 6-month structured program aiming at a weight loss of 5-10% of the original body weight. It included six structured outpatient visits and four

telephone consultations with a pre-trained intervention coach. Daily dietary energy intake was reduced by 600 kcal and was maintained at a minimum of 1200 kcal/day. Physical activity was stimulated to a level of 10 000 steps a day and at least 30 minutes of exercise two to three times a week. Behavioral changes were facilitated by motivational counseling. After the 6-month program or when the weight loss of 5-10% had been achieved, women started with appropriate infertility treatment if they were not yet pregnant. Women in the control group started with appropriate infertility treatment immediately after randomization. Appropriate infertility treatment was based on the Dutch reproductive medicine guidelines[23] and could consist of expectative management, ovulation induction, intrauterine insemination, IVF or ICSI depending of the diagnosis the couple received after the infertility workup.

Women were followed for 24 months after randomization. If a woman conceived within 24 months but her pregnancy ended thereafter, monitoring was continued until the end of pregnancy.

Subgroup assignment

In the protocol of the LIFEstyle study, subgroup analyses were predefined, based on frequently used cut-off values in the literature: age (≥ 36 years versus < 36 years), anovulatory versus ovulatory status, BMI (≥ 35 kg/m^2 versus < 35 kg/m^2) and waist-hip (WH) ratio (≥ 0.8 versus < 0.8). All of the subgroup variables were determined at baseline. For the current analyses, women were assigned to these subgroups and outcomes were calculated and tabulated. In addition, the interaction between subgroup parameters and effect of the lifestyle intervention was explored further by categorizing women in quartiles according to their age, BMI and WH ratio and repeating the analyses. The cut-off points of the quartile distribution are shown in Supplementary Table S1.

Outcomes

Three main outcomes were analyzed. The first, was the primary outcome of the LIFEstyle study, i.e. the vaginal birth rate of healthy singletons at term within 24 months after randomization, referred to here as 'healthy live births'. A child was considered healthy if it was born alive at term without major congenital anomalies. The second was overall live birth rate (live births independent of gestational age, mode of delivery and health) following conception within the 24-month follow-up period (the delivery could be after 24 months) and, the third was natural conception rate, defined as natural conceptions of an ongoing pregnancy conceived within 24 months after randomization. Ongoing pregnancy was defined as a viable pregnancy of at least 10 weeks gestation. Live birth rates included pregnancies resulting from both treatment dependent and natural conceptions.

Statistical analysis

The baseline characteristics of women were summarized for each subgroup using frequencies and rates for categorical data, and means (for normally distributed data) or medians (for non-normally distributed data) with SDs or interquartile ranges for continuous variables. Differences between baseline variables in the intervention and control group in the particular subgroups were assessed using the Student's *t*-test, Mann Whitney *U* or Chi square test where appropriate.

The outcomes in the subgroups were calculated as rates, percentages and crude (cOR) and adjusted odds ratios (aOR) with the accompanying 95% CI. We adjusted for maternal age (years), baseline BMI (kg/m^2), smoking (yes/no), ovulatory status (anovulatory/ovulatory), duration of infertility (months), nulliparity (yes/no) and non-completion of the intervention program (yes/no). Logistic regression analyses with randomization group (intervention or control group), subgroup and the interaction between randomization group and subgroup were performed with adjustment for confounders. A significant interaction between a subgroup factor and the lifestyle intervention was defined as a *P*-value <0.1. Interaction effects were visually presented as forest plots of aORs and respective CIs for each subgroup. Analyses were performed using the Statistical Package for the Social Sciences version 22 (IBM Corporation, Armonk, NY, USA), graphs were produced using GraphPad Prism (Graphpad Software, Inc., La Jolla, CA, USA).

Ethical approval

The study protocol of the LIFEstyle study was approved by the Medical Ethics Committee (MEC; 2008.284) of the University Medical Centre Groningen and the board of directors of each participating center. Written informed consent was obtained for all women who agreed to participate in the study. The LIFEstyle study was registered in the Dutch trial register (NTR 1530).

Results

Between June 2009 and June 2012 we randomized 577 women, of whom 290 women were randomized in the lifestyle intervention preceding infertility treatment arm (nine were lost to follow-up and one withdrew informed consent) and 287 women to the prompt infertility treatment arm (one was lost to follow-up and two withdrew informed consent). So, in total data of 280 women in the intervention group and data of 284 women in the control group were available for analysis.

Baseline characteristics

The number of anovulatory women ≥ 36 years in the intervention group was significantly lower than the number of anovulatory women ≥ 36 years in the control group. Women < 36 years in the intervention group had a significantly longer duration of infertility than in the control group. Anovulatory women in the intervention group were significantly more often nulliparous than anovulatory women in the control group (Table 1). Women with a BMI ≥ 35 kg/m^2 in the intervention group tried to conceive significantly longer, they were less often anovulatory and they were more often diagnosed with unexplained infertility than women with a BMI ≥ 35 kg/m^2 in the control group. Women with a WH ratio ≥ 0.8 in the intervention group were less often anovulatory than in the control group. Women with a WH ratio < 0.8 in the intervention group were more often Caucasian and were more often anovulatory than women in the control group (Table 2). Women did not differ substantially in any other respect. Rate of non-completers of the intervention ranged from 17.6% to 22.1% in the different subgroups.

