

CLINICAL STUDY

Decreased ovarian function is associated with obesity in very long-term female survivors of childhood cancer

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Abstract

Objective: Obesity and gonadal dysfunction are known major side effects of treatment in adult childhood cancer survivors (CCS). In the general population, obesity has a negative influence on female fertility. We aimed to evaluate whether obesity and serum insulin are associated with decreased ovarian reserve markers in CCS.

Design: Retrospective single-center cohort study.

Methods: Data of 191 female survivors of childhood cancer were analyzed. Median follow-up time was 18.8 (2.3–48.8) years. Outcome measures were serum anti-Müllerian hormone (AMH) and total follicle count (FC). Potential risk factors were: BMI; body composition measures, determined by dual-energy X-ray absorptiometry (total fat percentage, lean body mass, and visceral fat percentage); and fasting insulin.

Results: Lower serum AMH was found in obese subjects (β (%) -49 , $P=0.007$) and in subjects with fasting insulin in the highest tertile (β (%) -43 , $P=0.039$). Total fat percentage tends to be associated with serum AMH (β (%) -2.1 , $P=0.06$). Survivors in the highest tertile of insulin had significantly lower FC than survivors in the lowest tertile (β -6.3 , $P=0.013$). BMI and other measures of body composition were not associated with FC. Correlation between serum AMH and antral follicle count (AFC) was $\rho=0.32$ ($P=0.08$).

Conclusions: Obesity and insulin resistance are associated with gonadal damage, as reflected by decreased AMH and reduced FC in adult survivors of childhood cancer. In contrast to its highly predictive value for AFC in the healthy female population, serum AMH does not seem to correlate as well with AFC in CCS.

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Introduction

The prevalence of obesity has increased dramatically since the 1970s (1). It is a well-described risk factor for diabetes, hypertension, heart disease, stroke, and cancer (2). In healthy women, high BMI also affects reproductive health (3, 4) as reflected by impaired ovulatory function and a lower pregnancy rate (5). In childhood cancer survivors (CCS), obesity is a major side effect, occurring in 9–30%, and mainly depending on previous treatment (6, 7). In adult female CCS, the risk of obesity is increased by 50% when compared with the general population (8).

Gonadal dysfunction is an important side effect of cancer treatment in CCS. Alkylating agents and

abdominal radiotherapy, in particular, can have a deleterious effect on ovarian function (9). To determine ovarian reserve, anti-Müllerian hormone (AMH) was identified as a reliable serum marker (10). It is produced by the granulosa cells of small growing follicles, reflects the size of the primordial follicle pool in the ovaries, and is an indicator of a woman's reproductive capacity (11). It is stable during and between menstrual cycles, in contrast to FSH (12, 13, 14), and is considered to be a valued marker for ovarian reserve, as it corresponds well with antral follicle count (AFC), which reflects reproductive status (10).

In their late reproductive years, the gonadal function of women seems to be affected by obesity (15, 16). Insulin resistance, which occurs at a higher frequency

in adult CCS, may affect granulosa cell function (17, 18). The association between obesity, insulin resistance, and gonadal function, which are more prevalent in CCS than in the normal population, has never been explored. Therefore, the aim of this study was to determine the association between BMI, body composition, insulin levels, and ovarian reserve as reflected by AMH and AFC in a substantial single center cohort of female adult survivors of childhood cancer.

Materials and methods

Subjects

A retrospective single-center cohort study was performed among female survivors that visited our late-effects outpatient clinic for long-term CCS. Inclusion criteria were age ≥ 18 and < 50 years and female CCS diagnosed and treated between 1964 and 2005, who had both serum AMH levels and BMI determined at the same moment at least 5 years after cessation of cancer treatment. Exclusion criteria were ovariectomy, polycystic ovary syndrome (PCOS), and AMH $> 5 \mu\text{g/l}$ (Supplementary Figure 1, see section on supplementary data given at the end of this article). One hundred and five survivors visited the gynecological outpatient clinic for screening. In the remaining 180 CCS, we were not able to distinguish between PCOS and non-PCOS subjects because data on hyperandrogenism (clinical and biochemical) and total follicle counts (FC; transvaginal ultrasound) were not available. As we were not able to use the Rotterdam criteria in these remaining cases, and Dewailly *et al.* (19) suggested a cutoff limit of AMH $> 5 \mu\text{g/l}$ to define PCOM, we used this marker and cutoff limit for the presence of PCOM as one of the criteria of PCOS and excluded these survivors.

