Preconception Leptin and Fecundability, Pregnancy, and Live Birth Among Women With a History of Pregnancy Loss

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Context: With the increase of obesity, it is imperative to understand the neuroendocrine mechanisms, including the neuroendocrine hormone leptin, by which obese or overweight women are at increased risk for subfertility and infertility.

Objective: The objective was to examine associations between preconception serum leptin concentrations, fecundability, pregnancy, and live birth.

Design: Secondary analysis of a prospective cohort among women with prior pregnancy losses.

Setting: The study was conducted at four US medical centers (2006 to 2012).

Intervention: Not available.

Materials and Methods: Preconception serum leptin concentrations were measured at baseline, and women were followed for up to six menstrual cycles, and throughout pregnancy if they conceived. Discrete Cox proportional hazard regression models were used to assess fecundability odds ratios (FORs) and log-binomial regression to estimate risk ratios (RRs) for pregnancy and live birth. Models were adjusted for age, physical activity, treatment arm, and adiposity, either by measured waist-to-hip ratio or body mass index (BMI).

Results: High leptin concentrations were associated with decreased fecundability (FOR 0.72, 95% CI 0.58, 0.90), reduced risk of pregnancy (RR 0.87, 95% CI 0.78, 0.96) and live birth (RR 0.76, 95% CI 0.65, 0.89) comparing the upper to the lower tertile. However, adjustment for BMI in lieu of waist-to-hip ratio nullified observed associations.

Conclusions: In women with a history of pregnancy loss, relations between higher preconception leptin and fecundability were attenuated after adjustment for BMI, although not after adjustment for other

Abbreviations: BMI, body mass index; FOR, fecundability odds ratio; RR, risk ratio; TTP, time to pregnancy.

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markers of adiposity. Leptin may serve as a complementary marker of adiposity for assessment of obesity and reproductive outcomes.

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Freeform/Key Words: leptin, adiposity, fecundability, live birth, pregnancy

The obesity epidemic has increased to unprecedented numbers in the past few decades [1, 2]. Among women, the prevalence of overall obesity has increased substantially from 2005 to 2014 [2], with alarming estimates that two out of three women were overweight or obese in 2013 [1]. With the increase of obesity, it is imperative to understand the mechanisms by which women are at increased risk for numerous health conditions, including subfertility and infertility [3–5].

Obesity causes changes in body composition and the neuroendocrine axis, including the hormone leptin [6]. Leptin is a hormone produced by adipocytes that plays a crucial role in regulating appetite and metabolism [6]. In addition to communicating energy storage and food intake to the brain, leptin has been proposed as a marker of adipose abundance [7, 8]. However, unlike other surrogates of adiposity such as waist-to-hip ratio (WHR) and body mass index (BMI), leptin has been associated with specific adipose deposits in the body [9]. In women there is a stronger association between leptin and subcutaneous fat as opposed to central or visceral fat [9–11]. In a recent meta-analysis, increased early pregnancy leptin concentrations were reported in women who subsequently developed gestational diabetes [12] despite similarities in body mass index [11]. In all, these studies suggest that leptin may serve as a unique and valuable marker for adiposity.

By facilitating energy-intensive processes such as oogenesis and ovulation, leptin also plays an important role in human reproduction [13]. Specifically, a threshold concentration of leptin is needed for the onset of puberty and maintenance of the menstrual cycle. Critically low concentrations or abnormal signaling are associated with delayed puberty and decreased fertility [14]. Correction of leptin concentrations using replacement doses has also been shown to reverse menstrual irregularity and central amenorrhea [15]. This reversal is likely through leptin's effect on the hypothalamic-pituitary-ovarian axis, as leptin stimulates luteinizing hormone secretion from the pituitary [16–18] and activates receptors for gonadotropin-releasing hormones in the hypothalamus [15]. Taken together, this suggests the importance of investigating the relation between adiposity, leptin, and human reproduction.

