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Anti-Müllerian Hormone Levels and Cardiometabolic Disturbances by Weight Status Among Men in the 1999 to 2004 National Health and Nutrition Examination Survey

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Context: Serum anti-Müllerian hormone level (AMH) and body mass index may be jointly associated with cardiometabolic risk.

Objectives: Examine the contribution of AMH to cardiometabolic disturbances by weight status among US adult men.

Design: Cross-sectional analysis using data from the 1999 to 2004 waves of the National Health and Nutrition Examination Survey.

Setting: Multistage probability sampling of the noninstitutionalized US population.

Participants: US men aged ≥18 years. Final analytic sample sizes ranged from 517 to 1063 participants.

Main Outcome and Exposure Measures: Cardiometabolic disturbances (metabolic syndrome and its components, insulin resistance, diabetes, and chronic inflammation) and AMH were obtained from trained staff and nurses in a mobile examination center or during in-home visits.

Results: AMH was directly associated with insulin resistance among obese men [OR 1.08 (95% CI 1.00, 1.15); $P = 0.046$; N = 146], whereas AMH was inversely associated with waist circumference (WC) among obese men [OR 0.95 (95% CI 0.91, 0.99); $P = 0.049$; N = 146]. An inverse relationship was also observed between categorical AMH and diabetes status [medium vs low AMH; OR 0.19 (95% CI 0.043, 0.84); $P = 0.030$; N = 145] among obese men, with a strong inverse relationship also detected among overweight men [high vs low AMH; OR 0.011 (95% CI 0.0004, 0.27); $P = 0.007$; N = 193]. An inverse relationship between continuous AMH and diabetes [OR 0.75 (95% CI: 0.59, 0.93); $P = 0.011$; N = 193] was also detected among overweight men.

Conclusions: AMH was associated with specific cardiometabolic risk factors, including WC, diabetes status, and insulin resistance, in overweight and obese US men.

Abbreviations: AMH, anti-Müllerian hormone level; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; FG, fasting glucose; HDL-C, high-density lipoprotein cholesterol; HbA_{1c}, glycated Hb; HOMA-IR, homeostatic model assessment of insulin resistance; MEC, mobile examination center; MetS, metabolic syndrome; NHANES, National Health and Nutrition Examination Survey; PCOS, polycystic ovary syndrome; SBP, systolic blood pressure; TG, triglyceride; WC, waist circumference.

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The anti-Müllerian hormone (AMH), also known as Müllerian-inhibiting substance, is a homodimeric glycoprotein belonging to the TGF-β superfamily with a molecular weight of 140 kDa [1–4]. The AMH gene is located on chromosome 19 and composed of 2750 nucleotide bases, with established target organs being the Müllerian ducts in males and the gonads in both sexes [3, 4]. The gene encoding for the AMH receptor (comprised of two transmembrane proteins, AMHR1 and AMHR2) is located on chromosome 12 [4] and is exclusively expressed in target organs [3]. AMH is well known for its role in sex differentiation [3]. Sertoli cells begin producing AMH in the fifth week of embryonic development among males, causing the regression of the Müllerian ducts, whereas Leydig cells produce testosterone and promote the differentiation of Wolffian ducts into epididymides, vasa deferentia, and seminal vesicles [4, 5]. Among females, secretion of AMH by granulosa cells does not start until the 36th week of gestation. In the absence of AMH, the Müllerian ducts differentiate into the oviducts, uterus, and upper vagina [3, 5]. AMH exhibits sexually dimorphic behavior in which it plays a major role in male but not female development until puberty [6, 7]. Although testicular production of AMH starts to decline after puberty, ovarian production of AMH is initiated at puberty and ceases at menopause, with adult men and women exhibiting comparable AMH levels [6, 8] and AMH production becoming sexually dimorphic again among the elderly [9, 10].

Since the late 1990s, several ELISA kits have been developed for the quantification of AMH in serum, plasma, and follicular fluid, with a sensitivity of ~1 ng/mL [4]. Unlike other sex hormones, the half-life of AMH in blood exceeds 1 day [11]. Accordingly, a wide range of clinical applications has recently been developed in which serum levels of AMH are considered a biomarker of ovarian age among women [3]. For instance, serum AMH levels have been shown to predict age at menopause [5] as well as fertility after ovarian surgery or cancer therapy [5, 12]. However, the primary target population for AMH testing has been women experiencing infertility [5, 13]. Whereas low serum AMH levels are predictive of diminished ovarian reserve [13], high serum AMH levels are predictive of polycystic ovary syndrome (PCOS) among women [1, 5, 14]. Thus, excessively low or high serum AMH levels can adversely affect response to controlled ovarian stimulation in the context of assisted reproductive technologies [3, 5].

Although inconclusive, current evidence suggests that obesity and its associated cardiometabolic disturbances may be problematic in the context of low and high AMH levels, particularly among women. In fact, women having low serum AMH levels associated with diminished ovarian reserve may exhibit signs and symptoms generally associated with menopause, including obesity and its associated cardiometabolic disturbances [15–20]. By contrast, PCOS, a condition associated with high serum AMH levels, is the most frequent endocrine disorder among women of reproductive age, with an estimated prevalence of 5% to 10% [21, 22], and a substantial proportion of women diagnosed with PCOS are overweight/obese (40% to 70%) and/or insulin resistant (50% to 70%) [23–26].

In recent years, there has been a dramatic increase in the prevalence of obesity in the United States. Obese individuals are at an increased risk of experiencing metabolic syndrome (MetS), a cluster of cardiometabolic disturbances [27] that typically include abdominal adiposity, elevated blood pressure, hyperglycemia, elevated triglycerides (TGs), and reduced level of high-density lipoprotein cholesterol (HDL-C) [28]. Other MetS conditions include inflammation, such as high levels of C-reactive protein (CRP), and various measures of insulin resistance, such as the homeostatic model assessment of insulin resistance (HOMA-IR). MetS

has been consistently associated with increased risks of type 2 diabetes, cardiovascular disease, and all-cause mortality [29].

Although biologically plausible, evidence linking serum AMH level to obesity-related cardiometabolic disturbances remains unsettled among men. In fact, AMH is considered a biomarker for male fertility through spermatogenesis [30]. Few studies have suggested a detrimental effect of obesity on various indicators of male fertility, including semen parameters (sperm concentration, total count, morphology, and motility), as well as hormonal biomarkers such as testosterone and SHBG [30–32]. Although circulating AMH is known to decline with age among men, recent studies have associated serum AMH level with alternative functions besides gonadal development, including the cardiovascular system [6, 33]. In fact, AMHR2 was shown to be expressed in the cardiovascular system [6, 34], and high serum AMH levels have been linked to reduced risk of cardiovascular disease among elderly men [6, 10]. Thus, serum AMH level may be a useful screening tool that can predict cardiometabolic disturbances among obese and nonobese men.

The purpose of this study was to examine the relationships between serum AMH levels and specific cardiometabolic disturbances among adult US men who participated in the 1999 to 2004 National Health and Nutrition Examination Survey (NHANES). We hypothesized an inverse relationship between serum AMH levels and prevalence of cardiometabolic disturbances irrespective of weight status.

