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Ovarian markers and irregular menses among women with type 1 diabetes

in the Epidemiology of Diabetes Interventions and Complications study

Running title: Ovarian markers and type 1 diabetes

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Lifescan (Milpitas, CA), Medtronic Diabetes (Minneapolis, MN), Nipro Home Diagnostics (Ft. Lauderdale, FL), Nova Diabetes Care (Billerica, MA), Omron (Shelton, CT), Perrigo Diabetes Care (Allegan, MI), Roche Diabetes Care (Indianapolis, IN) , and Sanofi-Aventis (Bridgewater NJ).

Summary

Objective Women with type 1 diabetes have increased risk of infertility compared to women without diabetes even after adjustment for irregular menses, but etiologies are incompletely understood. Our aim was to examine the prevalence of abnormalities in ovarian markers consistent with polycystic ovary syndrome in women with type 1 diabetes and associations with irregular menses and diabetes-specific variables.

Design, Patients, and Measurements We conducted a secondary analysis of women in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC), a randomized trial and observational follow-up of intensive insulin therapy for type 1 diabetes. We included women with anti-Müllerian hormone (AMH) measurements among women not using oral contraceptives (n=187). Initial AMH and testosterone measures were performed between EDIC years 1-4. History of irregular menses was assessed annually.

Results The median age of women was 35 (interquartile ratio 29, 40) years; 133 (35%) had elevated AMH and 62 (17%) reported irregular menses. Twelve percent of women had relative elevations in total testosterone. In multivariable models, lower insulin dosages were associated with higher AMH concentrations (p=0.0027), but not diabetes duration, glycemic control, body mass index, or irregular menses. Neither irregular menses nor diabetes-specific variables were associated with testosterone concentrations.

Conclusions Among women with type 1 diabetes in their thirties, abnormalities in ovarian markers are common and not associated with irregular menses, and thus may partially account for decreased fecundity in women with type 1 diabetes.

Keywords: fertility, ovary, type 1 diabetes, women

Women with type 1 diabetes have a higher risk of infertility compared to women without diabetes, even after accounting for the higher prevalence of irregular menses among women with type 1 diabetes[1-4]. This higher risk has been attributed to suboptimal glycemic control[4, 5] as well

as the higher prevalence of autoimmune diseases such as thyroid disorders among women with type 1 diabetes[6]. Women with type 1 diabetes may also have a high prevalence of polycystic ovary syndrome (PCOS), suggested by elevated levels of ovarian markers such as anti-Müllerian hormone (AMH)[7] and testosterone which are made by the gonads[8].

Few studies have examined these ovarian markers among women with type 1 diabetes after their adolescence and early twenties. For several reasons, it is important to examine ovarian markers and predictors of such markers at older ages. In general, women are at increased risk of infertility as they age, particularly in their thirties or their 4th decade of life [9]. Due to years of intensive insulin therapy, women with type 1 diabetes may be particularly vulnerable to obesity as they age[10]. Longer duration of diabetes and prolonged exposure to elevations in glucose could also adversely affect the ovary through deposition of advanced glycation end-products[11]. Exogenous insulin may also have gonadotropic effects[12], particularly because supraphysiologic systemic insulin levels are significantly higher in persons with type 1 diabetes due to the need to achieve physiologic levels in the portal circulation[13].

Therefore, due to previous reports noting increased risk for infertility among women with type 1 diabetes as well as prior reports noting high prevalence of PCOS among women with type 1 diabetes[13], we examined the prevalence of elevations in AMH and testosterone and associations with irregular menses and diabetes-specific variables. We conducted a secondary analysis of a large population of well-characterized reproductive-age women (n=379 women) with type 1 diabetes with an average of 35 years. We used data from participants in Epidemiology of Diabetes Interventions and Complications (EDIC) study[14], which is the ongoing observational follow-up to the Diabetes Control and Complications Trial (DCCT)[15]. We hypothesized that irregular menses as well as longer diabetes duration, higher body mass index (BMI), poorer glycemic control, and higher insulin dosage would be associated with abnormal concentrations in ovarian markers.