Few previous studies have examined the role of leptin concentrations as a marker of adiposity on human reproduction, particularly on fecundability and live birth. Past studies have typically been limited in comparing leptin concentrations between fertile women and women with unexplained infertility. These studies tended to find higher concentrations of leptin, whether measured in serum or ovarian follicular fluid, and lower endometrial leptin expression among women with infertility [19–23]. Higher follicular fluid concentration of leptin was associated with lower likelihood of live birth among women undergoing *in vitro* fertilization [24], whereas another study found an association between higher nonfasting plasma leptin levels and improved likelihood of live birth in women with recurrent pregnancy loss [25].

No prior studies have evaluated preconception leptin concentrations among women not undergoing fertility treatment and not all studies have accounted for other surrogates of adiposity. Thus, our objective was to evaluate the association between preconception serum leptin concentrations and fecundability, pregnancy, and live birth among women attempting natural pregnancy while accounting for other markers of adiposity.

1. Methods

A. Study Design

Of 1228 women who were enrolled in the Effects of Aspirin in Gestation and Reproduction Trial, this analysis included 1053 women who did not withdraw from the study prior to

pregnancy (n = 128) and did not have missing serum leptin measurement (n = 30), BMI (n = 11), or WHR ratio (n = 6) data (Fig. 1). Details regarding the trial have been published previously [26]. Study participants were 18 to 40 years, had one or two documented prior pregnancy losses, self-reported regular menstrual cycles (*i.e.,* 21 to 42 days in length), and were attempting pregnancy without the use of fertility treatment.

Participants completed questionnaires on demographics, lifestyle factors, and medical and reproductive history at a baseline visit prior to randomization. Height and weight were measured and used to calculate BMI (kg/m^2). WHR and sum of skinfolds were measured at the baseline visit using standardized protocols. Participants were followed for up to six menstrual cycles or throughout pregnancy for those who became pregnant. The institutional review board at each study site (Salt Lake City, Utah; Denver, Colorado; Buffalo, New York; Scranton, Pennsylvania) and data coordinating center approved the trial protocol and all participants provided written informed consent prior to enrollment. The trial was registered on ClinicalTrials.gov (#NCT00467363).

B. Analysis of Serum Leptin Concentrations

Preconception serum samples were collected at baseline, which was scheduled to occur during days two to four of the menstrual cycle for each participant. It is important to note that not all samples were collected when the participant was fasting. Leptin concentrations were measured using ELISA (R&D Systems, Inc., Minneapolis, MN) [27]. Interassay laboratory coefficients of variation were 3.3% and 3.2% at mean concentrations of 64.5 and 621 pg/mL, respectively, for lyophilized manufacturer's controls, and 16.9% for an in-house pooled serum control.