1. Materials and Methods

A. Database

NHANES is a series of nationally representative surveys conducted by the National Center for Health Statistics to assess the health and nutritional status of the noninstitutionalized US population. Demographic, socioeconomic, and health data were collected by trained staff and nurses in a mobile examination center (MEC) or during in-home visits. Anthropometric, physiological, and laboratory measurements were collected for all or, in some cases, a subgroup of study participants. Informed consent was obtained for all participants, and the institutional review board of the National Center for Health Statistics approved all protocols for the NHANES [35]. NHANES became a continuous surveillance system in 1999. For these analyses, we combined the 1999 to 2000, 2001 to 2002, and 2003 to 2004 NHANES datasets in which serum AMH level was assessed and subsequently applied a series of selection criteria to fulfill the study purpose.

B. Study Sample

The source population consisted of 31,126 1999 to 2004 NHANES participants (9965 from the 1999 to 2000 wave, 11,039 from the 2001 to 2002 wave, and 10,122 from the 2003 to 2004 wave). Of those, a total of 8091 were considered study eligible after exclusion of females (n = 15,942) and participants <18 years of age (n = 14,065). An additional 7028 participants were excluded for missing data on serum AMH levels, resulting in a final analytic sample ranging from 517 to 1,063 depending on the cardiometabolic outcome of interest. A CONSORT diagram reflecting the sample selection criteria is shown in Fig. 1. When comparing study-eligible men ≥18 years of age (sample 1) to those study analyzed with sample sizes ranging between 1063 (sample 2) and 517 (sample 3), no statistically significant differences in most basic sociodemographic, lifestyle, or health characteristics (age, race, education, marital status, poverty-income ratio, smoking status, and weight status) were observed, with noteworthy differences in alcohol consumption between sample 1 and sample 3 only [36].

C. Exposure

Using surplus specimens, the NHANES 1999 to 2000, 2001 to 2002, and 2003 to 2004 collected data on serum AMH level (nanograms per milliliter), which were analyzed using the

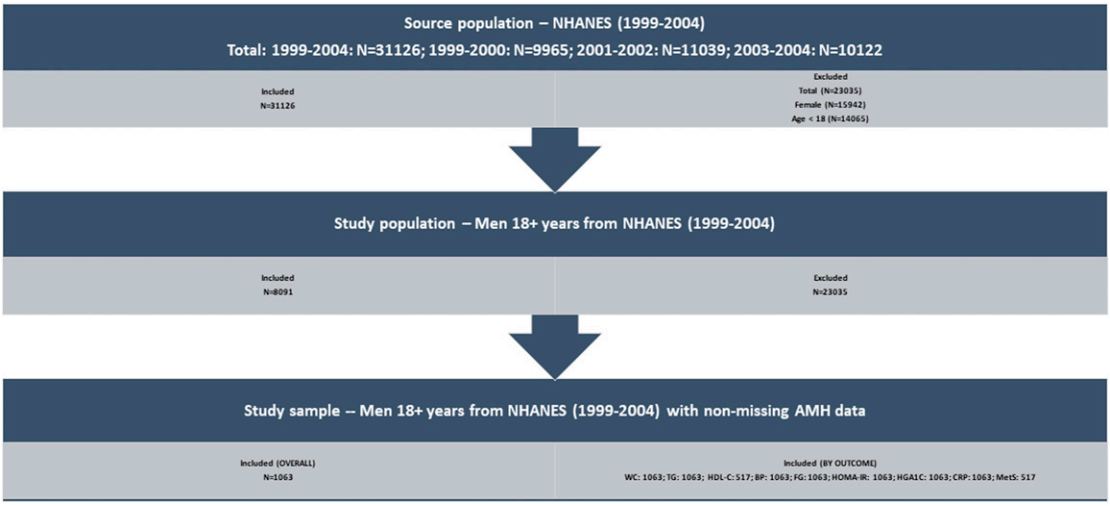

Figure 1. Participant flow chart, 1994–2004 NHANES.

Beckman Coulter AMH Gen II ELISA (Beckman Coulter, Brea, CA), an assay applied clinically in Europe and for research purposes in the United States, with previously reported intra- and interplate reproducibility [37]. We operationalized serum AMH as a continuous measure as well as a categorical measure with cutoffs based on the interquartile range as low (<3.6), medium (3.6 to <11.5), and high (≥11.5).

D. Outcomes

Weight, height, WC, systolic blood pressure (SBP), and diastolic blood pressure (DBP) measurements were collected for all MEC participants during physical examination. Laboratory measurements were performed in subsamples of MEC participants. These included blood lipids (HDL-C and TGs), diabetes profile (glucose, insulin, and glycated Hb [HbA_{1c}]), and CRP. Glucose, insulin, HDL-C, and TG levels were obtained on a subsample of MEC participants after an overnight fast. Self-reported treatments for hypertension, dyslipidemia, or hyperglycemia were obtained through questionnaire data.

The primary outcomes of interest were MetS components, MetS, insulin resistance, diabetes, and chronic inflammation. MetS was defined according to the updated National Cholesterol Education Program Adult Treatment Panel III criteria (2005 revision) as follows [38]: (i) elevated blood pressure (≥130/85 mm Hg) or treatment of hypertension; (ii) increased WC (>40 inches); (iii) elevated fasting glucose (FG) levels (≥100 mg/dL) or treatment of hyperglycemia; (iv) reduced HDL-C level (<50 mg/dL) or treatment of dyslipidemia; and (v) elevated TG levels (≥150 mg/dL) or treatment of dyslipidemia. Each of these MetS criteria was considered as a metabolic disturbance, and MetS was among men who satisfied at least three of those five criteria [38, 39]. Insulin resistance was assessed using the HOMA-IR, which was calculated from fasting levels of insulin and glucose, using the formula [fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5], analyzed as a continuous variable and further categorized based on the cutoff point of 2.5 [40]. Individuals were identified as having diabetes if their HbA_{1c} was ≥6.5% [41]. Chronic inflammation was measured using CRP by latex-enhanced nephelometry and analyzed as a dichotomous variable based on the cutoff point of one suggesting clinically raised CRP [42].

E. Covariates

Sociodemographic, lifestyle, and health-related characteristics were identified as *a priori* confounders for the hypothesized relationships based on the literature. Sociodemographic characteristics included age (in years; 18 to 24, 25 to 29, 30 to 34, 35 to 39, 40 to 44, 45 to

49, 50 to 54, 55 to 59, 60 to 64, 65 to 96, and 70 or older), race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, or other), education (less than high school, high school, and more than high school), marital status (married/living with partner or other), and poverty-income ratio (<100%, 100% to <200%, and ≥200%).

Lifestyle and health-related factors were defined as smoking status (nonsmoker, ex-smoker, or current smoker), alcohol consumption (≥12 glasses in past 12 months) (yes or no), physical activity (walking/bicycling, tasks around home/yard, and moderate activity or vigorous activity in the past 30 days) (yes or no), and weight status [body mass index (BMI) categories]. BMI was calculated as weight (kilograms) divided by height squared (m^2) and categorized using to the World Health Organization definition as underweight/normal weight (<25 kg/m^2), overweight (25 to 29.9 kg/m^2), and obese (≥30 kg/m^2). BMI categories for underweight and normal-weight participants were combined due to sample size limitations. BMI was identified as a measure of weight status and a potential confounder and/or effect modifier for the hypothesized relationships.