Materials and Methods

Population and Setting

The DCCT and EDIC studies have been described in detail. Briefly, the DCCT was a multicenter, randomized clinical trial designed to compare the impact of intensive vs. conventional diabetes treatment on the development and progression of early microvascular complications of type 1 diabetes[16]. From 1983-1989, 1,441 patients (including 680 women) were enrolled at 29 centers. The goal of intensive therapy was to maintain glycemic control as close to the non-diabetic range as possible using ≥ 3 daily insulin injections or an insulin pump, with dose adjustment guided by frequent self-monitoring of blood glucose. Conventional treatment consisted of 1-2 daily insulin injections without stipulated target glucose concentrations. The DCCT included a primary prevention cohort and a secondary intervention cohort. The primary prevention cohort consisted of 726 subjects with no retinopathy, urinary albumin excretion rate < 40 mg/24 hours, and diabetes duration of 1–5 years at DCCT baseline. The secondary intervention cohort consisted of 715 subjects who had non-proliferative retinopathy, urinary albumin excretion rate ≤ 200 mg/24 hours, and diabetes duration of 1–15 years. Individuals were excluded from participating in the DCCT if they were hypertensive, taking any blood pressure or lipid-lowering medications, or had a history of symptomatic ischemic heart disease or symptomatic peripheral neuropathy. Informed consent was obtained from all participants through the institutional review boards at participating centers.

Beginning in 1994 and continuing to the present, clinical, behavioral, and biochemical endpoints have been obtained annually during EDIC by history, physical exam, and laboratory testing. Variables include smoking, BMI, waist circumference, insulin dosage, medication use, and hemoglobin A1c (HbA1c).   The EDIC standardized annual history included a detailed interview regarding menstrual patterns or discontinuation of menses, gynecologic surgeries, and use of exogenous hormones, particularly oral contraceptive pill (OCP) use[17]. At each of these annual interviews, women were asked "Since the last visit, has the patient had any changes to the conditions mentioned below?" "Irregular menses" was specifically listed as a condition, and women were classified as having any history of irregular menses (yes/no).

Under a separate ancillary study mechanism, a sample of 415 women who had not undergone gynecologic surgery were selected for measurement of AMH. Strategies for sample selection have been previously described [18] (Supplementary Figure 1). Women underwent premenopausal

AMH measurement as close to possible at EDIC baseline and another AMH measurement at EDIC year 10 or prior to menopause; a subgroup of 50 women underwent up to 4 measures prior to menopause. For the purposes of this report, we examined the subset of women not using OCPS who had an AMH measurement and a testosterone measurement obtained during EDIC years 1-4, when women were on average 36-38 years old.

Measurement of AMH concentrations was conducted by the EDIC Central Biochemistry Laboratory at the University of Minnesota (Minneapolis, MN) using a modified second generation enzyme-linked immunosorbent assay from Beckman Coulter (Webster, TX). The limit of quantification and limit of detection for the Gen II AMH assay are 1.14 pmol/l and 0.571 pmol/l, respectively. The modified Gen II assay includes a pre-dilution step which avoids interference with active complement binding [18]. In the EDIC laboratory, the coefficients of variation were 8.1% at a mean concentration of 23.46 pmol/l and 4.2% at a mean concentration of 59.26 pmol/l. For values less than the limit of quantification but above the lower limit of detection, SoftMax Pro software (Sunnyvale, CA) was used to plot values, fit a cubic regression curve, and create splines which were then used to calculate AMH concentrations.

Total serum testosterone was quantitated at the EDIC CBL using a rapid liquid chromatography-tandem mass spectrophotometry assay with a limit of detection of 0.0817 nmol/l. The assay was certified by the Centers for Disease Control Hormone Standardization Program (http://www.cdc.gov/labstandards/hs.html). Inter-assay coefficients of variation were 3.0% at 6.576 nmol/l and 2.6% at 28.53 nmol/l.