Outcome measures (AMH/FC)

Serum samples were taken randomly during the menstrual cycle as AMH has been shown not to fluctuate during the menstrual cycle or during OCP use (12). AMH was measured using an in-house double-antibody ELISA (commercially available through Beckman-Coulter) (10, 20). The intra- and interassay coefficients of variation were < 10 and $< 5\%$ respectively (10, 20). A subset of patients ($n = 91$) underwent a standardized gynecological examination. Clinical examination was performed after an overnight fasting period on a random day and included menstrual history, current cycle length, cycle regularity, height, and weight. Transvaginal ultrasonography was performed to assess ovarian volume and total FC for both ovaries and to exclude other genital abnormalities. FC was called AFC if the FC was performed during the follicular phase (days 2–5) or during amenorrhea. PCOS was diagnosed according to the revised Rotterdam 2003

criteria (21). The presence of polycystic ovaries was defined as ≥ 12 follicles in one or both ovaries and/or increased ovarian volume ($> 10 \text{ ml}$), without the presence of a cyst ($> 10 \text{ mm}$) (22).

Obesity variables

Follow-up data of the most recent visit included the following variables: weight, height, BMI calculated from height and weight (23), and waist:hip ratio (WHR), as measured by waist circumference divided by hip circumference (24). Serum insulin (pmol/l) was measured using a chemiluminescence-based immunoassay (Immulite 2000, Siemens DPC, Los Angeles, CA, USA) after an overnight fasting period. Glucose levels were measured using a Hitachi 917 analyzer (Roche Diagnostics). Insulin resistance was determined by calculation of the homeostasis model assessment (HOMA) score as plasma glucose (mmol/l) \times plasma insulin (mU/l)/22.5 (25). Lean body mass (kg) and percentage of body fat were measured by dual-energy X-ray absorptiometry (DXA, Lunar Prodigy, GE Healthcare, Madison, WI, USA). Visceral fat percentage was calculated from intra-abdominal fat (kg) and total fat (kg) measured by DXA (26). Waist, WHR, and visceral fat percentage were not analyzed in the subset of survivors treated with abdominal radiotherapy because of impairment of local body fat and the frequent occurrence of scoliosis.

Potential confounders

Data concerning treatment protocols, disease, and patient characteristics were retrieved from our local database and were completed using the medical records where necessary. Follow-up time was defined as the time since cessation of treatment. Among patients exposed to alkylating agents, the alkylating agent dose (AAD) score was calculated by determining the drug dose tertile distribution in our entire cohort of survivors and adding the tertile scores (1, 2, and 3) for each of the alkylating agents given to a particular patient as described previously by Green *et al.* and Tucker *et al.* (27, 28). An AAD score of zero was assigned to patients not exposed to any alkylating agent.

Statistical analysis

To examine the associations between obesity variables and AMH or FC we used univariate and multiple linear regression analyses. In all multiple linear regression models, age, age at diagnosis, total body irradiation, abdominal radiotherapy, and AAD score were included as potential confounders. The analyses were performed in several steps. First, BMI, body composition, and insulin were entered as continuous variables. Secondly, to evaluate whether there was an exponential

association with AMH, squared variables of BMI, body composition measures, and insulin were added to the relevant model. Subsequently, all variables were divided into quintiles or tertiles (depending on sample size) and added to the relevant model as dummy variables, with the lowest quintile/tertile as reference category. Additionally, BMI was divided into four categories: $\geq 30 \text{ kg/m}^2$ (obese), $25\text{--}30 \text{ kg/m}^2$ (overweight), $18\text{--}25 \text{ kg/m}^2$ (normal weight), and $< 18 \text{ kg/m}^2$ (underweight) and added to the model as dummy variables with normal weight as reference category. Associations are expressed as standardized regression coefficients because this measure allows direct comparison of the strengths of associations between different determinants. The distribution of AMH was normalized by $^{10}\log$ transformation to improve the plots of the residual analyses and expressed as percentage. *P* values < 0.05 (two-tailed) are considered statistically significant. SPSS 17.0 software (SPSS) was used for statistical analysis.