F. Statistical Analysis

All analyses were conducted using STATA version 15 (StataCorp, College Station, TX), and the NHANES recommended fasting sample weights for the period of 1999 to 2004. First, we summarized the sociodemographic, lifestyle, health, and cardiometabolic characteristics of the study population by weight status using means ± SEM and median ± interquartile range for continuous variables or frequencies and percentages for categorical variables. Second, we examined bivariate relationships between serum AMH levels and cardiometabolic characteristics by weight status. Third, we examined the relationship between continuous and categorical serum AMH levels and each of the selected cardiometabolic characteristics using multivariable logistic regression models with and without stratification by weight status. We adjusted for *a priori* confounders and considered weight status as a potential confounder and/or effect modifier. ORs were computed with 95% CIs using logistic (svy:logit) regression models, taking sampling weights into consideration. These weights were defined to represent the US civilian, noninstitutionalized population while accounting for oversampling of certain age and ethnic groups as well as interview nonresponse. In fully adjusted regression models that did not stratify by weight status, two-way interactions of AMH-by-BMI were evaluated to assess effect modification by weight status of the relationship between serum AMH levels and the selected outcomes. Two-sided statistical tests were performed at an α level of 0.05.

2. Results

The study sample consisted of up to 1063 eligible US men (356 underweight/normal weight, 409 overweight, and 298 obese) who participated in the 1999 to 2004 NHANES and had complete AMH data, with (mean ± SEM) age of (42.9 ± 0.7) years. As shown in Table 1, there were no statistically significant differences in the study sample's distribution by weight status for most of the selected baseline characteristics including age, race/ethnicity, poverty-income ratio, smoking status, alcohol consumption, and physical activity. By contrast, higher BMI was significantly associated with lower educational attainment and greater likelihood of being married or living with a partner. As expected, a positive and noteworthy relationship was observed between weight status and MetS as well as several MetS components, including WC, TGs, HDL-C, hypertension, and hyperglycemia. Although study participants with higher BMI were significantly more likely to have HOMA-IR ≥2.5, no statistically significant relationships were found between BMI and diabetes or CRP. Finally, there were no remarkable differences between categorical BMI in terms of AMH levels, although a decreasing trend in AMH levels was observed when plotted against BMI using a locally weighted scatterplot smoothing curve (Table 1 and Fig. 2).

Table 1. Demographic, Lifestyle, Health, and Cardiometabolic Characteristics by BMI Groups: 1999–2004 NHANES

	Total (n = 1063) (%)	Underweight/Normal Weight (n = 356) (%)	Overweight (n = 409) (%)	Obese (n = 298) (%)
Age, y				
Mean ± SEM	42.9 ± 0.7	41.1 ± 1.1	43.2 ± 1.4	44.6 ± 1.4
P value			0.025	
18–24	15.2	19.9	15.3	9.7
25–29	9.9	10.5	10.4	8.6
30–34	9.5	7.7	8.9	12.3
35–39	12.9	14.7	13.4	10.2
40–49	16.1	15.9	15.0	17.5
50–59	18.8	16.9	18.1	21.9
60–69	11.6	8.5	12.1	14.7
70+	5.9	5.7	6.7	5.1
P value			0.67	
Race/ethnicity				
Mexican American	7.9	7.4	8.7	7.6
Other Hispanic	4.8	3.0	5.7	5.7
Non-Hispanic white	71.0	68.6	73.5	71.4
Non-Hispanic black	10.9	13.1	9.8	9.9
Other	5.1	7.9	2.2	5.3
P value			0.25	
Education				
Less than high school	22.1	21.5	21.0	24.0
High school	24.4	21.5	27.4	24.5
More than high school	53.5	57.0	51.6	51.5
P value			0.0050	
Marital status				
Married/living with partner	67.9	58.0	68.3	77.9
Other	32.1	41.6	31.7	22.1
P value			0.0050	
Poverty-income ratio				
<100%	11.1	14.7	9.9	8.4
100% to <200%	21.5	20.3	21.7	22.8
≥200%	67.3	64.9	68.4	68.9
P value			0.57	
Smoking status				
Never smoker	44.3	40.3	43.8	49.6
Ex-smoker	27.2	25.6	29.5	26.2
Current smoker	28.4	34.1	26.7	24.1
P value			0.47	
Alcohol consumption (≥12 glasses in past 12 mo)				
Yes	41.1	38.1	40.9	44.9
No	58.9	61.9	59.1	55.1
P value			0.45	
Physical activity				
Yes	89.8	89.3	89.9	90.4
No	10.2	10.7	10.1	9.6
P value			0.93	
MetS				
Yes (3+)	43.9	22.1	42.6	66.4
No (0–2)	56.1	77.9	57.3	33.6
P value			<0.0001	
WC >40 inches				
Yes	41.2	3.1	37.7	89.6
No	58.8	96.8	62.3	10.4
P value			<0.0001	

(*Continued*)

Table 1. Demographic, Lifestyle, Health, and Cardiometabolic Characteristics by BMI Groups: 1999–2004 NHANES (Continued)

	Total (n = 1063) (%)	Underweight/Normal Weight (n = 356) (%)	Overweight (n = 409) (%)	Obese (n = 298) (%)
TG ≥150 mg/dL or Rx				
Yes	46.1	34.0	50.6	54.7
No	53.9	65.9	49.3	45.3
P value			0.0008	
HDL-C <40 mg/dL or Rx				
Yes	49.2	34.9	51.1	61.3
No	50.7	65.1	48.9	38.7
P value			0.0067	
SBP >130 or DBP >85 mm Hg or Rx				
Yes	23.7)	19.4	23.6	28.8
No	76.3	80.6	76.3	71.2
P value			0.095	
FG ≥100 mg/dL or Rx				
Yes	41.0	33.0	39.7	51.7
No	59.0	66.9	60.3	48.3
P value			0.029	
HOMA-IR				
≥2.5	69.2	56.0	67.1	87.3
<2.5	30.8	43.9	32.9	12.7
P value			<0.0001	
Diabetes				
Yes (HbA_{1c} ≥6.5%)	6.6	4.1	4.6	(11.8
No (HbA_{1c} <6.5%)	93.4	95.9	95.4	(88.2
P value			0.077	
CRP				
Mean ± SEM	0.37 ± 0.04	0.21 ± 0.03	0.41 ± 0.09	0.52 ± 0.11
P value			0.001	
≥1.0	5.9	3.9	6.4	7.5
<1.0	94.1	96.0)	93.6	92.5
P value			0.45	
AMH				
Mean ± SEM	8.7 ± 0.5	9.6 ± 0.7	8.2 ± 0.6	8.3 ± 1.0
P value			0.28	
Low	24.7	21.8	24.7	28.3
Medium	50.2	48.7	50.6	51.5
High	25.0	29.5	24.7	20.2
P value			0.51	

Column percentages may not add to 100% due to rounding.
Abbreviation: Rx, prescription.

Table 2 presents the relationships between serum AMH levels and cardiometabolic characteristics in the overall study sample. When defined as a continuous variable or categorized based on cutoffs from the interquartile range, serum AMH level was not significantly related to the selected cardiometabolic characteristics in multivariable logistic regression models. However, these relationships appear to be dependent on BMI, as shown in Tables 3 and 4.