Statistical Analysis
We examined the proportion of women with elevated AMH, elevated testosterone concentrations, and histories of irregular menses, as well as combinations of these reproductive abnormalities. Table 1 shows the women's baseline characteristics. There are currently no guidelines or recommendations regarding absolute cutpoints for elevations in AMH [7] or testosterone [19], only that the testosterone cutpoint should reflect the upper 2.5-5th percentile of the distribution for women in the specific study population, which by definition precludes estimates of prevalence. Therefore, we used values identified as consistent with polycystic ovary

morphology (PCOM) obtained from another study of women with type 1 diabetes which used the same AMH assay as our study. In the study by Lebkowska et al[20], an AMH concentration of 26.70 pmol/l had the greatest area under the curve for PCOM (0.85, 95% CI 0.73, 0.97) with 90.2% sensitivity and 70.3% specificity compared to transvaginal ultrasound. In that study, elevations in testosterone concentrations were defined as 3.05 nmol/l, with an interquartile range of 1.74-3.82 nmol/l[20]. Therefore, in our analysis, we examined the proportion of women who exceeded 1.74 nmol/l and 3.05 nmol/l of total testosterone.

We examined the unadjusted associations between the initial AMH/testosterone concentrations in EDIC years 1-4 and other variables measured concurrently (Table 2). Multivariable models were used to examine the adjusted associations between risk factors and AMH or testosterone (Supplemental Tables 1 and 2). AMH and testosterone concentrations were log transformed for analysis. The percent change in AMH or testosterone concentration per unit increase in concurrent risk factor or the percent difference in the mean AMH concentration is presented. HbA1c levels and total daily insulin dose were evaluated as time-weighted variables defined as the running arithmetic mean up to the point of the dependent variable measurement. All analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Results

Table 1 presents the participant characteristics at EDIC baseline. Women had a median age of 35 years (interquartile range 29-40 years). The average diabetes duration was 14 years. Approximately one in five women reported current cigarette use. The average BMI was consistent with overweight. Approximately 21% of women reported a history of irregular menses and over 1/3 had elevated AMH concentrations defined as ≥ 26.70 pmol/l. Twenty-three (12.2%) had testosterone concentrations ≥ 1.74 nmol/l and 5 (2.7%) had concentrations ≥ 3.05 nmol/l. Sixteen percent of women had at least 2 reproductive abnormalities, and only 7 women had irregular menses, elevations in AMH, and elevations in testosterone.

Figure 1 shows AMH by age and Figure 2 shows testosterone concentrations by age. Table 2 presents the mean initial AMH and testosterone concentrations in EDIC years 1-4 by tertiles of each concurrent risk factor. In these unadjusted comparisons, lower age, higher testosterone, and

irregular menses were associated with higher AMH concentrations. Higher tertiles of AMH were associated with higher testosterone concentrations. AMH and testosterone concentrations were not significantly associated with tertiles of BMI, waist circumference, insulin dosage, or glycemic control.

Supplemental Tables 1 and 2 presents risk factor associations with AMH and testosterone concentrations in multivariable linear regression models. Factors associated with higher AMH concentrations included younger age, not smoking, lower insulin dosages, and higher testosterone concentrations. Significant risk factors for testosterone included current smoking and higher AMH concentrations. Neither AMH nor testosterone concentrations were associated with irregular menses in multivariable models. In multivariable logistic regression models, the only variable associated with the presence of at least 2 reproductive abnormalities was age, with older age correlating with a lower odds (odds ratio [OR] 0.87, 95% CI 0.80, 0.94).