Results

Gonadal function

The cohort consisted of 425 female survivors of childhood cancer, of whom 292 visited the late-effects

outpatient clinic. Seven survivors were excluded because of previous one-sided or two-sided ovariectomy ($n=6$ and $n=1$ respectively) and two because they were > 50 years old. Sixteen survivors were clinically diagnosed with PCOS by a gynecologist and were therefore excluded. Another 76 survivors were excluded because their AMH levels were $> 5 \mu\text{g/l}$. Finally, 191 female survivors were included in the analysis.

Clinical characteristics and treatment details of the total cohort of female CCS of our center and the survivors included in the study are shown in Table 1. The included sample is representative for the total cohort of female CCS of our center.

At the time of inclusion, 42 of 191 included survivors (22.0%) had regular menstrual cycles, whereas eight survivors (4.2%) had an oligo- or amenorrhea. One survivor had shortening of mean menstrual cycle length (1.3%). No data on menstrual cycles were available in one survivor (1.3%) who delivered recently and in one survivor (1.3%) who was pregnant. In 23 of 191 survivors (30.3%), data on menstrual cycle at the time of screening were not available. All other survivors used oral contraceptive pills (99/191, 51.8%) or were under hormonal replacement therapy (16/191: 8.4%) at the time of follow-up.

Median AMH level of the total group was 1.7 (range 0.1–4.9) $\mu\text{g/l}$. Median total FC was 10 (0–25).

Table 1 Representativeness of included survivors compared with the total group of female adult CCS and survivors in whom AMH was measured.

	Total group of adult female CCS ($n=425$)		Female CCS with AMH measurement ($n=292$)		Included survivors in this study ^a ($n=191$)	
Age at diagnosis (years)	7.3 (0–17.9)		6.2 (0–16.8)		6.3 (0–16.2)	
Age at follow-up (years)	NA		26.5 (17.7–57.4)		27.1 (17.7–50.0)	
Follow-up time (years)	NA		17.8 (2.3–48.8)		18.8 (2.3–48.8)	
Diagnosis <i>n</i> (%)						
ALL and T-NHL	128 (30)		91 (31)		52 (27)	
Acute myeloid leukemia	9 (2)		9 (3)		7 (4)	
B-cell non-Hodgkin lymphoma	25 (6)		18 (6)		12 (6)	
Hodgkin lymphoma	29 (7)		21 (7)		18 (9)	
Bone tumor	22 (5)		14 (5)		14 (7)	
Wilms tumor	47 (11)		41 (14)		31 (16)	
Neuroblastoma	32 (8)		29 (10)		18 (9)	
Germ cell tumor	12 (3)		8 (3)		2 (1)	
Malignant mesenchymal tumor	35 (8)		27 (9)		17 (9)	
Brain tumor	52 (12)		18 (6)		13 (7)	
Other	34 (8)		16 (5)		7 (4)	
Therapy <i>n</i> (%)	<i>n</i> (%)	TCD (Gy)	<i>n</i> (%)	TCD (Gy)	<i>n</i> (%)	TCD (Gy)
Abdominal radiotherapy	27 (7)	25 (10–71)	24 (8)	25 (10–62)	20 (11)	25 (10–60)
Total body irradiation	13 (3)	8 (7–15)	16 (5)	8 (6–12)	11 (6)	12 (7–12)
AAD score						
0	237 (56)		150 (51)		93 (49)	
1	47 (11)		35 (12)		19 (10)	
2	47 (11)		39 (13)		26 (14)	
3	70 (17)		55 (19)		42 (22)	
4	9 (2)		5 (2)		5 (3)	
≥ 5	15 (4)		8 (2)		6 (3)	

NA, not applicable; CCS, childhood cancer survivors; AMH, anti-Müllerian hormone; ALL, acute lymphoblastic leukemia; T-NHL, T-cell non-Hodgkin lymphoma; TCD, total cumulative dose; AAD, alkylating agent dose.

^aAfter exclusion of ovariectomized subjects ($n=7$), PCOS subjects as classified according to the revised PCOS Rotterdam criteria ($n=16$), AMH $> 5 \mu\text{g/l}$ ($n=76$) if subjects were not classified according to the revised PCOS Rotterdam criteria since information about follicle count and hyperandrogenism was not available, and women > 50 years ($n=2$). Data are expressed as median (range) or frequencies (%).

Table 2 Univariate and multivariate linear regression analyses illustrating the influence of BMI, measures of body composition, and insulin on AMH levels.