Table 3 displays the relationships between serum AMH level defined as a continuous variable and cardiometabolic characteristics by weight status. These BMI-stratified analyses were consistent with the overall analysis when the outcome of interest was MetS, TGs, HDL-C, hypertension, hyperglycemia, or CRP. By contrast, a significant AMH-by-BMI interaction ($P < 0.0001$) was observed in the context of WC, in which a significant and inverse relationship was observed between serum AMH level and likelihood of WC >40 inches among obese men alone [OR 0.95 (95% CI 0.91, 0.99); $P = 0.049$; N = 146]. Similarly, a significant and inverse relationship [OR 0.75 (95% CI 0.59, 0.93); $P = 0.011$; N = 193] was observed between

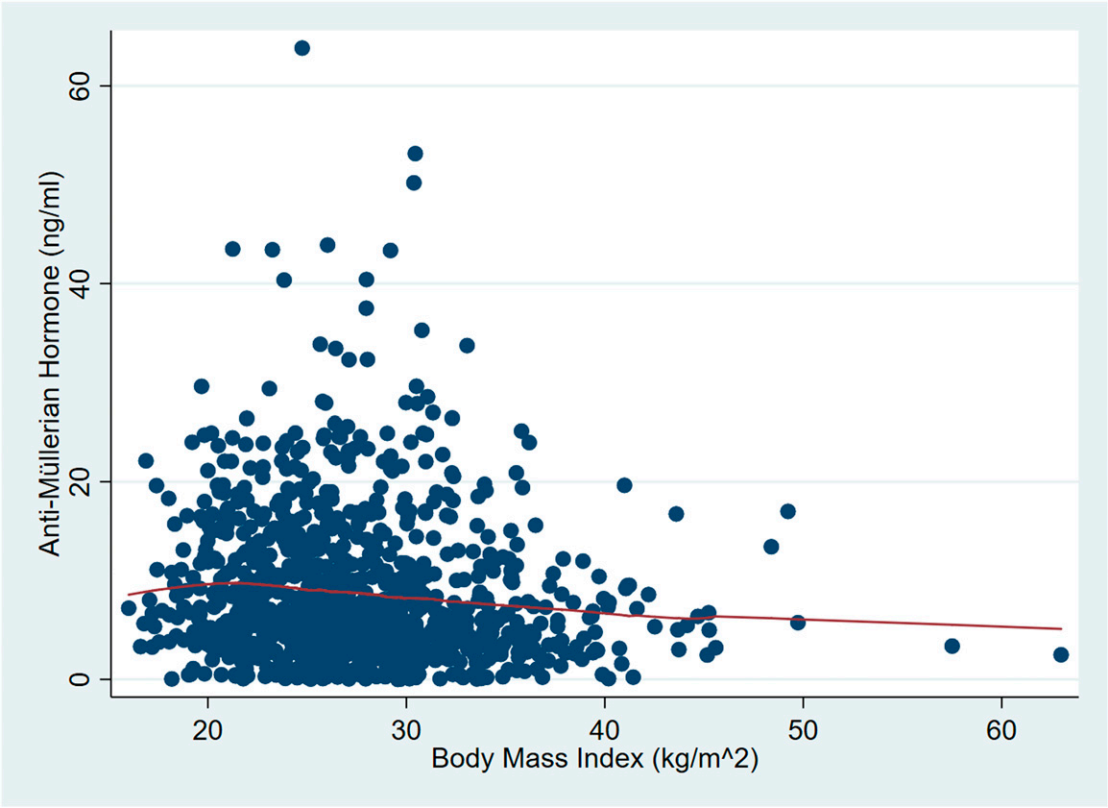

Figure 2. Locally weighted scatterplot smoothing plot for serum AMH level by BMI, 1999–2004 NHANES.

serum AMH levels and diabetes (HbA_{1c} ≥6.5%) among overweight men alone. Finally, a direct and significant relationship was observed between serum AMH level and the likelihood of HOMA-IR ≥2.5 [OR 1.08 (95% CI 1.00, 1.15); $P = 0.046$; N = 146] among obese men alone.

Table 4 displays the relationships between serum AMH level defined as a categorical variable (low, medium, and high) and cardiometabolic characteristics by BMI. Statistically significant AMH-by-BMI interactions were observed in the context of WC and diabetes. Consistent with the continuous outcome, a significant and inverse relationship was observed when comparing medium vs low serum AMH levels on the likelihood of diabetes among obese men [OR 0.19 (95% CI 0.043, 0.84); $P = 0.030$; N = 145] and when comparing high vs low serum AMH levels on the likelihood of diabetes among overweight men [OR 0.011 (95% CI 0.0004, 0.27); $P = 0.007$; N = 193].

3. Discussion

A. Key Findings

Using the 1999 to 2004 NHANES data, we examined the cross-sectional relationships between serum AMH levels and selected cardiometabolic characteristics among adult US men after taking into account weight status and adjusting for a range of potential confounders. Our analyses suggested that as serum AMH levels increased, the odds of abdominal obesity decreased, whereas the odds of insulin resistance increased among obese men. Moreover, as serum AMH levels increased, the odds of diabetes decreased among overweight and obese men; relationships that did not apply to men classified as under- or normal weight. Irrespective of weight status, serum AMH level was uncorrelated with other MetS components, including hypertension, HDL-C, TGs, hyperglycemia, or chronic inflammation. These

Table 2. Relationships Between Serum AMH and Cardiometabolic Characteristics: 1999–2004 NHANES

	AMH (n = 1063)				
	Continuous			Medium vs Low	High vs Low
	n	Mean ± SEM	aOR[a] (95% CI)	aOR[a] (95% CI)	aOR[a] (95% CI)
MetS					
Yes (3+)	249	7.9 ± 1.0	1.00 (0.96, 1.04)	0.76 (0.35, 1.66)	0.93 (0.31, 2.75)
No (0–2)	268	9.1 ± 0.8	Reference	Reference	Reference
WC >40 inches					
Yes	417	7.3 ± 0.6	0.98 (0.94, 1.01)	1.28 (0.55, 2.98)	1.00 (0.39, 2.59)
No	646	9.7 ± 0.6	Reference	Reference	Reference
TG ≥150 mg/dL or Rx					
Yes	686	8.3 ± 0.8	1.00 (0.97, 1.04)	1.00 (0.55, 1.84)	0.82 (0.39, 1.75)
No	377	9.1 ± 0.5	Reference	Reference	Reference
HDL-C <40 mg/dL or Rx					
Yes	282	8.7 ± 0.9	1.01 (0.98, 1.05)	1.32 (0.61, 2.83)	1.52 (0.72, 3.21)
No	235	8.5 ± 0.8	Reference	Reference	Reference
SBP >130 or DBP >85 or Rx					
Yes	294	7.7 ± 0.9	0.97 (0.94, 1.01)	0.98 (0.53, 1.81)	0.68 (0.36, 1.29)
No	769	9.0 ± 0.5	Reference	Reference	Reference
FG ≥100 mg/dL or Rx					
Yes	460	7.2 ± 0.5	0.97 (0.93, 1.00)	1.00 (0.60, 1.66)	0.75 (0.39, 1.42)
No	603	9.7 ± 0.7	Reference	Reference	Reference
HOMA-IR					
≥2.5	610	8.9 ± 0.9	1.01 (0.97, 1.06)	1.73 (0.69, 4.28)	1.53 (0.57, 4.09)
<2.5	225	8.1 ± 0.7	Reference	Reference	Reference
Diabetes					
Yes (HbA_{1c} ≥6.5%)	81	7.1 ± 1.5	0.99 (0.93, 1.06)	0.39 (0.12, 1.28)	0.96 (0.32, 2.90)
No (HbA_{1c} <6.5%)	982	8.8 ± 0.5	Reference	Reference	Reference
CRP, mg/dL					
≥1.0	68	6.6 ± 1.0	0.95 (0.88, 1.01)	Reference	Reference
<1.0	995	8.8 ± 0.5	Reference	1.49 (0.55, 4.04)	0.39 (0.07, 2.09)