Healthy live birth rate

Within 24 months after randomization, the frequency of healthy live births was lower in the total intervention group compared to the control group: 76 (27.1%) versus 100 (35.2%) (OR 0.69, 95% CI 0.48-0.98, Table 3). There were no significant differences between intervention and control groups in the healthy live birth rate in women ≥36 or <36 years, with anovulatory or ovulatory status or in women with a BMI ≥ 35 or < 35 kg/m^2. In women with a WH ratio < 0.80 the frequency of healthy live births was lower in the intervention group (4/30, 13.3%) compared to the control group (18/43, 41.9%). However this difference disappeared after adjusting for confounders (aOR 0.28, 95% CI 0.08-1.04). WH ratio showed a significant interaction with the effect of lifestyle intervention on healthy live birth rate (P = 0.05). This resulted in a lower healthy live birth rate in women with a WH ratio < 0.8 who received the lifestyle intervention compared to women with a WH ratio ≥ 0.8 who received the lifestyle intervention. The effect of the intervention on the rate of the healthy live births was not altered by maternal age, ovulation status or BMI. There were no linear trends in the ORs by quartiles of age, BMI and WH ratio (Supplementary Table S2).

Overall live birth rate

The overall rate of live births conceived within 24 months after randomization were comparable in the total randomized intervention and control group (OR 0.82 95% CI 0.59-1.14, Table 4). The cOR and aOR of overall live births were not significantly different between the intervention and control group in any of the subgroups. In addition, the effect of the lifestyle intervention on overall live births was not altered by maternal age, ovulation status, BMI or WH ratio, nor were any linear trends in OR observed by quartiles of age, BMI and WH ratio (Supplementary Table S3).

Table 1. Baseline characteristics of women according to age and ovulatory status.

Characteristic	Age ≥36 years		Age <36 years		Anovulatory women		Ovulatory women	
	Intervention (n=34)	Control (n=29)	Intervention (n=246)	Control (n=255)	Intervention (n=123)	Control (n=140)	Intervention (n=157)	Control (n=144)
Age, mean (SD), years	37.3 (0.9)	37.1 (0.9)	28.8 (3.8)	28.9 (4.0)	28.3 (4.1)	28.6 (4.5)	31.0 (4.5)	30.9 (4.3)
Caucasian[a], No (%)	30 (88)	22 (76)	219 (89)	224 (87.8)	112 (91)	121 (86)	137 (87)	125 (87)
BMI, median (IQR), kg/m^2	36.9 (34.3-38.9)	37.0 (33.5-38.3)	36.0 (33.4-38.6)	36.0 (33.4-38.6)	36.3 (33.2-38.6)	36.8 (34.5-38.8)	36.3 (33.4-38.8)	35.4 (33.1-38.0)
Education, No (%)								
Primary school (4-12 years)	2 (5.8)	1 (3.4)	14 (5.7)	8 (3.1)	6 (4.9)	5 (3.6)	10 (6.4)	4 (2.8)
Secondary Education	12 (35)	8 (28)	55 (22)	55 (22)	30 (24)	35 (25)	37 (24)	28 (19)
Intermediate Vocational Education	11 (32)	13 (45)	118 (48)	118 (46)	57 (46)	63 (45)	72 (46)	68 (47)
Higher Vocational Education and University	8 (24)	5 (17)	47 (19)	64 (25)	21 (17)	31 (22)	34 (22)	38 (26)
Current smoker, No (%)	7 (21)	4 (14)	65 (26)	56 (22)	35 (29)	28 (20)	37 (24)	32 (22)
Nulliparous, No (%)	21 (62)	16 (55)	197 (80)	199 (78)	104 (85)[§]	101 (72)[§]	114 (73)	114 (79)
Trying to conceive since, median (IQR), months	35 (16-49)	38 (21-67)	22 (14-34)[§]	18 (13-29)[§]	16 (11-31)	15 (11-24)	24 (18-40)	24 (16-38)
Diagnostic category of infertility, No (%)*								
Anovulation	6 (18)[§]	12 (41)[§]	117 (48)	128 (50)	123 (100)	140 (100)	0	0
-of which PCOS [b]	4 (67)	8 (67)	89 (76)	96 (75)	93 (76)	104 (74)	0	0
Unexplained	15 (44)	9 (31)	69 (28)	68 (27)	0	0	84 (54)	77 (53)
Male factor	10 (29)	5 (17)	55 (22)	59 (23)	7 (5.7)	13 (9.3)	58 (37)	51 (35)
Tubal factor	3 (8.8)	4 (14)	9 (3.7)	12 (4.7)	1 (0.8)	3 (2.1)	11 (7.0)	13 (9.0)
Other	2 (5.9)	1 (3.4)	6 (2.4)	6 (2.4)	0	1 (0.7)	8 (5.1)	6 (4.2)
Non-completers intervention	6 (17.6)	NA	52 (21.1)	NA	26 (21.1)	NA	32 (20.4)	NA

[a] Non-Caucasian women were mostly of Mediterranean or Caribbean ethnicity IQR: interquartile range, PCOS: polycystic ovary syndrome

[§] Significant difference between intervention and control group ($p<0.05$), using Student's t-test for normally distributed continuous data, Mann Whitney U non-normally distributed continuous data or Chi square test for categorical data.