	AMH (%) <i>n</i> =191 (122) ^a					
	Univariate model			Multivariate model		
	β (%)	95% CI	<i>P</i> value	β (%)	95% CI	<i>P</i> value
Obesity (BMI ≥ 30 kg/m ²)	-55*	-75; -18	0.009	-49*	-68; -17	0.007
Overweight (BMI 25–30 kg/m ²)	1	-35; 56	0.974	15	-20; 64	0.450
Underweight (BMI <18 kg/m ²)	-17	-68; 116	0.698	-21	-64; 76	0.568
BMI	-3.3	-6.8; 0.4	0.081	-2.0	-5.0; 1.0	0.191
Total fat percentage	-3.7*	-6.4; -1.0	0.009	-2.1	-4.2; 0.1	0.06
Lean body mass (kg)	2.0	-11.3; 5.2	0.22	0.4	-2.2; 3.0	0.76
Waist circumference (cm) ^b	-2.0*	-3.7; -0.2	0.025	-0.5	-1.8; 0.9	0.511
Waist:hip ratio ^b	-2.5*	-4.3; -0.2	0.037	0.1	-1.8; 2.6	0.894
Visceral fat percentage ^b	-13*	-22; -2	0.02	-1	-10; 8	0.79
Insulin	-0.4	-0.9; 0.1	0.132	-0.2	-0.6; 0.3	0.48
Insulin 2nd tertile (24–54 pmol/l)	2	-47; 96	0.952	-8	-45; 55	0.761
Insulin 3rd tertile (>54 pmol/l)	-54*	-76; -12	0.019	-43*	-67; -3	0.039
HOMA	-13	-28; 5	0.135	-6	-18; 9	0.387
HOMA 2nd tertile (0.58–1.40)	17	-40; 127	0.636	-8	-46; 9	0.753
HOMA 3rd tertile (>1.40)	-45	-71; 8	0.080	-41	-65; 0.3	0.051

AMH, anti-Müllerian hormone; HOMA, homeostasis model assessment. **P*<0.05.

^aNumber of available DXA scans.

^bFor the dependent variables, waist, waist:hip ratio, and visceral fat percentage, survivors treated with abdominal radiotherapy are excluded from the analysis (*n*=19). Corrected for age, age at diagnosis, total body irradiation, abdominal radiotherapy, and alkylating agent dose (AAD) score.

The Spearman correlation coefficient (ρ) between AMH and AFC was 0.32 (*P*=0.08) in survivors who were screened during the follicular phase or during amenorrhea. FSH was significantly inversely correlated with AMH (ρ =-0.30, *P*<0.001) and was significantly higher in survivors with AMH <0.1 μ g/l compared with survivors with AMH >0.1 μ g/l (20.9 vs 4.9 U/l).

Influence of obesity, body composition, and insulin on AMH

Twenty subjects (10%) were defined as obese (BMI ≥ 30 kg/m²), 25 (13%) as overweight (BMI 25–30 kg/m²), seven (4%) as underweight (BMI <18 kg/m²), and 121 (63%) had normal weight (Supplementary Table 1, see section on supplementary data given at the end of this article). AMH levels were significantly inversely associated with obesity (BMI ≥ 30 kg/m²), high fasting insulin (>54 pmol/l), total fat percentage, waist circumference, WHR, and visceral fat percentage but not with HOMA and lean body mass (Table 2). After adjustment for confounders (age, age at diagnosis, treatment with abdominal or total body irradiation, and AAD score) obesity (BMI ≥ 30 kg/m²) and high fasting insulin (>54 pmol/l) remained significantly associated with AMH (β (%) -48, *P*=0.008, and β (%) -43, *P*=0.039, respectively) (Table 2 and Figs 1 and 2a and b).