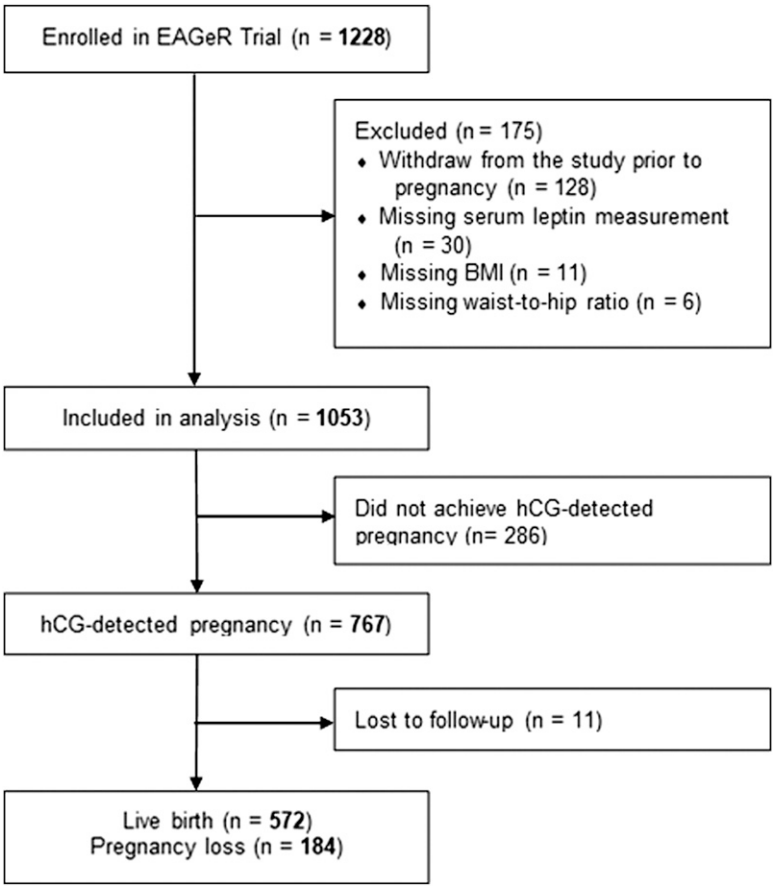

Figure 1. Flow chart of participant inclusion and pregnancy outcomes.

C. Outcome Assessment

Time to pregnancy (TTP), pregnancy, and live birth were the outcomes of this analysis. TTP was defined as the number of menstrual cycles until a positive pregnancy test over up to six consecutive cycles of follow-up [28, 29]. To document pregnancy precisely, participants had frequent study visits and early ultrasound [29]. Urine pregnancy tests sensitive to 25 mIU/mL human chorionic gonadotropin (hCG) were conducted at clinic visits scheduled at the end of each cycle (Quidel Quickvue, Quidel Corporation, San Diego, CA). Additionally, free β-hCG was measured after study completion in daily first-morning urine samples collected on the last 10 days of each participant's first and second menstrual cycles, and in urine samples collected at the end of each cycle. An early ultrasound at 6.5 weeks' gestation was performed for clinical confirmation of pregnancy. Live birth status was obtained by participants' report or via chart abstraction by trained staff.

D. Statistical Analysis

Participants' demographics and reproductive history were examined by tertile of preconception leptin concentrations using χ^2 tests and t-tests as appropriate. Fecundability odds ratios (FORs) and 95% CIs were estimated to evaluate the association between leptin concentrations and TTP using Cox proportional hazard regression models for discrete survival time, accounting for left truncation (cycles trying before study entry) and right censoring. Models evaluated leptin by tertiles, whereby the middle and upper tertiles were compared with the lower tertile, adjusted for age, physical activity, treatment arm, and WHR. Additionally, models were adjusted for BMI, waist circumference, hip circumference, sum of skinfolds (triceps, subscapular, suprailiac) and weight. Because there is a short-term leptin response to food intake [30], additional models were adjusted for fasting status at time of leptin assessment to account for the use of nonfasting serum samples. Because leptin is produced by adipocytes, it is correlated with body fat mass [31] but may have higher expression from subcutaneous versus visceral adipose depots [9]. Although BMI is frequently used to categorize obesity, prior studies have noted that it might not reflect central adiposity accurately [32]. In several studies, WHR has been shown to predict mortality and cardiovascular risk associated with obesity better [33, 34]. Thus, main analytic models were adjusted for WHR, and sensitivity analyses adjusted for other markers of adiposity, including BMI, to help to understand leptin's effect on fecundability outside of its relation with various markers of adiposity.

Weighted log-binomial regression models were used to estimate risk ratios (RRs) and 95% CIs for leptin tertiles and pregnancy and live birth. Models were adjusted for the same factors listed above (age, physical activity, treatment arm, and WHR), and similar subanalyses were done to evaluate the role of fasting status and other markers of adiposity. In pregnancy outcome analyses, inverse probability weights were used to account for potential selection bias by exclusion of women who withdrew from the study. In live birth outcome analyses, weights were applied to account for selection bias due to study withdrawal and restriction to women who had achieved hCG-detected pregnancy [35]. Probability of withdrawal and pregnancy weights were constructed separately because determinants of withdrawal and pregnancy may differ. To specify the models, variables were selected that were thought likely to influence leptin and withdrawal or pregnancy. Withdrawal weights were based on leptin, treatment arm, age, and number of prior losses; pregnancy weights additionally included parity, marital status, and an interaction term for parity and age. SAS version 9.4 (SAS Institute, Cary, NC) was used for all statistical analysis.