*More than 1 diagnostic category is possible in same woman

[b] The denominator is the number of women with anovulatory infertility

Table 2. Baseline characteristics of women according to BMI and waist-hip (WH) ratio.

Characteristic	BMI ≥ 35 kg/m²		BMI <35 kg/m²		Waist-hip ratio ≥0.8		Waist-hip ratio <0.8	
	Intervention (n=175)	Control (n=180)	Intervention (n=104)	Control (n=103)	Intervention (n=242)	Control (n=240)	Intervention (n=30)	Control (n=43)
Age, mean (SD), years	29.9 (4.4)	29.8 (4.7)	29.7 (4.6)	29.7 (4.4)	29.8 (4.4)	29.8 (4.7)	30.0 (5.2)	29.6 (3.9)
Caucasian[a], No (%)	154 (88)	155 (86)	94 (90)	90 (87)	211 (87)	210 (88)	30 (100)[§]	35 (81)[§]
BMI, median (IQR), kg/m²	37.8 (36.5-39.3)	37.9 (36.3-39.2)	32.7 (31.6-33.8)	32.5 (31.6-33.8)	36.2 (33.4-38.7)	36.0 (33.3-38.4)	36.5 (34.2-38.8)	36.9 (34.4-39.0)
Education, No (%)								
Primary school (4-12 years)	14 (8.0)	6 (3.3)	2 (1.9)	3 (2.9)	15 (6.2)	7 (2.9)	1 (3.3)	2 (4.7)
Secondary Education	37 (21)	40 (22)	30 (29)	22 (21)	56 (23)	52 (22)	9 (30)	10 (23)
Intermediate Vocational Education	81 (46)	84 (47)	47 (45)	47 (46)	114 (47)	113 (47)	11 (37)	18 (42)
Higher Vocational Education and University	35 (20)	43 (24)	20 (19)	26 (25)	45 (19)	56 (23)	8 (27)	13 (30)
Current smoker, No (%)	35 (20)	32 (18)	37 (36)	28 (27)	60 (25)	50 (21)	9 (30)	10 (23)
Nulliparous, No (%)	138 (79)	133 (74)	79 (76)	82 (80)	184 (76)	180 (75)	27 (90)	35 (81)
Trying to conceive since, median (IQR), months	24 (16-41)[§]	19 (14-32)[§]	17 (13-30)	19 (13-35)	22 (14-36)	19 (13-31)	24 (15-49)	17 (13-34)
Diagnostic category of infertility, No (%)*								
Anovulation	74 (42)[§]	101(56)[§]	48 (46)	38 (37)	102 (42)[§]	125 (52)[§]	17 (57)[§]	14 (33)[§]
-of which PCOS [b]	53 (72)	71 (70)	40 (83)	32 (84)	80 (78)	92 (74)	10 (59)	11 (79)
Unexplained	58 (33)[§]	40 (22)[§]	26 (25)	37 (36)	73 (30)	63 (26)	9 (30)	14 (33)
Male factor	34 (19)	40 (22)	31 (30)	24 (23)	60 (25)	53 (22)	3 (10)	11 (26)
Tubal factor	9 (5.1)	8 (4.4)	3 (2.8)	8 (7.8)	10 (4.1)	13 (5.4)	2 (6.7)	3 (7.0)
Other	8 (4.6)	5 (2.8)	0	2 (1.9)	7 (2.9)	4 (1.7)	0	3 (7.0)
Non-completers intervention	34 (19.4)	NA	23 (22.1)	NA	51 (21.1)	NA	6 (20.0)	NA

[§]Significant difference between intervention and control group p<0.05, using Student's t-test for normally distributed continuous data, Mann Whitney U non-normally distributed continuous data or Chi square test for categorical data.
*More than 1 diagnostic category is possible in same woman
[b] The denominator is the number of women with anovulatory infertility

Natural conception rate

The rate of natural conceptions resulting in an ongoing pregnancy conceived within 24 months in the total randomized population was significantly higher in women who received the lifestyle intervention (OR 1.83, 95% CI 1.21-2.76, Table 5). This effect remained in women who were <36 years (aOR 2.17 95% CI 1.37-3.42), women who were anovulatory (aOR 4.15, 95% CI 2.04-8.44), women who had a BMI ≥ 35.0 kg/m^2 (aOR 1.93, 95% CI 1.12-3.34), women with a BMI < 35 kg/m^2 (aOR 3.20, 95% CI 1.42-7.22) and women with a WH ratio ≥ 0.8 (aOR 2.43, 95% CI 1.50-3.96). The effect of the lifestyle intervention on the rate of natural conceptions showed a significant interaction with maternal ovulatory status (P = 0.02). These data Indicate that anovulatory women who received the lifestyle intervention had a significantly increased natural conception rate compared to ovulatory women who received the lifestyle intervention. For the other subgroups the effect of the lifestyle intervention was not significantly altered, nor were any linear trends observed when women were divided in quartiles of age, BMI and WH ratio (Supplementary Table S4).