Influence of obesity, body composition, and insulin on FC

There were no significant associations between FC and BMI or body composition measures (Table 3). Survivors

with insulin and HOMA in the highest tertile had significantly lower FC than others. After adjustment for confounders, no linear or exponential association between FC and BMI or measures of body composition was found. FC did not differ significantly between quintiles of BMI or body composition (data not shown). Subsequently, FC did not differ between BMI categories. However, there were only five obese subjects with available FCs (data not shown). Insulin was

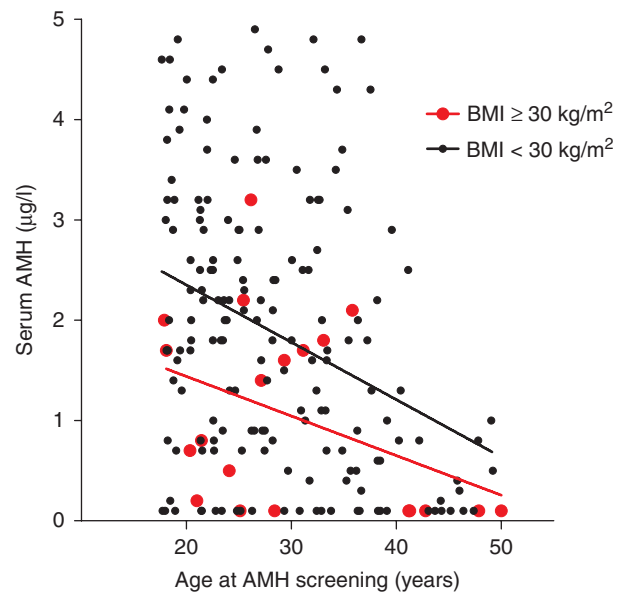


Figure 1 Anti-Müllerian hormone (AMH) in obese (BMI ≥ 30 kg/m²) survivors compared with nonobese survivors.

significantly associated with FC, i.e. survivors with an insulin level in the highest tertile (>48 pmol/l) had significantly lower FC counts when compared with survivors with insulin levels <25 pmol/l ($\beta = -6.3$, $P=0.013$) (Table 3 and Fig. 2c).

Discussion

The current study shows that diminished ovarian reserve, as reflected by low AMH and low FC, was independently associated with obesity and high insulin levels in female CCS. Our results show that obesity is indeed independently associated with decreased AMH in female CCS (6, 29, 30). In the current study, total and visceral fat percentages were not associated with gonadal function, although we observed a trend to a negative association between total fat percentage and AMH levels. This may be due to a size effect or to the fact that we measured total fat percentage and not the fat distribution in different compartments. In fact, for that purpose, abdominal computed tomography, which is the gold standard for measuring intra-abdominal fat mass, is preferred over DXA (31).

Although no oral glucose tolerance tests were performed and therefore some cases in the prediabetic state might have been missed, none of the subjects were diagnosed and treated for diabetes mellitus, based on fasting glucose levels and/or medical history. Therefore, we evaluated fasting insulin levels as a measure for insulin resistance and related these to ovarian reserve markers. The negative association between fasting insulin levels and AMH was previously described in the reproductive-age women without PCOS (16) and was confirmed by our study among CCS.

We identified obesity to be negatively associated with ovarian reserve as assessed by AMH levels. There is no linear association between AMH and BMI, but above a certain threshold (in this case, BMI 30 kg/m²), AMH is significantly lower compared with normal weight subjects. The explanation for this nonlinear association could be that only above a certain threshold of BMI do metabolic changes occur, including insulin resistance, leptin resistance, and elevated levels of adipokines. These factors could play a role in affecting the pituitary–gonadal axis and damaging granulosa cells, although this hypothesis has never been proved. In this study, all subjects with obesity were evaluated, including one survivor treated for craniopharyngioma and one survivor treated with high-dose brain tumor irradiation (> 35 Gy). In these subjects, hypothalamic obesity could not be excluded. To our knowledge, no other studies that assess a possible link between obesity and AMH have been performed in female CCS. In the general population, only one study among three has shown an association in women of reproductive age (15, 16, 32).

Based on these results, we hypothesize that obesity influences the degree of gonadal damage in female CCS. However, it is also conceivable that impaired gonadal function may lead to the development of adiposity and insulin resistance. In animal models, it is known that estradiol has an inhibitory effect on food intake via anorexigenic peptides that decrease meal size. In ovariectomized rats, the removal of estrogens leads to changes in meal size, obesity (33, 34), increased leptin