Discussion

As women age, they are more likely to report difficulties with fertility, with fecundity decreasing gradually after 32 years of age and more rapidly after 37 years of age[9]. Few reports have examined abnormalities in ovarian markers in women with type 1 diabetes in this age range, although as many as a third of younger women with type 1 diabetes may have PCOM and androgen abnormalities[13]. In a relatively large cohort of well-phenotyped women with type 1 diabetes with a median age of 35 years, we found that AMH elevations affected over 1/3 of the population, consistent with the presence of PCOM. Elevations in testosterone were considerably less common. Although almost one in five women reported irregular menses, irregular menses were not correlated with ovarian markers. Therefore, elevations in AMH are common and could potentially explain decreased fecundity in women with type 1 diabetes, apart from irregular menses.

We also found that factors associated with higher AMH included younger age, higher testosterone concentrations, and not currently smoking as has been reported in other studies[21]. In contrast to other reports, we note that lower insulin doses over time were also correlated with higher AMH concentrations, suggesting that in this late-reproductive age population, higher

exogenous insulin does not act as a gonadotropin and thus does not result in elevations in ovarian markers. Previous reports conflict[20, 22]. We may have found different results due to the relatively older age of our population and our use of time-weighted variables, which accounts for longitudinal changes in insulin dosing over time, as opposed to previous cross-sectional analyses. We also included a significantly larger number of women than in previous reports, which included less than women with type 1 diabetes[20, 22].

The factors associated with higher testosterone concentration included higher AMH and a history of smoking, which was likely due to the fact that women using OCPs were excluded; thus, women who had testosterone measurement were younger and already more likely to smoke than women who did not have testosterone measurement. Due to the presence of insulin receptors on the ovary and associations between elevations in testosterone with PCOM, others have hypothesized that exogenous insulin may act as a gonadotropin in type 1 diabetes. In EDIC women, insulin did not appear to be associated with elevated testosterone concentrations[23]. This may be because the presence of significant elevations in testosterone was rare in EDIC women. In addition, previous reports note that among women with known hyperandrogenemia, androgen levels decline as women age particularly beginning in the 4th decade of life[24, 25], which may also account for the lower androgen levels.

Neither AMH nor testosterone elevations were correlated with the prevalence of irregular menses, even though histories of irregular menses were common in the EDIC population and among other cohorts of women with type 1 diabetes[26, 27]. Thus, irregular menses may independently contribute to decreased risk of fecundity apart from these pathways. Multiple endocrinologic disorders can result in irregular menses, including disorders that are more common in women with type 1 diabetes than in women without diabetes; we have previously reported that the risk of hypothyroidism was high in EDIC, with approximately 25% of women noting hypothyroidism by the 18th year of EDIC follow-up[6]. Others have noted that women with type 1 diabetes have abnormal gonadotropin releasing hormone pulse generation which may also result in irregular menstrual cycles[28].

Strengths of this report include its large and well-characterized population of women with type 1 diabetes who were of older age than in previous reports examining ovarian markers. We used an AMH assay with higher sensitivity and precision than presented in previous studies that used first generation assays, which may be less subject to issues of performance and interpretation than transvaginal ultrasound[7]. Testosterone was performed with mass spectrometry, generally considered more sensitive than other methods[29], an issue particularly important in women. This report also has several limitations. The assessment of reproductive abnormalities was not a primary objective of the EDIC study, and thus women were not asked to record cycle length with menstrual diaries. EDIC did not conduct transvaginal ultrasounds to assess ovarian morphology nor clinical assessments of hyperandrogenism such as measurement of Ferriman-Gallwey scores. Difficulties with fertility and actual thyroid hormone levels were not assessed. There are no universal cutpoints for abnormal levels of AMH and testosterone[19]. We examined cutpoints corresponding with the 25th percentile of total testosterone as well as with the 50th percentile compared to previous reports in younger populations, and thus it is unlikely that lower cutpoints would lead to a significantly higher prevalence of hyperandrogenism[30]. Testosterone was assessed only in women not using OCPs, and it is possible that clinical hyperandrogenemia was masked among the 14% of women who were OCP users or that testosterone concentrations were less elevated among women who chose not to use OCPs, thus underestimating the proportion of women with hyperandrogenemia.