Discussion

The predefined subgroup analyses from a multicenter RCT showed that age, ovulatory status and BMI did not significantly influence the effect of a 6-month lifestyle intervention in terms of rate of healthy live births within 24 months and the rate of overall live births following conception within 24 months after randomization. WH ratio had a significant interaction with lifestyle intervention on healthy live birth rate, resulting in a lower healthy live birth rate in women with a WH ratio <0.8. WH ratio had no interaction regarding overall live birth rate or on natural conception rate. The chance of a natural conception of an ongoing pregnancy in women who received a lifestyle intervention increased significantly in anovulatory obese infertile women compared to ovulatory women.

Three different outcome measures were chosen for this analysis. Healthy live birth rate within 24 months was the primary outcome of the LIFEstyle study and was chosen to integrate potential advantages of weight loss. The outcome overall live birth conceived within 24 months, irrespective of term or mode of delivery, was chosen as this represents the outcome of major interest for patients and is in line with recent recommendations in the CONSORT statement.[24] In addition, due to a longer duration of follow-up, this outcome describes the efficacy of lifestyle intervention on a broader time frame. We consider our third outcome, natural conception resulting in an ongoing pregnancy within 24 months, as an important outcome in infertility trials. It is not uncommon for couples to conceive naturally prior to or in between treatment cycles. It is important to distinguish this outcome from treatment success, since a natural conception may prevent unnecessary infertility

treatments and prevent unnecessary medicalization of reproduction. Moreover, in many countries couples have to contribute financially to their infertility treatments, and costs involved may pose a great burden to couples. Natural conception decreases these costs and prevents complications associated with infertility treatment.

A strength of our study is that the analyses were based on predefined subgroups from the large LIFEstyle RCT. In this trial a heterogeneous group of obese infertile women, with a broad spectrum of infertility diagnosis, were included. Due to this heterogeneity it was possible to investigate differences in effectiveness of a lifestyle intervention in clinically meaningful subgroups of obese infertile women. In addition, the analysis of the interaction between subgroups and intervention or control group was based on common methodology of subgroup analysis.[25] The analyses in subgroups were predefined in the study protocol and the choice of subgroups was based on evidence of potential heterogeneity of treatment effect of the lifestyle intervention based on the literature and pathophysiological or biological hypotheses.

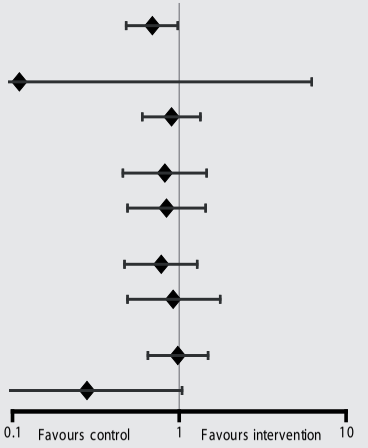
Several drawbacks of the current analysis should be considered. This study was a subgroup analysis of a RCT and sample size determination of the trial was based on the primary outcome (vaginal birth of a healthy singleton). The trial was not powered to detect subgroup effects or interactions. In addition, interaction tests are likely to be underpowered and a true effect may therefore be overlooked.[26] As a result, our findings should be interpreted with some caution, as there may be interaction effects of subgroups that we were not able to confirm due to the above-mentioned disadvantages. Although the definition of the subgroups was based on justified cut-off values, these cut-off values could still be viewed as more or less arbitrary. To account for uncertainty of the cut-off values of the dichotomized analyses, we performed logistic regression analyses on the subgroups that are based on continuous variables (age, BMI, WH ratio) and divided these in quartiles. These exploratory analyses did not show a linear trend in the ORs of the different quartiles, indicating that continuous analysis of our results did not provide alternations in the interpretation of our results.

Sneed and co-workers previously showed that the negative impact of obesity on infertility treatment outcomes diminishes in women above 36 years of age.[27] We did not find this effect in our analysis, possibly due to a low number of women included above the age of 36, leading to lack of power.

The current finding that anovulatory women benefit specifically from a lifestyle intervention preceding infertility treatment in terms of natural conception rates is in line with earlier

Table 3. Rate of healthy live births* within 24 months after randomisation in the subgroups including the p-values for interaction.