2. Results

Women with preconception leptin concentrations in the upper tertile tended to have higher BMI, lower incomes, and were more likely to have a high school education or less, whereas

women with concentrations in the lower tertile report consuming alcohol more frequently in the year prior to randomization (Table 1). No differences in fasting glucose levels were observed across the three tertiles of leptin ($P = 0.07$), although differences in the fasting homeostatic model assessment of insulin resistance were observed ($P < 0.0001$) (Table 1).

In this population, 767 of 1053 (72.8%) achieved hCG-detected pregnancy and 572 of 1042 (54.9%) had a live birth (11 of 767 women who achieved hCG-detected pregnancy were lost to follow-up). Women in the upper tertile of leptin concentrations had reduced fecundability, or a longer TTP, compared with those in the lower tertile (FOR 0.72, 95% CI 0.58, 0.90) after adjusting for age, physical activity, treatment arm, and WHR (Table 2). Similar results were observed when the model was additionally adjusted for fasting status (FOR 0.75, 95% CI 0.61,0.93). However, results were attenuated after adjusting for BMI in place of WHR (FOR 0.94, 95% CI 0.69, 1.28). A similar pattern was observed between the upper tertile of leptin and a reduction in achieving pregnancy (RR 0.87, 95% CI 0.78, 0.96) after adjusting for age, physical activity, treatment arm, and WHR (Table 3). Similar results were also observed for achieving pregnancy after additionally adjusting for fasting status (RR 0.87 95% CI 0.79, 0.96). Women in the upper tertile of leptin concentrations were also observed to have lower live birth rates (RR 0.76, 95% CI 0.65, 0.89) during the six cycles of follow up after adjusting for age, physical activity, treatment arm, WHR, and fasting status. Again, the results were attenuated after adjusting for BMI in place of WHR both for likelihood of pregnancy (RR 1.02, 95% CI 0.88, 1.18) and live birth (RR 0.91, 95% CI 0.74, 1.13) in the overall cohort.

BMI was measured at baseline and women who became pregnant during the study had BMI and other anthropometrics measured again at four weeks of pregnancy. Mean BMI increased by 0.31 points between baseline and four weeks of pregnancy (from 25.2 to 25.6 kg/m^2), and the average weight change was 0.86 kg (1.9 pounds).

When evaluating other markers of adiposity, leptin correlated was most strongly with BMI and was least correlated with WHR (Table 4). Furthermore, the upper tertile of leptin concentrations were not associated with fecundability, or a longer TTP, after adjusting for age, physical activity, treatment arm, and waist circumference, hip circumference, sum of skinfolds, or weight (Table 5). Similarly, women with the upper tertile of leptin concentrations were not observed to have a reduction in achieving pregnancy or live birth after adjusting for the aforementioned factors (Table 6).

3. Discussion

A. Principal Findings

In women with a history of prior pregnancy loss, higher preconception leptin concentrations were associated with decreased fecundability and reduced probability of pregnancy and live birth. These findings appear to be explained by leptin's relation with higher BMI rather than other measurements such as WHR. These findings propose the potential use of leptin as a complementary marker of adiposity in continued efforts to discern the link between obesity and poor reproductive outcomes.

Our findings are generally consistent with research evaluating the association between leptin and reproductive health outcomes, although no previous studies have specifically evaluated fecundability or preconception concentrations among fertile populations. Studies comparing leptin concentrations between fertile and infertile women tended to show higher leptin concentrations among women with infertility [19, 20]. These findings are consistent with ours as we similarly observed a decrease in fecundability with higher leptin concentrations prior to adjustment for adiposity. Direct comparison with these studies is not possible because they did not adjust for adiposity.