Finally, it is unknown whether the relationships between AMH, testosterone, irregular menses, and PCOS differ between women with and without type 1 diabetes. We did not conduct a comparison between women with and without diabetes. In a previous report, we noted that women in EDIC had lower AMH concentrations than women in a control group, and that these lower concentrations were primarily attributable to a slightly lower prevalence of elevated AMH concentrations[18]. Aside from the comparison of women in EDIC with a control group, two previous reports have noted that women with type 1 diabetes had slightly lower AMH concentrations than women without type 1 diabetes[30, 31]. Reports have conflicted as to whether testosterone concentrations differ between women with and without type 1 diabetes. Lebkowska et al noted that testosterone levels were similar among 37 women with type 1 diabetes vs. 42 women without diabetes by PCOS status,[20] and Soto et al[32] and Codner et al[22] found that

testosterone levels were similar in 28 women with type 1 diabetes and 18 women without diabetes. In contrast, Salonia et al found that testosterone concentrations were higher among women with type 1 diabetes (n=50) than women without type 1 diabetes (n=47),[33] and Escobar-Morreale et al also noted that testosterone concentrations were higher among women with type 1 diabetes without PCOS (n=52) compared to controls (n=18).[34]

We conclude that the prevalence of abnormalities in ovarian markers, specifically elevations in AMH, is common in women with type 1 diabetes at an age where they are at increased risk for infertility. Irregular menses are also common although not associated with AMH, and elevations in testosterone are relatively uncommon. In conjunction with previous reports noting decreased fecundity in women with type 1 diabetes, are findings can be used to guide pregnancy planning and fertility management in women with type 1 diabetes in their thirties. Additional investigation of the reasons for decreased fecundity among women with type 1 diabetes should be conducted, particularly regarding abnormal AMH production.

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Figure 1. Scatterplot of age (years) vs. anti-Müllerian hormone (AMH) concentrations (pmol/l).

Figure 2. Scatterplot of age (years) vs. total testosterone concentrations (nmol/l).

	Women with AMH and testosterone measures (n=187)
Table 1. Participant characteristics at EDIC baseline among women with anti-Müllerian hormone (AMH) and testosterone measurements. Means ± standard deviations or N (%) shown.	
Age (years)	35.4 ± 6.9
Duration of diabetes (years)	14.4 ± 5.2
Baseline oral conceptive use (n, %)	5 (2.8)*
Current smoking (n, %)	29 (16.0)
Irregular menses (n, %)	37 (20.8)
Body mass index (kg/m^2)	26.5 ± 4.2
Waist circumference (cm)	80.1 ± 9.3
Time-weighted insulin dose (units/kg/day)	0.67 ± 0.18
Time-weighted HbA1c (%)	8.1 ± 1.3
AMH (pmol/l)	28.7 ± 32.1
AMH $\geq$ 26.7 pmol/l (n, %)	68 (36.4)
AMH $\geq$ 26.7 pmol/l AND irregular menses (n, %)	17 (9.1)
Testosterone (nmol/l)	1.20 ± 1.02
Testosterone $\geq$ 1.74 nmol/l (n,%)	23 (12.2)

Table 2. Mean ± SD initial anti-Müllerian hormone (AMH) and testosterone concentrations in EDIC years 1-4 by concurrent risk factors. P-values in bold type indicate p<0.05.