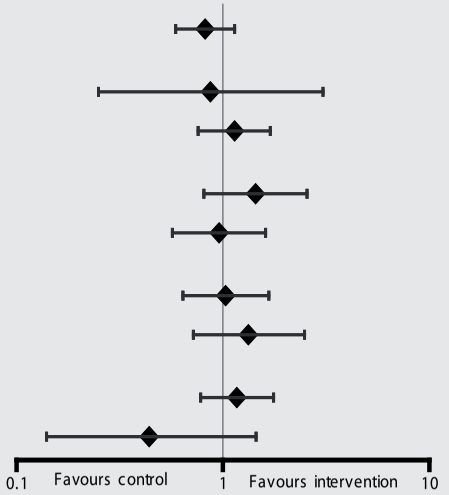
Subgroup factor	Rate (%) of healthy live births<24 months		OR_{crude} (95% CI)	$OR_{adjusted}$ (95%CI) [a]	OR (95%CI)[b]	$p_{interaction}$ [a]
	Intervention	Control				
Overall effect	76/280 (27)	100/284 (35)	0.69 (0.48-0.98)	NA		
Age (years)						0.12
≥36	1/34 (2.9)	5/29 (17)	0.15 (0.02-1.33)	0.11 (0.00-6.23)[c]		
<36	75/246 (31)	95/255 (37)	0.74 (0.51-1.07)	0.90 (0.60-1.34)		
Ovulatory status						0.91
Anovulatory	37/123 (30)	52/140 (37)	0.73 (0.44-1.22)	0.82 (0.46-1.46)		
Ovulatory	39/157 (25)	48/144 (33)	0.66 (0.40-1.09)	0.84 (0.49-1.44)		
BMI (kg/m^2)						0.62
≥35.0	43/175 (25)	61/180 (34)	0.64 (0.40-1.01)	0.78 (0.47-1.28)		
<35.0	33/104 (32)	39/103 (38)	0.76 (0.43-1.35)	0.92 (0.49-1.76)		
Waist-hip ratio						0.05
≥0.80	70/242 (29)	82/240 (34)	0.78 (0.53-1.15)	0.98 (0.65-1.49)		
<0.80	4/30 (13)	18/43 (42)	0.21 (0.06-0.72)	0.28 (0.08-1.04)		



* vaginal live birth at term without major congenital anomalies, OR: odds ratio, CI: confidence interval
a Using logistic regression analysis with correction for baseline variables age, BMI, smoking, ovulatory status, duration of infertility, nulliparity and non-completer of intervention status when this was not a subgroup factor.
b For the overall effects the crude OR is plotted, for all subgroups the adjusted OR is plotted
c not able to adjust for non-completer status due to low number of occurrence

Table 4. Rate of overall live births* conceived within 24 months after randomisation in the subgroups including the p-values for interaction

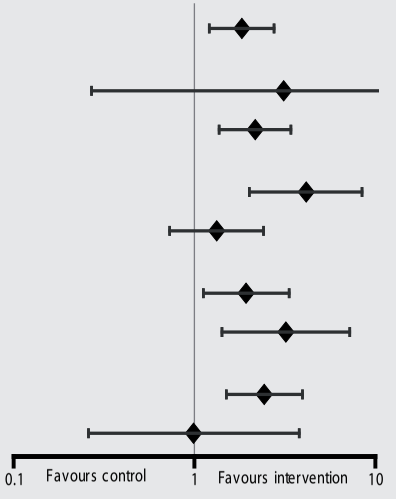
Subgroup factor	Rate (%) of live births conceived within 24 months		OR_{crude} (95% CI)	$OR_{adjusted}$ (95%CI) [a]	OR (95%CI)[b]	$p_{interaction}$ [a]
	Intervention	Control				
Overall effect	149/280 (53)	165/284 (58)	0.82 (0.59-1.14)	NA		
Age (years)						0.66
≥36	10/34 (29)	10/29 (35)	0.79 (0.27-2.29)	0.87 (0.25-3.06)		
<36	139/246 (57)	155/255 (61)	0.84 (0.59-1.20)	1.14 (0.76-1.70)		
Ovulatory status						0.40
Anovulatory	72/123 (59)	83/140 (59)	0.97 (0.59-1.59)	1.44 (0.81-2.56)		
Ovulatory	77/157 (49)	82/144 (57)	0.73 (0.46-1.15)	0.96 (0.57-1.61)		
BMI (kg/m²)						0.25
≥35.0	89/175 (51)	107/180 (59)	0.71 (0.46-1.08)	1.03 (0.64-1.67)		
<35.0	60/104 (58)	58/103 (56)	1.06 (0.61-1.83)	1.33 (0.72-2.49)		
Waist-hip ratio						0.27
≥0.80	131/242 (54)	138/240 (58)	0.87 (0.61-1.25)	1.17 (0.78-1.76)		
<0.80	13/30 (43)	27/43 (63)	0.45 (0.18-1.17)	0.44 (0.14-1.45)		



* live births independent of gestational age, mode of delivery and health
[a]Using logistic regression analysis with correction for baseline variables age, BMI, smoking, ovulatory status, duration of infertility, nulliparity and non-completer of intervention status when this was not a subgroup factor.
[b]For the overall effects the crude OR is plotted, for all subgroups the adjusted OR is plotted

Table 5. Rate of natural conceptions within 24 months after randomisation including the p-values for interaction