Some previous studies among women undergoing infertility treatment suggest an association between leptin concentrations, pregnancy rates, and live birth. One group examined follicular fluid leptin concentrations in women undergoing infertility treatment, finding that higher concentrations of follicular fluid leptin were associated with a lower likelihood of

Table 1. The EAGeR Trial: Demographics and Characteristics by Tertile of Baseline Leptin

		Leptin Tertile[a]			
	Total	**Tertile 1**	**Tertile 2**	**Tertile 3**	***P***
N	1053	368	346	339	
Age, yr: Mean ± SD	28.9 (4.7)	29.2 (4.6)	28.8 (4.6)	28.7 (5)	0.3187
BMI, kg/m^2: Mean ± SD	26 (6.4)	21.1 (2.3)	24.9 (3.7)	32.4 (6.2)	0
Race					
White	1006 (95.5)	349 (94.8)	332 (96)	325 (95.9)	0.7478
Other	47 (4.5)	19 (5.2)	14 (4)	14 (4.1)	
Education					
≤High school	125 (11.9)	32 (8.7)	37 (10.7)	56 (16.5)	0.0047
>High school	928 (88.1)	336 (91.3)	309 (89.3)	283 (83.5)	
Household income[b]					
≥$100,000	434 (41.3)	140 (38)	162 (46.8)	132 (39.1)	<0.0001
$75,000–$99,999	136 (12.9)	68 (18.5)	42 (12.1)	26 (7.7)	
$40,000–$74,999	155 (14.7)	56 (15.2)	51 (14.7)	48 (14.2)	
$20,000–$39,999	258 (24.5)	77 (20.9)	78 (22.5)	103 (30.5)	
≤$19,999	69 (6.6)	27 (7.3)	13 (3.8)	29 (8.6)	
Employed[b]					
Yes	783 (75.6)	261 (72.3)	262 (76.8)	260 (77.8)	0.1943
No	253 (24.4)	100 (27.7)	79 (23.2)	74 (22.2)	
Time from last loss to randomization (mo)[b]					
≤4 mo	567 (54.7)	205 (56)	191 (56.3)	171 (51.7)	0.8049
5–8 mo	197 (19)	73 (19.9)	59 (17.4)	65 (19.6)	
9–12 mo	79 (7.6)	26 (7.1)	25 (7.4)	28 (8.5)	
>12 mo	193 (18.6)	62 (16.9)	64 (18.9)	67 (20.2)	
Previous pregnancies, not including losses					
0					
1	445 (42.3)	144 (39.1)	153 (44.2)	148 (43.7)	0.2204
2	376 (35.7)	125 (34)	128 (37)	123 (36.3)	
3	213 (20.2)	90 (24.5)	60 (17.3)	63 (18.6)	
Number of previous live births					
0	478 (45.4)	154 (41.8)	162 (46.8)	162 (47.8)	0.3269
1	385 (36.6)	137 (37.2)	129 (37.3)	119 (35.1)	
2	190 (18)	77 (20.9)	55 (15.9)	58 (17.1)	
Smoking in past y[b]					
Never	934 (89.1)	331 (90.2)	307 (89.5)	296 (87.6)	0.149
<6 times/wk	70 (6.7)	26 (7.1)	24 (7)	20 (5.9)	
Daily	44 (4.2)	10 (2.7)	12 (3.5)	22 (6.5)	
Alcohol consumption in past y[b]					
Often	24 (2.3)	16 (4.4)	5 (1.5)	3 (0.9)	0.0355
Sometimes	315 (30.2)	105 (28.6)	104 (30.8)	106 (31.5)	
Never	703 (67.5)	246 (67)	229 (67.8)	228 (67.7)	
Fasting glucose[c]	80.9 ± 12.6	77.8 ± 17.9	79.5 ± 8.9	83.3 ± 10.4	0.0719
Fasting insulin[c]	61.2 ± 40.5	41.5 ± 22.6	44.5 ± 18.2	90.6 ± 56.2	<0.0001
Fasting HOMA-IR[c]	1.7 ± 1.2	1.1 ± 0.6	1.2 ± 0.5	2.6 ± 1.8	<0.0001