Abbreviations: aOR, adjusted OR; Rx, prescription.
[a]Adjusted for sociodemographic, lifestyle, and health-related characteristics.

findings suggest that serum AMH level may serve as a useful biomarker of protective benefits against diabetes as well as risk of insulin resistance, especially hyperinsulinemia, in the context of overweight and obesity. Although the positive relationship between serum AMH levels and insulin resistance and the absence of a similar relationship between AMH and the lipid profile may be counterintuitive, it is plausible that the decline in serum AMH level is a marker of aging, which is often accompanied by chronic disease development, including a decline in β-cell function, leading to onset of diabetes. Furthermore, the measure used to define diabetes (HbA_{1c}) is determined by chronically high glucose levels, whereas the measures used to define insulin resistance (HOMA-IR) and lipid profile (HDL-C and TGs) were acutely evaluated. Given these differences, it is likely that the level of AMH is more strongly associated with chronic rather than acute biological phenomena. Additional studies are needed to validate these study findings.

B. Previous Studies: Literature Review

To date, a limited number of studies have examined the inverse relationship between AMH and obesity-related cardiometabolic disorders. In a cross-sectional study of men aged 22 to 61 years, Andersen *et al.* [30] reported an inverse relationship between BMI and serum AMH levels. A prospective cohort study of 43 severely obese men 20 to 59 years of age found that weight loss was linked to an increase in total sperm count, semen volume, and testosterone, SHBG, and AMH levels [31]. Unlike these two studies, we did not observe a considerable

Table 3. Relationships Between Serum AMH (Continuous) and Cardiometabolic Characteristics by Weight Status: 1999–2004 NHANES

		aOR (95% CI)[a]		
	N	Underweight/Normal Weight	Overweight	Obese
MetS				
Yes (3+)	517	0.96 (0.86, 1.07)	0.97 (0.89, 1.05)	1.05 (0.98, 1.11)
No (0–2)		Reference	Reference	Reference
P interaction = 0.16				
WC >40 inches				
Yes	1063	0.95 (0.86, 1.06)	0.99 (0.93, 1.04)	0.95 (0.91, 0.99)
No		Reference	Reference	Reference
P interaction < 0.0001				
TG ≥150 mg/dL or Rx				
Yes	1063	1.03 (0.96, 1.11)	1.01 (0.95, 1.07)	1.01 (0.97, 1.06)
No		Reference	Reference	Reference
P interaction = 0.98				
HDL-C <40 mg/dL or Rx				
Yes	517	0.99 (0.89, 1.11)	0.99 (0.94, 1.04)	1.05 (0.99, 1.10)
No		Reference	Reference	Reference
P interaction = 0.19				
SBP >130 or DBP >85 or Rx				
Yes	1063	0.99 (0.87, 1.11)	0.98 (0.92, 1.04)	0.97 (0.93, 1.02)
No		Reference	Reference	Reference
P interaction = 0.63				
FG ≥100 mg/dL or Rx				
Yes	1063	0.95 (0.90, 1.01)	0.98 (0.92, 1.03)	0.97 (0.92, 1.01)
No		Reference	Reference	Reference
P interaction = 0.89				
HOMA-IR				
≥2.5	1063	1.03 (0.93, 1.14)	1.01 (0.94, 1.07)	1.08 (1.00, 1.15)
<2.5		Reference	Reference	Reference
P interaction = 0.76				
Diabetes				
Yes (HbA_{1c} ≥6.5%)	1063	1.02 (0.81, 1.29)	0.75 (0.59, 0.93)	1.00 (0.94, 1.07)
No (HbA_{1c} <6.5%)		Reference	Reference	Reference
P interaction = 0.82				
CRP, mg/dL				
≥1.0	1063	Reference	Reference	Reference
<1.0		0.73 (0.51, 1.05)	0.98 (0.88, 1.09)	1.05 (0.98, 1.12)
P interaction = 0.061				

Abbreviations: aOR, adjusted OR; Rx, prescription.
[a]Adjusted for sociodemographic, lifestyle, and health-related characteristics.

difference in serum AMH levels by BMI categories, although we did observe a decreasing trend in serum AMH levels by BMI. Inconsistent findings may be ascribed to differences in study design, populations, and sampling strategies.

Current evidence implies that the relationship between serum AMH levels and cardiometabolic disorders may be bidirectional, with obesity affecting reproductive hormones, including AMH, and AMH potentially linked to cardiovascular disease. In a cross-sectional study of 1337 men between the ages of 18 and 60 years diagnosed with male factor or mixed-factor infertility, Ventimiglia *et al.* [43] reported an inverse relationship between MetS with serum AMH level as well as other sex hormone biomarkers, including total testosterone, SHBG, and inhibin B. In a cross-sectional study of 153 older adult men aged 54 to 93 years, Dennis *et al.* [6] found that, in the absence of vascular disorders, serum AMH levels were inversely correlated with the ultrasonographically determined distal and midinfrarenal aortal diameters, suggesting AMH may be a novel putative regulator of the cardiovascular system. Zhao and Schooling [33] conducted a large case-control study involving Mendelian

randomization and found that genetically predicted AMH was inversely associated with coronary artery disease/myocardial infarction [OR 0.93 (95% CI 0.87, 0.99) per ng/mL log AMH].

Although our results suggested no noteworthy relationship between serum AMH levels and MetS components with the exception of WC among obese men, we found a consistent relationship between serum AMH levels and diabetes, a known risk factor for cardiovascular disease. It is plausible that serum AMH levels may correlate with other sex hormone biomarkers such as testosterone, estradiol, and SHBG, which have been linked to cardiometabolic risk factors such as type 1 and type 2 diabetes among men [44–53]. Because serum AMH levels are less prone to daily fluctuations than other sex hormones, it may be useful as a screening tool for cardiometabolic risk.

The link between AMH and diabetes and/or insulin resistance may operate through hypogonadism. Three categories of hypogonadism exist in men, including primary hypogonadism (testicular defects), secondary hypogonadism (hypothalamus/pituitary defects), and mixed hypogonadism (combination of the two) [54]. AMH is potentially an important marker for congenital secondary hypogonadism among adults and delayed puberty in adolescents [54]. In fact, around the time of puberty, Sertoli cells mature, whereas AMH levels decline due to an increased level of intratesticular testosterone, which inhibits AMH production [55]. The relationship between insulin resistance and hypogonadism, as reflected by free testosterone levels, is shown to be bidirectional among men. In fact, free testosterone is a marker for developing insulin resistance, MetS, and/or type 2 diabetes [54], in which men who are hypogonadal appear to have increased risk for insulin resistance as measured by HOMA-IR and hyperglycemia [54]. Similarly, insulin resistance can suppress liver production of SHBG, resulting in increased levels of free T. Complex interrelationships among AMH, sex hormones, and cardiometabolic risk factors are presented in this study [56].