Testosterone ≥ 1.74 nmol/l AND AMH ≥ 26.7 pmol/l (n, %)						19 (10.1)
Testosterone ≥ 1.74 nmol/l AND AMH ≥ 26.7 pmol/l AND irregular menses (n, %)						7 (3.7)
Risk factor	AMH		p-value	Testosterone		p-value
Testosterone ≥ 1.74 nmol/l AND AMH ≥ 26.7 pmol/l AND irregular menses (n, %)	N=187	(pmol/l)		N=187	(nmol/l)	10 (5.3)
Age (years) Women with at least 2 of the following: AMH ≥ 26.7 pmol/l /l, testosterone ≥ 1.74 nmol/l or irregular menses (n, %)						30 (16.0)
20-29 years	N=37	47.9 + 35.9	**<0.0001**	N=37	1.46 + 0.92	0.17

*Women using OCPs at the time of AMH and testosterone measurement were excluded, although 5 women were using OCPs at baseline but not at subsequent visits when testosterone was measured.
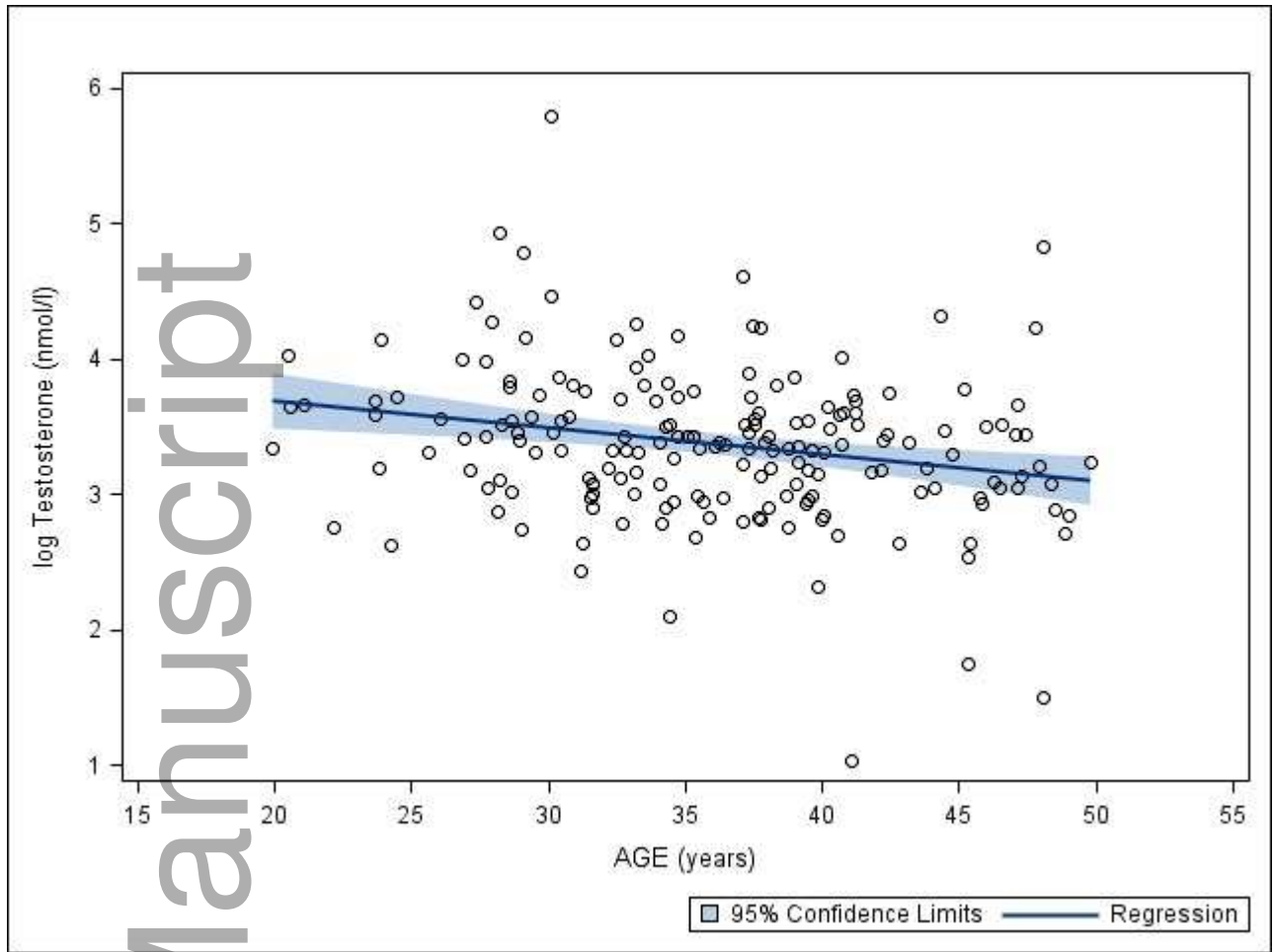
30-39 years	N=99	29.6 ± 31.5		N=99	1.19 ± 1.18	
40-50 years	N=51	12.9 ± 20.9		N=51	1.04 ± 0.67	
Duration of diabetes (years)						