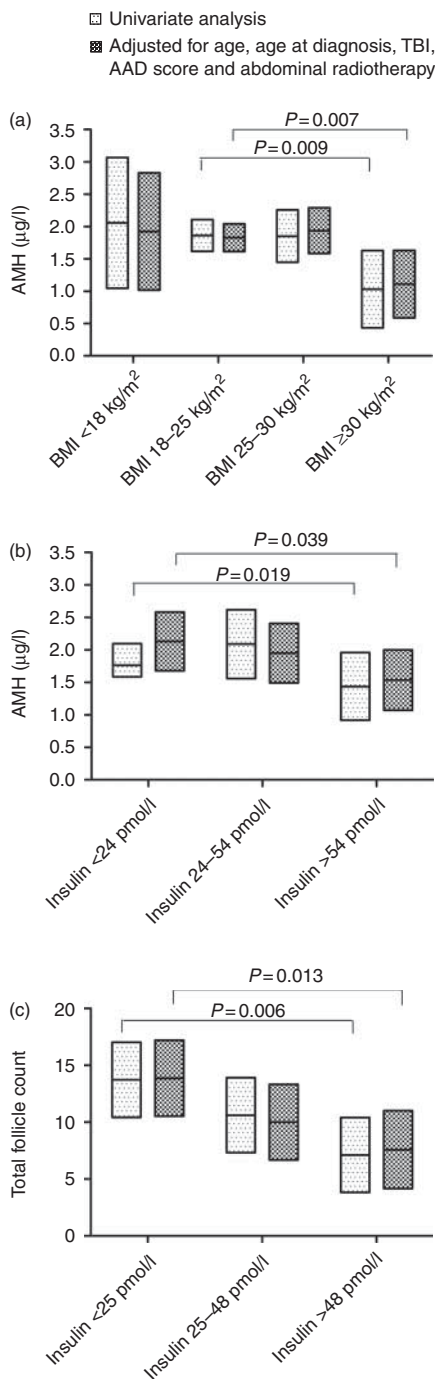


Figure 2 (a) Anti-Müllerian hormone (AMH) in four BMI categories, univariate and after adjustment for possible confounders expressed as mean (95% CI). (b) AMH in serum fasting insulin subgroups expressed as mean (95% CI). (c) Total antral follicle count in serum fasting insulin subgroups expressed as mean (95% CI).

Table 3 Multivariate linear regression analyses illustrating the lacking influence of BMI, measures of body composition, and insulin on total follicle count.

	Total follicle count $n=54$ (34) ^a					
	Univariate model			Multivariate model		
	β (%)	95% CI	<i>P</i> value	β (%)	95% CI	<i>P</i> value
Obesity (BMI ≥ 30 kg/m ²)	-0.6	-7.8; 6.6	0.867	-0.9	-8.0; 6.2	0.804
Overweight (BMI 25–30 kg/m ²)	-2.1	-7.7; 3.5	0.452	-2.1	-8.1; 3.8	0.471
Underweight (BMI < 18 kg/m ²)	-3.0	-13.9; 7.9	0.584	-5.4	-16.4; 5.6	0.330
BMI	-0.03	-0.43; 0.38	0.902	-0.01	-0.42; 0.40	0.978
Total fat percentage	-0.09	-0.44; 0.26	0.614	-0.06	-0.44; 0.32	0.747
Lean body mass (kg)	0.18	-0.15; 0.51	0.266	0.20	-0.17; 0.57	0.278
Waist circumference (cm) ^b	-0.02	-0.24; 0.21	0.890	0.04	-0.18; 0.26	0.732
Waist:hip ratio ^b	-0.06	-0.37; 0.25	0.696	0.05	-0.26; 0.35	0.759
Visceral fat percentage ^b	-0.58	-2.44; 1.28	0.529	-0.18	-2.10; 1.75	0.851
Insulin	-0.03	-0.07; 0.00	0.083	-0.02	-0.06; 0.01	0.192
Insulin 2nd tertile (25–48 pmol/l)	-3.1	-7.8; 1.5	0.186	-3.9	-8.6; 0.8	0.104
Insulin 3rd tertile (> 48 pmol/l)	-6.6*	-11.3; -2.0	0.006	-6.3*	-11.2; -1.4	0.013
HOMA	-0.98	-2.24; 0.27	0.121	-0.70	-2.00; 6.00	0.283
HOMA 2nd tertile (0.66–1.24)	-2.47	-7.22; 2.28	0.302	-3.42	-8.40; 1.57	0.175
HOMA 3rd tertile (> 1.24)	-5.06*	-9.95; -0.18	0.043	-5.01	-10.26; 0.23	0.061

HOMA, homeostasis model assessment. * $P < 0.05$.

^aNumber of available DXA scans.