C. Strengths and Limitations

This is a nationally representative cross-sectional study of US men to examine the evolving hypothesis linking serum AMH levels to cardiometabolic characteristics. With a relatively large sample, we were able to perform stratified analyses by weight status. However, study findings should be examined carefully and in light of several limitations. First, we retrospectively performed analyses of existing data that were collected for the purpose of public health surveillance and not for testing the hypotheses of interest in this study. Although NHANES is generally comprehensive in nature, not all NHANES participants had data collected on all NHANES components. In fact, subsamples were selected by NHANES staff to collect MEC data, and data available for each laboratory test were often conducted on a limited number of NHANES participants, with different sample sizes taken depending on whether the laboratory test was fasting or nonfasting. Of note, AMH level was determined among a subsample of 1999 to 2004 NHANES men with surplus biological samples available. Second, cross-sectional designs preclude the establishment of a temporal or causal relationship between the exposure and outcome of interest. For instance, it is not clear if AMH is protective against diabetes or if diabetes results in reduced AMH levels. Third, selection bias may have resulted from subsampling of the 1999 to 2004 NHANES participants based on the availability of exposure and outcome data. Despite the large initial sample, after applying eligibility criteria and subdividing the sample based on BMI, the comparisons groups were relatively small, and underweight men were underrepresented. It is worth noting, however, that smaller samples based on availability of exposure and outcome data did not differ on basic characteristics from the sample of men, ≥18 years. Therefore, selection bias, especially with regard to distribution by weight status, may not be problematic. Fourth, nondifferential misclassification is a plausible explanation and may have resulted in conservative measures of the association between the main exposure and outcome variables. Fifth, residual confounding may have biased the observed exposure-outcome relationships. Although we adjusted for established risk factors for cardiometabolic disorders, there may be other

Table 4. Relationships Between Serum AMH (Medium vs Low and High vs Low) and Cardiometabolic Characteristics by Weight Status: 1999–2004 NHANES

		aOR (95% CI)[a]		
	N	Underweight / Normal Weight	Overweight	Obese
Medium vs low				
MetS				
Yes (3+)	517	0.64 (0.12, 3.40)	0.37 (0.099, 1.34)	1.31 (0.22, 7.66)
No (0–2)		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.84; *P* interaction (ob vs u/n) = 0.34				
WC >40 inches				
Yes	1063	0.89 (0.24, 33.6)	1.62 (0.55, 4.79)	0.14 (0.012, 1.53)
No		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.59; *P* interaction (ob vs u/n) = 0.22				
TG ≥150 mg/dL or Rx				
Yes	1063	1.51 (0.42, 5.47)	0.97 (0.37, 2.59)	0.46 (0.16, 1.29)
No		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.79; *P* interaction (ob vs u/n) = 0.10				
HDL-C < 40 mg/dL or Rx				
Yes	517	2.44 (0.49, 12.18)	1.03 (0.29, 3.57)	0.69 (0.14, 3.50)
No		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.58; *P* interaction (ob vs u/n) = 0.48				
SBP >130 or DBP >85 or Rx				
Yes	1063	0.59 (0.20, 1.74)	1.04 (0.34, 3.17)	1.19 (0.26, 5.58)
No		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.24; *P* interaction (ob vs u/n) = 0.19				
FG≥100 mg/dl or Rx				
Yes	1063	0.69 (0.24, 1.96)	0.69 (0.26, 1.86)	1.76 (0.66, 4.72)
No		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.94; *P* interaction (ob vs u/n) = 0.33				
HOMA-IR				
≥2.5	1063	3.41 (0.77, 15.22)	1.34 (0.31, 5.78)	1.98 (0.43, 9.17)
<2.5		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.98; *P* interaction (ob vs u/n) = 0.98				
Diabetes				
Yes (HbA_{1c} ≥6.5%)	1063	0.014 (0.000011, 17.94)	0.59 (0.067, 5.33)	0.19 (0.043, 0.84)
No (HbA_{1c} <6.5%)		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.33; *P* interaction (ob vs u/n) = 0.84				
CRP, mg/dL				
≥1.0	1063	Reference	Reference	Reference
<1.0		5.56 (0.30, 103.22)	1.29 (0.21, 7.80)	2.29 (0.38, 13.77)
P interaction (ov vs u/n) = 0.25; *P* interaction (ob vs u/n) = 0.19				
High vs low				
MetS				
Yes (3+)	517	3.08 (0.60, 15.74)	0.55 (0.10, 2.94)	1.36 (0.23, 8.07)
No (0–2)		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.64; *P* interaction (ob vs u/n) = 0.83				

(*Continued*)

Table 4. Relationships Between Serum AMH (Medium vs Low and High vs Low) and Cardiometabolic Characteristics by Weight Status: 1999–2004 NHANES (Continued)

		aOR (95% CI)[a]		
	N	Underweight / Normal Weight	Overweight	Obese
WC >40 inches				
Yes	1063	7.16 (0.013, 4097.84)	1.18 (0.24, 5.81)	0.16 (0.013, 1.97)
No		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.18; *P* interaction (ob vs u/n) = 0.025				
TG ≥150 mg/dL or Rx				
Yes	1063	1.15 (0.32, 4.15)	1.18 (0.28, 4.98)	0.43 (0.11, 1.69)
No		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.62; *P* interaction (ob vs u/n) = 0.26				
HDL-C <40 mg/dL or Rx				
Yes	517	2.97 (0.64, 13.72)	0.99 (0.42, 2.33)	1.25 (0.24, 6.50)
No		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.48; *P* interaction (ob vs u/n) = 0.59				
SBP >130 or DBP >85 or Rx				
Yes	1063	0.36 (0.095, 1.33)	0.77 (0.18, 3.25)	0.78 (0.21, 2.83)
No		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.062; *P* interaction (ob vs u/n) = 0.084				
FG ≥100 mg/dL or Rx				
Yes	1063	0.85 (0.33, 2.2)	0.52 (0.17, 1.57)	0.89 (0.27, 3.00)
No		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.44; *P* interaction (ob vs u/n) = 0.96				
HOMA-IR				
≥2.5	1063	3.19 (0.77, 15.23)	1.32 (0.32, 5.38)	2.91 (0.74, 11.52)
<2.5		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.94; *P* interaction (ob vs u/n) = 0.85				
Diabetes				
Yes (HbA_{1c} ≥6.5%)	1063	1.19 (0.043, 33.07)	0.011 (0.0004, 0.27)	1.18 (0.32, 4.37)
No (HbA_{1c} <6.5%)		Reference	Reference	Reference
P interaction (ov vs u/n) = 0.034; *P* interaction (ob vs u/n) = 0.99				
CRP, mg/dL				
Greater than or equal to median (0.15)	1063	Reference	Reference	Reference
Less than median (0.15)		—	0.64 (0.21, 7.80)	1.71 (0.29, 10.24)
P interaction (ov vs u/n) = 0.74; *P* interaction (ob vs u/n) = —				

Abbreviations: ob, obese; ov, overweight; Rx, prescription; u/n, underweight/normal weight.
[a]Adjusted for sociodemographic, lifestyle, and health-related characteristics.

unobserved confounders at play. In particular, we were unable to adjust for serum FSH, an adipogenic gonadotropin that is inversely correlated with AMH, when examining the relationship between AMH and the cardiometabolic risk factors of interest because during these three waves of NHANES data collection (1999 to 2000, 2001 to 2002, and 2003 to 2004), data on FSH were collected only among women, ≥35 years of age, whereas AMH data were collected on men. Finally, given the large number of outcomes and stratified analyses that can lead to multiple testing potentially necessitating adjustment for false discovery rate, the role of chance cannot be ruled out as an explanation for observed statistically significant results.