Values are mean ± SD or n (%) as indicated.
This study included 1053 women who did not withdraw from the study prior to pregnancy (n = 128) and did not have missing leptin (n = 30), BMI (n = 11), or WHR (n = 6) data.
Abbreviations: EAGeR, Effects of Aspirin in Gestation and Reproduction Trial; HOMA-IR, homeostatic model assessment of insulin resistance.
[a]Tertile 1 < 11.22 ng/mL; tertile 2 = 11.22–26.24 ng/mL; tertile 3 > 26.25 ng/mL.
[b]Among the 1053 study participants, data were missing for income (n = 1), employment (n = 17), time from last loss to randomization (n = 17), smoking (n = 5), and alcohol consumption (n = 11).
[c]Values are geometric mean ± SD.

having a live birth after adjusting for waist circumference and insulin resistance [24]. This study did not adjust for BMI so it is unknown whether the association would remain after adjustment for an alternative marker of adiposity. It is also different from ours, as it

Table 2. FORs and 95% CIs[a] for Preconception Leptin Tertiles[b] and Time to Pregnancy

	Tertile 1	Tertile 2	Tertile 3
Unadjusted	Reference	1.04 (0.86,1.27)	0.73 (0.59,0.90)
Model 1	Reference	1.05 (0.86,1.28)	0.73 (0.59,0.89)
Model 2	Reference	1.04 (0.85,1.28)	0.72 (0.58,0.90)
Model 3	Reference	1.14 (0.92,1.41)	0.94 (0.69,1.28)
Model 4	Reference	1.05 (0.86,1.29)	0.75 (0.61,0.93)

Model 1: Adjusted for age, physical activity, and treatment arm.
Model 2: Adjusted for age, physical activity, treatment arm, and WHR.
Model 3: Adjusted for age, physical activity, treatment arm, and BMI.
Model 4: Adjusted for age, physical activity, treatment arm, and fasting status.
[a]Calculated using Cox proportional hazard regression models, accounting for left truncation and right censoring.
[b]Reference is tertile 1 <11.22 ng/mL; tertile 2 = 11.22 –26.24 ng/mL; tertile 3 > 26.25 ng/mL.

evaluated infertile women and examined leptin concentrations in follicular fluid instead of serum. The study suggests that the mechanism by which elevated intrafollicular leptin concentrations impact reproduction is through its association with lower oxygen tension in follicles. This is hypothesized to affect the maturation and development of oocytes negatively [36]. Another study evaluating women who were undergoing *in vitro* fertilization found that relatively higher baseline nonfasting serum leptin concentrations were associated with a lower likelihood of pregnancy, although they did not evaluate the likelihood of live birth [22]. Although many samples in our study were collected in subjects who were not fasting, we observed similar results to this prior study. It is also important to note that we maintained similar results after additional adjustments for fasting status. Last, this study evaluated the leptin to BMI ratio, finding links between the leptin to BMI ratio and pregnancy, which also points to the complex and interconnected nature of leptin, BMI, and pregnancy.

There is biological plausibility to support a link between preconception leptin and fecundability. It has been shown that leptin is associated with some changes in reproductive hormones over the menstrual cycle and could potentially be associated with ovulation [37]. Thus, leptin concentrations may affect ovulation through these mechanisms that could subsequently influence timing of pregnancy. Elevated leptin concentrations also appear to affect oocyte development negatively [22], which could provide another potential explanation

Table 3. RRs and 95% CIs[a] for Preconception Leptin Tertiles[b] and Pregnancy and Live Birth

	Pregnancy[c] (n = 767/1053)			Live Birth[d] (n = 572/1042)		
	Tertile 1	Tertile 2	Tertile 3	Tertile 1	Tertile 2	Tertile 3
Unadjusted	Reference	1.03 (0.95, 1.12)	0.85 (0.77, 0.93)	Reference	1.00 (0.89, 1.13)	0.77 (0.67, 0.89)
Model 1	Reference	1.03 (0.96, 1.12)	0.86 (0.78, 0.94)	Reference	1.01 (0.89, 1.14)	0.78 (0.68, 0.90)
Model 2	Reference	1.04 (0.96, 1.12)	0.87 (0.78, 0.96)	Reference	1.00 (0.88, 1.13)	0.76 (0.65, 0.89)
Model 3	Reference	1.08 (0.99, 1.17)	1.02 (0.88, 1.18)	Reference	1.06 (0.93, 1.20)	0.91 (0.74, 1.13)
Model 4	Reference	1.04 (0.96, 1.12)	0.87 (0.79, 0.96)	Reference	1.01 (0.89, 1.14)	0.79 (0.69, 0.91)