Tertile 1 (6-11)	N=50	26.8 ± 27.6	0.59	N=50	1.32 ± 1.57	0.61
Tertile 2 (11-17)	N=64	32.1 ± 33.9		N=64	1.16 ± 0.81	
Tertile 3 (17-26)	N=73	27.1 ± 33.5		N=73	1.15 ± 0.63	
Oral conceptive use						
Yes	-	-	-	-		--
No	N=187	28.7 ± 32.1		N=187	1.20 ± 1.02	
Current smoking						
Yes	N=28	19.9 ± 27.4	0.12	N=28	1.27 ± 0.62	0.68
No	N=159	30.2 ± 32.7		N=159	1.19 ± 1.07	
History of irregular menses						
Yes	N=49	40.1 ± 44.5	**0.004**	N=49	1.26 ± 0.82	0.62
No	N=136	25.0 ± 25.4		N=136	1.18 ± 1.08	
Body mass index (kg/m^2)						
Tertile 1 (18.9-23.7)	N=49	31.4 ± 3185	0.79	N=49	1.00 ± 0.43	0.26
Tertile 2 (23.7-26.7)	N=62	27.5 ± 34.0		N=62	1.27 ± 1.41	
Tertile 3 (26.7-42.1)	N=76	27.9 ± 31.1		N=76	1.28 ± 0.89	
Waist circumference (cm)						
Tertile 1 (59.8-73.7)	N=46	20.7 ± 26.0	0.14	N=46	0.97 ± 0.44	0.08
Tertile 2 (73.8-81.0)	N=62	32.5 ± 34.2		N=62	1.14 ± 0.49	
Tertile 3 (81.1-117.2)	N=79	30.4 ± 33.2		N=79	1.38 ± 1.45	