Subgroup factor	Rate (%) of natural conception of an ongoing pregnancy <24months		OR_{crude} (95% CI)	$OR_{adjusted}$ [a] (95%CI)	OR (95%CI)[b]	$p_{interaction}$ [a]
	Intervention	Control				
Overall effect	73/280 (26)	46/284 (16)	1.83 (1.21-2.76)	NA		
Age (years)						0.56
≥36	5/34 (15)	1/29 (3.5)	4.83 (0.53-44.0)	3.12 (0.27-35.9)[c]		
<36	68/246 (28)	45/255 (18)	1.78 (1.16-2.73)	2.17 (1.37-3.42)		
Ovulatory status						0.02
Anovulatory	35/123 (29)	16/140 (11)	3.08 (1.61-5.91)	4.15 (2.04-8.44)		
Ovulatory	38/157 (24)	30/144 (21)	1.21 (0.71-2.09)	1.33 (0.73-2.41)		
BMI (kg/m^2)						0.37
≥35.0	47/175 (27)	33/180 (18)	1.64 (0.99-2.71)	1.93 (1.12-3.34)		
<35.0	26/104 (25)	13/103 (13)	2.31 (1.11-4.80)	3.20 (1.42-7.22)		
Waist-hip ratio						0.38
≥0.80	67/242 (28)	38/240 (16)	2.04 (1.30-3.18)	2.43 (1.50-3.96)		
<0.80	5/30 (17)	8/43 (19)	0.88 (0.26-2.99)	0.99 (0.26-3.80)[d]		



[a]Using logistic regression analysis with correction for baseline variables age, BMI, smoking, ovulatory status, duration of infertility, nulliparity and non-completer of intervention status when this was not a subgroup factor.
[b]For the overall effects the crude OR is plotted, for all subgroups the adjusted OR is plotted
[c]not able to adjust for non-completer and smoking status due to low number of occurrence
[d]not able to adjust for non-completer status and nulliparity due to low number of occurrence

studies, although evidence on this topic is still limited. In a Cochrane review, Moran et al. concluded that lifestyle interventions in women with PCOS result in improvements in body composition, hyperandrogenism and insulin resistance.[28] However, they found insufficient data in the literature for assessing reproductive outcomes, such as ovulation and birth rates. Legro et al. recently reported data from an open-label, randomized trial of 149 women with PCOS and a BMI between 27 and 42 kg/m^2. They showed that treatment dependent ovulation rates significantly increased when women with PCOS received lifestyle modification for 16 weeks before starting ovulation induction, compared to women who received an oral contraceptive pill for 16 weeks before starting ovulation induction (60% vs. 46%, $P < 0.05$). Live birth rates did not differ significantly (26% vs. 12%, $P = 0.13$). The rate of natural conceptions was not evaluated, since infertility treatments were consecutive.[29]

The choice of BMI at baseline of 35 kg/m^2 as a cut-off value for subgroup analysis was based on the International Classification of obesity in Class I (BMI 30.00-34.99 kg/m^2) and Class II (BMI 35.00-39.99).[30] In addition, a BMI of ≥ 35 kg/m^2 is commonly advised as a cut-off value to withhold infertility treatment.[31] Our results do not show a significant difference in treatment effect of a lifestyle intervention in women with a BMI of ≥ 35 kg/m^2 compared to women with a BMI < 35 kg/m^2 on reproductive outcomes. Therefore, these results do not substantiate the strict BMI cut-off values used in some countries to withhold infertility treatment in order to improve reproductive outcomes.[15]

Zaadstra et al. showed that women with a WH ratio ≥ 0.8 have a significantly decreased pregnancy rate after artificial insemination with donor sperm compared to women with a WH ratio <0.8.[32] According to the WHO, a WH ratio ≥ 0.85 in women increases metabolic risks.[33] We found that in women with a WH < 0.8 a 6-month lifestyle intervention resulted in a lower healthy live birth rate compared to women with a WH ≥ 0.8. This might be an incidental finding, as it was not reproduced for the other outcome measures such as overall live births. An alternative explanation could be that women with a WH ratio < 0.8 (and therefore a normal centripetal fat distribution) do not benefit from weight loss, which is mainly peripheral. This finding should be investigated further, as it might implicate that women with a normal WH ratio do not benefit from lifestyle intervention in terms of healthy live birth rates.

In conclusion, we did not identify a subgroup in which lifestyle intervention did increase the healthy live birth rate. However, lifestyle intervention increased the natural conception rate in anovulatory, but not in ovulatory, obese infertile women. Our finding that lifestyle intervention leads more often to natural conception in anovulatory obese women could be used in the counseling of these women, but this requires further research using an appropriately powered study in order to confirm this finding.