Model 1: Adjusted for age, physical activity, and treatment arm.
Model 2: Adjusted for age, physical activity, treatment arm, and WHR.
Model 3: Adjusted for age, physical activity, treatment arm, and BMI.
Model 4: Adjusted for age, physical activity, treatment arm, and fasting status.
[a]Calculated using inverse probability weighted log-binomial regression.
[b]Reference is: tertile 1 < 11.22 ng/mL; tertile 2 = 11.22–26.24 ng/mL; tertile 3 > 26.25 ng/mL.
[c]Weights account for withdrawal prior to pregnancy (n = 128).
[d]Weights account for withdrawal after hCG-pregnancy (n = 11), withdrawal and nonpregnancy (n = 118), and nonpregnancy only (n = 286).

Table 4. Correlations Between Leptin and Markers of Adiposity

	Leptin	WHR	BMI	Waist Circumference	Hip Circumference	Sum of Skinfolds	Weight
Leptin	1.0	0.352	0.815	0.754	0.780	0.612	0.777
Waist-to-hip ratio		1.0	0.452	0.708	0.255	0.354	0.429
Body mass index			1.0	0.895	0.899	0.679	0.946
Waist circumference				1.0	0.859	0.660	0.902
Hip circumference					1.0	0.652	0.923
Sum of skinfolds						1.0	0.667
Weight							1.0

of our findings of decreased fecundability. Elevated leptin concentrations have also been shown to be strongly associated with obesity in different populations [38]. Obesity in turn has been associated with decreased fecundability, decreased ovarian responsiveness, and poorer oocyte quality [5]. The differing results we observed between models adjusted for BMI and WHR suggest an intricate relation between leptin and reproduction that is likely not independent of adiposity, although may not be explained by central adiposity as compared with overall body mass. Similarly, other markers of adiposity, including waist circumference and sum of skinfolds, also had high correlations with leptin. In addition, the association between leptin and fecundability also was attenuated following adjustment for these markers of adiposity. Interestingly, we found that the correlation between leptin and WHR among women in this study was 0.35, whereas the correlation with BMI was 0.8. The higher correlation between leptin and BMI perhaps suggests that leptin is a better marker of overall body mass than central adiposity or other measures in this cohort, and that the reproductive outcomes studied here are more sensitive to overall body mass than central adiposity. The interplay between leptin and neuroendocrine hormones necessary for oogenesis and ovulation are only partly explained by metabolism and future studies are needed to disentangle the complicated relationships between leptin, adiposity, and pregnancy.

Our study had several strengths including the prospective design of the parent trial and careful, detailed assessment of time to pregnancy by trained personnel. Leptin concentrations increase as pregnancy progresses [39], therefore, our assessment of preconception leptin concentrations is less prone to measurement error because of variability during pregnancy than studies relying on early pregnancy measures as markers of preconception status. We also assessed preconception WHR and BMI, which were measured by trained personnel. Additionally, we were able to compare models adjusted for WHR, BMI, and other markers of adiposity to help understand leptin's effect on reproductive outcomes outside of its relation with these markers of adiposity. Interestingly, the findings remain consistent when adjusted

Table 5. FORs and 95% CI[a] for Preconception Leptin Tertiles[b] and Time to Pregnancy, Adjusting for Multiple Markers of Adiposity

	Tertile 1	Tertile 2	Tertile 3
Model 1	Reference	1.10 (0.89, 1.36)	0.85 (0.64, 1.14)
Model 2	Reference	1.14 (0.92, 1.41)	0.92 (0.69, 1.25)
Model 3	Reference	1.14 (0.92, 1.41)	0.87 (0.67, 1.14)
Model 4	Reference	1.09 (0.88, 1.35)	0.83 (0.62, 1.11)