Despite these limitations, this study is among the largest to have examined the hypothesized relationships between AMH and various cardiometabolic disorders.

D. Conclusions and Future Studies

In the context of overweight and obese adult men, serum AMH levels may be predictive of specific cardiometabolic characteristics, including WC, diabetes status, and insulin resistance. Prospective cohort studies are needed to evaluate serum AMH levels as well as genetic markers for AMH and their ability to predict future development of cardiometabolic disorders.

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References and Notes

1. Pellatt L, Rice S, Mason HD. Anti-Müllerian hormone and polycystic ovary syndrome: a mountain too high? *Reproduction*. 2010;**139**(5):825–833.
2. Jost A. Recherches sur la differenciation sexuelle de l'embryon de lapin. *Arch Anat Microsc Morphol Exp*. 1946;**36**:271–315.
3. Karkanaki A, Vosnakis C, Panidis D. The clinical significance of anti-Müllerian hormone evaluation in gynecological endocrinology. *Hormones (Athens)*. 2011;**10**(2):95–103.
4. Hampl R, Šnajderová M, Mardešić T. Antimullerian hormone (AMH) not only a marker for prediction of ovarian reserve. *Physiol Res*. 2011;**60**(2):217–223.
5. Loh JS, Maheshwari A. Anti-Mullerian hormone--is it a crystal ball for predicting ovarian ageing? *Hum Reprod*. 2011;**26**(11):2925–2932.
6. Dennis NA, Jones GT, Chong YH, van Rij AM, McLennan IS. Serum anti-Mullerian hormone (AMH) levels correlate with infrarenal aortic diameter in healthy older men: is AMH a cardiovascular hormone? *J Endocrinol*. 2013;**219**(1):13–20.
7. Visser JA, Schipper I, Laven JS, Themmen AP. Anti-Müllerian hormone: an ovarian reserve marker in primary ovarian insufficiency. *Nat Rev Endocrinol*. 2012;**8**(6):331–341.
8. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WH. A validated model of serum anti-müllerian hormone from conception to menopause. *PLoS One*. 2011;**6**(7):e22024.
9. Chong YH, Campbell AJ, Farrand S, McLennan IS. Anti-Mullerian hormone level in older women: detection of granulosa cell tumor recurrence. *Int J Gynecol Cancer*. 2012;**22**(9):1497–1499.
10. Chong YH, Dennis NA, Connolly MJ, Teh R, Jones GT, van Rij AM, Farrand S, Campbell AJ, McLennan IS. Elderly men have low levels of anti-Müllerian hormone and inhibin B, but with high interpersonal variation: a cross-sectional study of the sertoli cell hormones in 615 community-dwelling men [published correction appears in *PLoS One*. 2013;8(10)]. *PLoS One*. 2013;**8**(8):e70967.
11. Griesinger G, Dafopoulos K, Buendgen N, Cascorbi I, Georgoulias P, Zavos A, Messini CI, Messinis IE. Elimination half-life of anti-Müllerian hormone. *J Clin Endocrinol Metab*. 2012;**97**(6):2160–2163.
12. Kelsey TW, Anderson RA, Wright P, Nelson SM, Wallace WH. Data-driven assessment of the human ovarian reserve. *Mol Hum Reprod*. 2012;**18**(2):79–87.
13. Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. *Endocr Rev*. 2009;**30**(5):465–493.
14. La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Artenisio AC, Stabile G, Volpe A. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). *Hum Reprod Update*. 2010;**16**(2):113–130.

15. Figueiredo Neto JA, Figuerêdo ED, Barbosa JB, Barbosa FF, Costa GR, Nina VJ, Nina RV. Metabolic syndrome and menopause: cross-sectional study in gynecology clinic. *Arq Bras Cardiol*. 2010;**95**(3): 339–345.
16. Jouyandeh Z, Nayebzadeh F, Qorbani M, Asadi M. Metabolic syndrome and menopause. *J Diabetes Metab Disord*. 2013;**12**(1):1.
17. Lin JW, Caffrey JL, Chang MH, Lin YS. Sex, menopause, metabolic syndrome, and all-cause and cause-specific mortality--cohort analysis from the Third National Health and Nutrition Examination Survey. *J Clin Endocrinol Metab*. 2010;**95**(9):4258–4267.
18. Mumusoglu S, Yildiz BO. Metabolic syndrome during menopause. *Curr Vasc Pharmacol*. 2018; (Sep):3.
19. Pu D, Tan R, Yu Q, Wu J. Metabolic syndrome in menopause and associated factors: a meta-analysis. *Climacteric*. 2017;**20**(6):583–591.
20. Stefanska A, Bergmann K, Sypniewska G. Metabolic syndrome and menopause: pathophysiology, clinical and diagnostic significance. *Adv Clin Chem*. 2015;**72**:1–75.
21. Brown MA, Chang RJ. Polycystic ovary syndrome: clinical and imaging features. *Ultrasound Q*. 2007; **23**(4):233–238.
22. Costello MF, Shrestha B, Eden J, Johnson NP, Sjoblom P. Metformin versus oral contraceptive pill in polycystic ovary syndrome: a Cochrane review. *Hum Reprod*. 2007;**22**(5):1200–1209.
23. Martínez-Bermejo E, Luque-Ramírez M, Escobar-Morreale HF. Obesity and the polycystic ovary syndrome. *Minerva Endocrinol*. 2007;**32**(3):129–140.
24. Moran LJ, Pasquali R, Teede HJ, Hoeger KM, Norman RJ. Treatment of obesity in polycystic ovary syndrome: a position statement of the Androgen Excess and Polycystic Ovary Syndrome Society. *Fertil Steril*. 2009;**92**(6):1966–1982.
25. Vrbikova J, Hainer V. Obesity and polycystic ovary syndrome. *Obes Facts*. 2009;**2**(1):26–35.
26. Garruti G, Depalo R, Vita MG, Lorusso F, Giampetruzzi F, Damato AB, Giorgino F. Adipose tissue, metabolic syndrome and polycystic ovary syndrome: from pathophysiology to treatment. *Reprod Biomed Online*. 2009;**19**(4):552–563.
27. Meigs JB. Epidemiology of the metabolic syndrome, 2002. *Am J Manag Care*. 2002;**8**(11 Suppl): S283–S292; quiz S293–286.
28. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F American Heart AssociationNational Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*. 2005;**112**(17): 2735–2752.
29. Hu G, Qiao Q, Tuomilehto J, Balkau B, Borch-Johnsen K, Pyorala K; DECODE Study Group. Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in non-diabetic European men and women. *Arch Intern Med*. 2004;**164**(10):1066–1076.
30. Andersen JM, Herning H, Aschim EL, Hjelmesæth J, Mala T, Hanevik HI, Bungum M, Haugen TB, Witczak O. Body mass index is associated with impaired semen characteristics and reduced levels of anti-Müllerian hormone across a wide weight range. *PLoS One*. 2015;**10**(6):e0130210.
31. Håkonsen LB, Thulstrup AM, Aggerholm AS, Olsen J, Bonde JP, Andersen CY, Bungum M, Ernst EH, Hansen ML, Ernst EH, Ramlau-Hansen CH. Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. *Reprod Health*. 2011;**8**(1):24.
32. Eisenberg ML, Kim S, Chen Z, Sundaram R, Schisterman EF, Louis GM. The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. *Hum Reprod*. 2015; **30**(2):493–494.
33. Zhao JV, Schooling CM. Endogenous androgen exposures and ischemic heart disease, a separate sample Mendelian randomization study. *Int J Cardiol*. 2016;**222**:940–945.
34. Ricci M, Mohapatra B, Urbiztondo A, Birusingh RJ, Morgado M, Rodriguez MM, Lincoln J, Vatta M. Differential changes in TGF-β/BMP signaling pathway in the right ventricular myocardium of newborns with hypoplastic left heart syndrome. *J Card Fail*. 2010;**16**(8):628–634.
35. Centers for Disease Control and Prevention. NCHS Research Ethics Review Board (ERB) Approval. Available at: www.cdc.gov/nchs/nhanes/irba98.htm. Accessed 27 November 2012.
36. Beydoun HA, Hossain S, Beydoun MA, Weiss J, Zonderman AB, Eid SM. Data from: Appendix Table 1. Comparison of study sub-samples on basic socio-demographic, lifestyle and health characteristics–1999-2004 NHANES. Figshare 2019. Accessed 12 February 2019. https://figshare.com/articles/Appendix_Table_1/7707452.
37. Wallace AM, Faye SA, Fleming R, Nelson SM. A multicentre evaluation of the new Beckman Coulter anti-Mullerian hormone immunoassay (AMH Gen II). *Ann Clin Biochem*. 2011;**48**(Pt 4):370–373.
38. Huang PL. A comprehensive definition for metabolic syndrome. *Dis Model Mech*. 2009;**2**(5-6):231–237.