Time-weighted insulin dose (units/kg/day)						
Tertile 1 (0.20-0.58)	N=61	30.7 ± 38.3	0.76	N=61	1.23 ± 1.46	0.95
Tertile 2 (0.58-0.74)	N=64	26.5 ± 29.3		N=64	1.17 ± 0.74	
Tertile 3 (0.74-1.39)	N=62	29.1 ± 28.5		N=62	1.21 ± 0.71	
Time-weighted hemoglobin A1c (%)						
Tertile 1 (5.6-7.3)	N=56	27.8 ± 31.0	0.62	N=56	1.19 ± 0.85	0.89
Tertile 2 (7.4-8.4)	N=65	26.4 ± 29.4		N=65	1.16 ± 0.58	
Tertile 3 (8.5-12.2)	N=66	31.7 ± 35.7		N=66	1.25 ± 1.42	
AMH (ng/ml)						
Tertile 1 (0.04-1.4)			--	N=60	0.90 ± 0.57	**<0.0001**
Tertile 2 (1.5-3.8)				N=63	1.05 ± 0.62	
Tertile 3 (3.9-23.5)				N=64	1.63 ± 1.44	
Testosterone (ng/dl)						
Tertile 1 (2.8-23.5)	N=61	16.1 ± 20.5	**<0.0001**			--
Tertile 2 (23.5-33.6)	N=59	22.3 ± 21.1				
Tertile 3 (33.6-329.7)	N=67	45.8 ± 40.6				
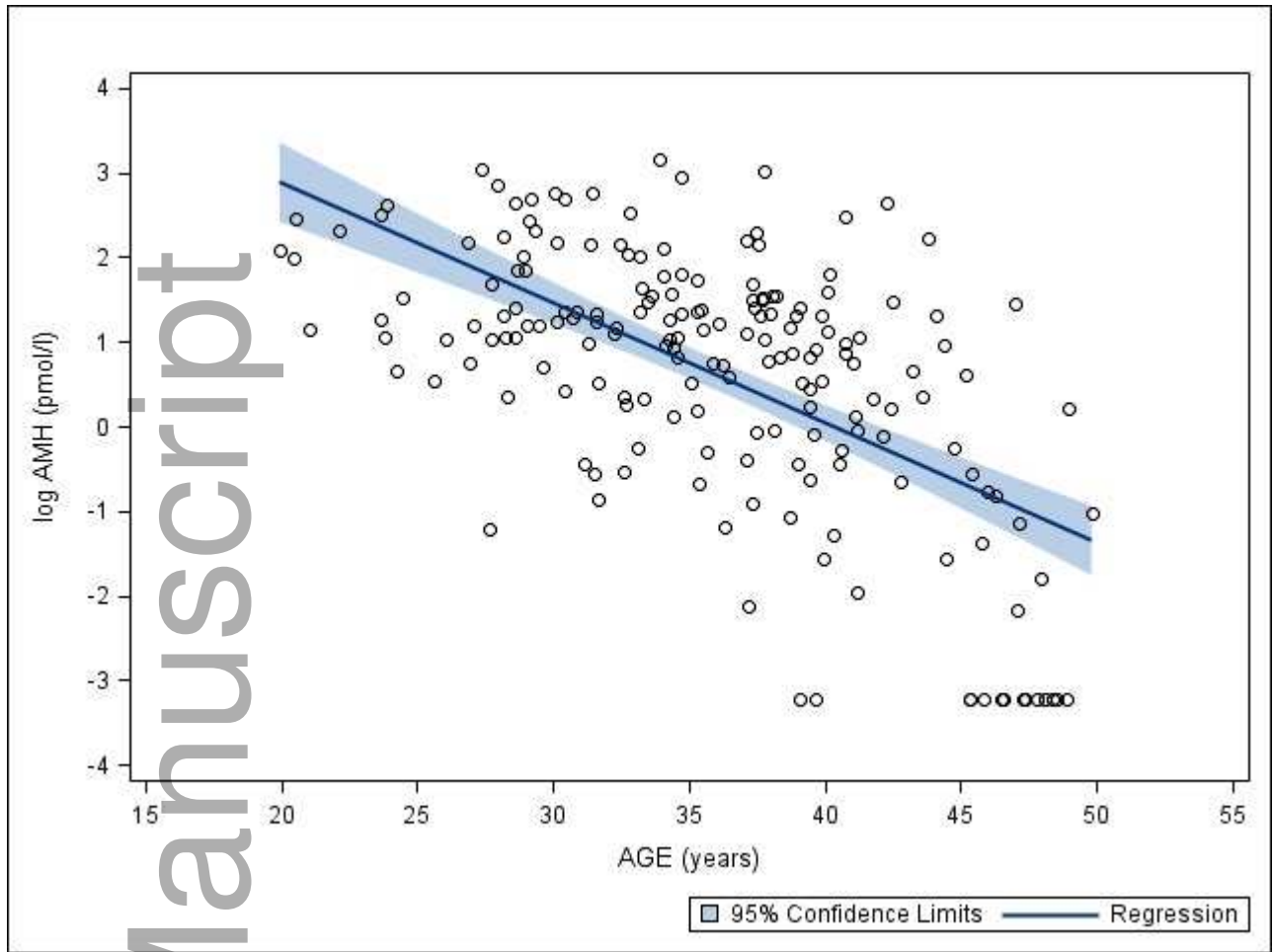
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