Model 1: Adjusted for age, physical activity, treatment arm, and waist circumference.
Model 2: Adjusted for age, physical activity, treatment arm, and hip circumference.
Model 3: Adjusted for age, physical activity, treatment arm, and sum of skinfolds (triceps, subscapular, suprailiac).
Model 4: Adjusted for age, physical activity, treatment arm, and weight.
[a]Calculated using Cox proportional hazard regression models, accounting for left truncation and right censoring.
[b]Reference is Tertile 1 <11.22 ng/mL; tertile = 11.22–26.24 ng/mL; tertile > 26.25 ng/mL.

Table 6. RRs and 95% CIs[a] for Preconception Leptin Tertiles[b] and Pregnancy and Live Birth, Adjusting for Multiple Markers of Adiposity

	Pregnancy[c] (n = 767/1053)			Live Birth[d] (n = 572/1042)		
	Tertile 1	Tertile 2	Tertile 3	Tertile 1	Tertile 2	Tertile 3
Model 1	Reference	1.07 (0.99, 1.17)	0.98 (0.85, 1.13)	Reference	1.07 (0.99, 1.17)	0.98 (0.85, 1.13)
Model 2	Reference	1.08 (1.00, 1.18)	0.99 (0.86, 1.14)	Reference	1.08 (1.00, 1.18)	0.99 (0.86, 1.14)
Model 3	Reference	1.08 (0.99, 1.17)	0.95 (0.84, 1.07)	Reference	1.08 (0.99, 1.17)	0.95 (0.84, 1.07)
Model 4	Reference	1.07 (0.99, 1.16)	0.98 (0.85, 1.12)	Reference	1.07 (0.99, 1.16)	0.98 (0.85, 1.12)

Model 1: Adjusted for age, physical activity, treatment arm, and waist circumference.
Model 2: Adjusted for age, physical activity, treatment arm, and hip circumference.
Model 3: Adjusted for age, physical activity, treatment arm, and sum of skinfolds (triceps, subscapular, suprailiac) – four missing observations for pregnancy analysis.
Model 4: Adjusted for age, physical activity, treatment arm, and weight.
[a]Calculated using inverse probability weighted log-binomial regression.
[b]Reference is Tertile 1 <11.22 ng/mL; Tertile 2 = 11.22 - 26.24 ng/mL; Tertile 3 > 26.25 ng/mL.
[c]Weights account for withdrawal prior to pregnancy (n = 128).
[d]Weights account for withdrawal after hCG-pregnancy (n = 11), withdrawal and nonpregnancy (n = 118), and nonpregnancy only (n = 286).

for WHR, often considered a better marker of central adiposity than BMI, although we observed attenuated findings after adjustment for BMI. Given that the women in our study were young, healthy, and had no prior diagnosis of infertility, our results may be more generalizable to a larger population of reproductive age women. Limitations of our study include the need for representation from women with varying ethnic backgrounds to address the ethnic variations associated with leptin and other markers of obesity and reproductive outcomes. Furthermore, many of our samples were taken when patients were not fasting. Although this collection method could have potentially affected our results, our results remained consistent when adjusting for fasting status. Another potential limitation is that leptin values were not measured in the menstrual cycle immediately prior to pregnancy. However, because changes in weight and BMI were minimal among women who conceived, it is unlikely that leptin levels would have had meaningful variation within the preconception period.

Overall, we observed associations between higher preconception leptin concentrations and reduced fecundability, pregnancy, and live birth. These associations appear to be explained by the strong relation between BMI and leptin rather than other obesity markers. Although there may not be a singular or simple surrogate for adiposity, there is a growing need to understand the pathways in which leptin and obesity decreases female fecundability and likelihood of pregnancy. Given the current obesity epidemic, understanding the impact that leptin and other markers of adiposity have on human reproduction is imperative.

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Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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