References

1. Eurostat. Eurostat newsrelease. 2011;172.
2. Flegal KM, Carroll MD, Kit BK,Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among us adults, 1999-2010. *JAMA*. 2012;307(5):491-497.
3. Ramlau-Hansen CH, Thulstrup AM, Nohr EA, Bonde JP, Sorensen TI, Olsen J. Subfecundity in overweight and obese couples. *Hum Reprod*. 2007;22(6):1634-1637.
4. Pasquali R, Patton L, Gambineri A. Obesity and infertility. *Curr Opin Endocrinol Diabetes Obes*. 2007;14(6):482-487.
5. van der Steeg JW, Steures P, Eijkemans MJ, et al. Obesity affects spontaneous pregnancy chances in subfertile, ovulatory women. *Hum Reprod*. 2008;23(2):324-328.
6. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab*. 1998;83(7):2361-2365.
7. Souter I, Baltagi LM, Kuleta D, Meeker JD, Petrozza JC. Women, weight, and fertility: The effect of body mass index on the outcome of superovulation/intrauterine insemination cycles. *Fertil Steril*. 2011;95(3):1042-1047.
8. Legro RS, Barnhart HX, Schlaff WD, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. *N Engl J Med*. 2007;356(6):551-566.
9. Rittenberg V, Seshadri S, Sunkara SK, Sobaleva S, Oteng-Ntim E, El-Toukhy T. Effect of body mass index on IVF treatment outcome: An updated systematic review and meta-analysis. *Reprod Biomed Online*. 2011;23(4):421-439.
10. Koning AM, Kuchenbecker WK, Groen H, et al. Economic consequences of overweight and obesity in infertility: A framework for evaluating the costs and outcomes of fertility care. *Hum Reprod Update*. 2010;16(3):246-254.
11. Pandey S, McLernon DJ, Scotland G, Mollison J, Wordsworth S, Bhattacharya S. Cost of fertility treatment and live birth outcome in women of different ages and BMI. *Hum Reprod*. 2014;29(10):2199-2211.
12. Ovesen P, Rasmussen S, Kesmodel U. Effect of prepregnancy maternal overweight and obesity on pregnancy outcome. *Obstet Gynecol*. 2011;118(2 Pt 1):305-312.
13. Weiss JL, Malone FD, Emig D, et al. Obesity, obstetric complications and cesarean delivery rate--a population-based screening study. *Am J Obstet Gynecol*. 2004;190(4):1091-1097.
14. Aune D, Saugstad OD, Henriksen T, Tonstad S. Maternal body mass index and the risk of fetal death, stillbirth, and infant death: A systematic review and meta-analysis. *JAMA*. 2014;311(15):1536-1546.
15. Pandey S, Maheshwari A, Bhattacharya S. Should access to fertility treatment be determined by female body mass index? *Hum Reprod*. 2010;25(4):815-820.
16. Vahratian A, Smith YR. Should access to fertility-related services be conditional on body mass index? *Hum Reprod*. 2009;24(7):1532-1537.
17. National Collaborating Centre for Women's and Children's Health (UK). 2013.
18. Practice Committee of the American Society for Reproductive Medicine. Obesity and reproduction: A committee opinion. *Fertil Steril*. 2015;104(5):1116-1126.
19. Mutsaerts MA, van Oers AM, Groen H, et al. Randomized trial of a lifestyle program in obese infertile women. *N Engl J Med*. 2016;374(20):1942-1953.
20. Mutsaerts MA, Groen H, ter Bogt NC, et al. The LIFESTYLE study: Costs and effects of a structured lifestyle program in overweight and obese subfertile women to reduce the need for fertility treatment and improve reproductive outcome. A randomised controlled trial. *BMC Womens Health*. 2010;10:22-6874-10-22.
21. The ESHRE Capri Workshop Group. Anovulatory infertility. *Hum Reprod*. 1995;10(6):1549-1553.
22. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod*. 2004;19(1):41-47.
23. Dutch Society of Obstetrics and Gynaecology (NVOG). Guidelines on reproductive medicine. http://nvog-documenten.nl/. Accessed March 10th, 2016.
24. Harbin Consensus Conference Workshop Group, Conference Chairs, Legro RS, et al. Improving the reporting of clinical trials of infertility treatments (IMPRINT): Modifying the CONSORT statement. *Hum Reprod*. 2014;29(10):2075-2082.

25. Rothwell PM. Treating individuals 2. subgroup analysis in randomised controlled trials: Importance, indications, and interpretation. *Lancet*. 2005;365(9454):176-186.
26. Brookes ST, Whitely E, Egger M, Smith GD, Mulheran PA, Peters TJ. Subgroup analyses in randomized trials: Risks of subgroup-specific analyses; power and sample size for the interaction test. *J Clin Epidemiol*. 2004;57(3):229-236.
27. Sneed ML, Uhler ML, Grotjan HE, Rapisarda JJ, Lederer KJ, Beltsos AN. Body mass index: Impact on IVF success appears age-related. *Hum Reprod*. 2008;23(8):1835-1839.
28. Moran LJ, Hutchison SK, Norman RJ, Teede HJ. Lifestyle changes in women with polycystic ovary syndrome. *Cochrane Database Syst Rev*. 2011;(7):CD007506. doi(7):CD007506.
29. Legro RS, Dodson WC, Kris-Etherton PM, et al. Randomized controlled trial of preconception interventions in infertile women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2015;100(11):4048-4058.
30. WHO. International classification of BMI. http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. Updated 2016. Accessed 1/14, 2016.
31. Balen AH, Anderson RA, Policy & Practice Committee of the BFS. Impact of obesity on female reproductive health: British fertility society, policy and practice guidelines. *Hum Fertil (Camb)*. 2007;10(4):195-206.
32. Zaadstra BM, Seidell JC, Van Noord PA, et al. Fat and female fecundity: Prospective study of effect of body fat distribution on conception rates. *BMJ*. 1993;306(6876):484-487.
33. World Health Organization. Waist circumference and Waist–Hip ratio: Report of a WHO expert consultation Geneva, 8–11 december 2008. 2011.