^bFor the dependent variables, waist, waist:hip ratio, and visceral fat percentage, survivors treated with abdominal radiotherapy are excluded from the analysis ($n=19$). Corrected for age, age at diagnosis, total body irradiation, abdominal radiotherapy, and AAD score.

sensitivity, and decreased insulin sensitivity (35). However, insulin resistance could also decrease granulosa cell function, which could lead to reduced ovarian function and therefore lower AMH levels (17, 18). Our hypothesis fits with the result of a study in type 2 diabetes mellitus (T2DM) patients, in which AMH levels were significantly lower than in healthy controls (36), which was possibly a result of insulin resistance in the T2DM patients. Furthermore, the fact that stringent glycemic control in diabetic patients improves menstrual cycles and fertility rates underlines the hypothesis that prolonged hyperglycemia and chronic complications of diabetes negatively affect ovarian reserve (37). Animal models have shown that ovulation was suppressed in hyperglycemic-hyperinsulinemic conditions, due to hypovascularization, follicular atresia, and eventually involution of ovaries, caused by glucotoxicity and the cytotoxic effect of obesity (38). It should, however, be stressed that due to the cross-sectional design of the current study, cause and effect could not be distinguished.

In 105/285 cases, PCOS diagnosis was verified to confirm the Rotterdam criteria, as these survivors also visited the gynecological outpatient clinic. However, in the remaining 180 CCS, we were not able to distinguish between PCOS and non-PCOS subjects because data on hyperandrogenism (clinical and biochemical) and total FC (transvaginal ultrasound) were not available. As we were not able to use the Rotterdam criteria in these remaining cases, and Dewailly *et al.* (19) suggested a cutoff limit of AMH > 5 $\mu\text{g/l}$ to define PCOM, we used this marker and cutoff limit for the presence of PCOM.

We recognize the limitation of this cutoff limit as we probably excluded more subjects than we would have done if we were able to exclude them based on the Rotterdam criteria. However, we believe that this is the best way to make our population as homogeneous as possible. Moreover, the remaining subjects were representative of the whole cohort of female CCS according to age, age at follow-up, diagnosis, and treatment. As we agreed that the use of the cutoff value is of limited accuracy, we also performed sub-analyses in the 105 cases that were classified based on the Rotterdam criteria. Sixteen survivors were diagnosed with PCOS and were therefore excluded from this analysis. We found a trend to an association between total fat percentage and AMH, although not significant ($P=0.053$). This is the same trend as found in our previous analyses. However, we did not find an association between BMI and AMH, which might be due to the underrepresentation of obese survivors in this subset ($n=5$). If we perform multivariate analyses in the whole group of survivors with AMH levels ($n=285$), we observe no significant correlations with obesity, which fits with the hypothesis that obese women with PCOS have raised AMH levels, while obese women without PCOS have lower AMH levels.

Despite the negative association between FC and serum insulin, we did not find an association between FC and obesity, in contrast to AMH. However, it should be stressed that FCs were available in only five obese subjects. So, power issues may be important. Larger cohorts are necessary to study this association in the future.

In healthy females, AFC and AMH correlate very well (39), but this study shows that this correlation is weak among CCS. This may be due to the fact that follicular AMH expression is lower in CCS treated with gonadotoxic therapies. To our knowledge, no large studies have been performed in female CCS in which the correlation has been studied. Therefore, we cannot draw any firm conclusions based on our small subset analyses regarding the real correlation between AFC and AMH. We believe that it is important to study this association prospectively in a large nationwide cohort, such as the DCOG LATER-VEVO study (40).

We did not correct our analyses for smoking and OCP use. Smoking is linked to ovarian aging in the general population (41). However, we did not find a significant difference in AMH levels between smokers and non-smokers. The fact that smoking significantly influences ovarian reserve in the general population but not in female CCS may be caused by the large effect of the gonadotoxic treatment that may overshadow the influence of smoking on ovarian reserve. Whether OCP use affects AMH levels is still a matter of debate. In our study, we did not find an association between OCP and AMH. Therefore, we did not include smoking and OCP use as confounders.

In conclusion, low serum AMH is associated with obesity and high insulin levels, and low FC with high insulin levels in a large cohort of adult female CCS. Furthermore, despite its highly predictive value for AFC in the healthy female population, serum AMH seems to correlate only weakly with AFC in CCS.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-13-0114>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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