Supplementary Table 1. Cut-off values of quartiles of maternal age, BMI, waist-hip ratio of women

Subgroup	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Maternal age (years)	≤26.4	26.4-29.7	29.7-33.3	>33.3
BMI (kg/bmi^2)	≤33.4	33.4-36.2	36.2-38.6	>38.61
Waist-hip ratio	≤0.82	0.82-0.86	0.86-0.91	>0.91

Supplementary Table 2. Healthy live birth rate in women according to quartile, by subgroup and randomisation group with adjustment for confounders

Subgroup	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*p*-value interaction
Age, aOR (95% CI)	0.92 (0.42-1.97)	0.76 (0.35-1.67)	1.78 (0.80-3.98)	0.32 (0.12-0.84)	0.08
-intervention group, rate (%)	24/67 (35.8)	21/74 (28.4)	22/69 (31.9)	9/70 (12.9)	
-control group, rate (%)	33/74 (44.6)	26/67 (28.8)	21/72 (29.2)	20/71 (28.2)	
BMI, aOR (95% CI)	0.87 (0.40-1.86)	1.17 (0.53-2.60)	0.38 (0.17-0.84)	1.19 (0.49-2.92)	0.06
-intervention group, rate (%)	24/72 (33.3)	19/67 (28.4)	15/71 (21.1)	18/69 (26.1)	
-control group, rate (%)	28/71 (39.4)	24/76 (31.6)	30/69 (43.5)	18/67 (26.9)	
Waist-hip ratio, aOR (95% CI)	0.84 (0.40-1.74)	1.51 (0.68-3.36)	0.54 (0.24-1.20)	0.70 (0.25-1.93)	0.65
-intervention group, rate (%)	20/78 (25.6)	24/68 (35.3)	20/71 (28.2)	10/55 (18.2)	
-control group, rate (%)	30/81 (37.0)	23/65 (35.4)	32/80 (40.0)	15/57 (26.3)	

Using logistic regression analysis with correction for baseline variables age, BMI, smoking, ovulatory status, duration of infertility, nulliparity and non-completer of intervention status when this was not a subgroup factor.

Supplementary Table 3. Live birth rate in women according to quartile, by subgroup and randomisation group with adjustment for confounders

Subgroup	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*p*-value interaction
Age, aOR (95% CI)	1.34 (0.30-3.01)	2.06 (0.91-4.66)	0.91 (0.41-2.01)	0.63 (0.30-1.33)	0.28
-intervention group, rate (%)	41/67 (61.2)	49/74 (66.2)	33/69 (47.8)	26/71 (36.6)	
-control group, rate (%)	49/74 (66.2)	39/76 (51.3)	43/72 (59.7)	34/71 (47.9)	
BMI, aOR (95% CI)	1.01 (0.48-2.10)	1.61 (0.69-3.72)	0.63 (0.29-1.39)	1.54 (0.67-3.56)	0.32
-intervention group, rate (%)	41/72 (56.9)	40/67 (59.7)	38/71 (53.5)	30/69 (43.5)	
-control group, rate (%)	43/71 (60.6)	43/76 (56.6)	46/69 (66.7)	33/67 (49.3)	
Waist-hip ratio, aOR (95% CI)	1.00 (0.50-2.01)	1.20 (0.53-2.76)	0.99 (0.47-2.06)	1.35 (0.52-3.47)	0.92
-intervention group, rate (%)	38/78 (48.7)	40/68 (58.8)	38/71 (53.5)	28/55 (50.9)	
-control group, rate (%)	48/81 (59.3)	41/65 (63.1)	47/80 (58.8)	29/57 (50.9)	

Using logistic regression analysis with correction for baseline variables age, BMI, smoking, ovulatory status, duration of infertility, nulliparity and non-completer of intervention status when this was not a subgroup factor.

Supplementary Table 4. Natural conception rate in women according to quartile, by subgroup and randomisation group with adjustment for confounders

Subgroup	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*p*-value interaction
Age, aOR (95% CI)	3.27 (1.38-7.73)	2.56 (1.06-6.16)	1.60 (0.61-4.21)	1.74 (0.62-4.88)	0.84
-intervention group, rate (%)	22/67 (32.8)	24/74 (32.4)	15/69 (21.7)	12/70 (17.1)	
-control group, rate (%)	14/74 (18.9)	12/67 (17.9)	12/72 (16.7)	8/71 (11.3)	
BMI, aOR (95% CI)	2.75 (1.01-7.52)	3.59 (1.46-8.79)	1.55 (0.67-3.59)	2.27 (0.83-6.15)	0.56
-intervention group, rate (%)	16/72 (22.2)	22/67 (32.8)	19/71 (26.8)	16/69 (23.2)	
-control group, rate (%)	9/71 (12.7)	11/76 (14.5)	14/69 (20.3)	12/67 (17.9)	
Waist-hip ratio, aOR (95% CI)	1.28 (0.55-2.98)	3.29 (1.34-8.09)	2.17 (0.83-5.67)	3.17 (1.05-9.59)	0.31
-intervention group, rate (%)	15/78 (19.2)	28/68 (41.2)	17/71 (23.9)	12/55 (21.8)	
-control group, rate (%)	16/81 (19.8)	13/65 (20.0)	10/80 (12.5)	7/57 (12.3)	

Using logistic regression analysis with correction for baseline variables age, BMI, smoking, ovulatory status, duration of infertility, nulliparity and non-completer of intervention status when this was not a subgroup factor.