39. ACOG Committee on Practice Bulletins--Gynecology. ACOG Practice Bulletin no. 108: polycystic ovary syndrome. *Obstet Gynecol*. 2009;**114**(4):936–949.
40. Zuk AM, Quiñonez CR, Saarela O, Demmer RT, Rosella LC. Joint effects of serum vitamin D insufficiency and periodontitis on insulin resistance, pre-diabetes, and type 2 diabetes: results from the National Health and Nutrition Examination Survey (NHANES) 2009-2010. *BMJ Open Diabetes Res Care*. 2018;**6**(1):e000535.
41. Real A, Ukogu C, Krishnamoorthy D, et al. Elevated glycohemoglobin HbA1c is associated with low back pain in nonoverweight diabetics. *Spine J*. 2019;**19**(2):225–231.
42. Wilkins J, Ghosh P, Vivar J, Chakraborty B, Ghosh S. Exploring the associations between systemic inflammation, obesity and healthy days: a health related quality of life (HRQOL) analysis of NHANES 2005-2008. *BMC Obes*. 2018;**5**(1):21.
43. Ventimiglia E, Capogrosso P, Colicchia M, Boeri L, Serino A, Castagna G, Clementi MC, La Croce G, Regina C, Bianchi M, Mirone V, Damiano R, Montorsi F, Salonia A. Metabolic syndrome in white European men presenting for primary couple's infertility: investigation of the clinical and reproductive burden. *Andrology*. 2016;**4**(5):944–951.
44. Al Hayek AA, Khader YS, Jafal S, Khawaja N, Robert AA, Ajlouni K. Prevalence of low testosterone levels in men with type 2 diabetes mellitus: a cross-sectional study. *J Family Community Med*. 2013; **20**(3):179–186.
45. Daka B, Langer RD, Larsson CA, Rosén T, Jansson PA, Råstam L, Lindblad U. Low concentrations of serum testosterone predict acute myocardial infarction in men with type 2 diabetes mellitus. *BMC Endocr Disord*. 2015;**15**(1):35.
46. Grossmann M, Gianatti EJ, Zajac JD. Testosterone and type 2 diabetes. *Curr Opin Endocrinol Diabetes Obes*. 2010;**17**(3):247–256.
47. Gyawali P, Martin SA, Heilbronn LK, Vincent AD, Taylor AW, Adams RJT, O'Loughlin PD, Wittert GA. The role of sex hormone-binding globulin (SHBG), testosterone, and other sex steroids, on the development of type 2 diabetes in a cohort of community-dwelling middle-aged to elderly men. *Acta Diabetol*. 2018;**55**(8):861–872.
48. Holt SK, Lopushnyan N, Hotaling J, Sarma AV, Dunn RL, Cleary PA, Braffett BH, Gatcomb P, Martin C, Herman WH, Wessells H; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Prevalence of low testosterone and predisposing risk factors in men with type 1 diabetes mellitus: findings from the DCCT/EDIC. *J Clin Endocrinol Metab*. 2014;**99**(9):E1655–E1660.
49. Joyce KE, Biggs ML, Djoussé L, Ix JH, Kizer JR, Siscovick DS, Shores MM, Matsumoto AM, Mukamal KJ. Testosterone, dihydrotestosterone, sex hormone-binding globulin, and incident diabetes among older men: the Cardiovascular Health Study. *J Clin Endocrinol Metab*. 2017;**102**(1):33–39.
50. Kelsey MM, Bjornstad P, McFann K, Nadeau K. Testosterone concentration and insulin sensitivity in young men with type 1 and type 2 diabetes. *Pediatr Diabetes*. 2016;**17**(3):184–190.
51. Kim C, Bebu I, Braffett B, Cleary PA, Arends V, Steffes M, Wessells H, Orchard T, Sarma AV; DCCT/EDIC Research Group. Testosterone and cardiac mass and function in men with type 1 diabetes in the Epidemiology of Diabetes Interventions and Complications Study (EDIC). *Clin Endocrinol (Oxf)*. 2016; **84**(5):693–699.
52. O'Reilly MW, Glisic M, Kumarendran B, et al. Serum testosterone, sex hormone-binding globulin and sex-specific risk of incident type 2 diabetes in a retrospective primary care cohort. *Clin Endocrinol (Oxf)*. 2019;**90**(1):145–154.
53. Svartberg J, Schirmer H, Wilsgaard T, Mathiesen EB, Njølstad I, Løchen ML, Jorde R. Single-nucleotide polymorphism, rs1799941 in the Sex Hormone-Binding Globulin (SHBG) gene, related to both serum testosterone and SHBG levels and the risk of myocardial infarction, type 2 diabetes, cancer and mortality in men: the Tromsø Study. *Andrology*. 2014;**2**(2):212–218.
54. Karakas SE, Surampudi P. New biomarkers to evaluate hyperandrogenemic women and hypogonadal men. *Adv Clin Chem*. 2018;**86**:71–125.
55. Rocha A, Iñiguez G, Godoy C, Gaete X, López P, Loreti N, Campo S, Rey RA, Codner E. Testicular function during adolescence in boys with type 1 diabetes mellitus (T1D): absence of hypogonadism and differences in endocrine profile at the beginning and end of puberty. *Pediatr Diabetes*. 2014;**15**(3): 198–205.
56. Beydoun HA, Hossain S, Beydoun MA, Weiss J, Zonderman AB, Eid SM. Data from: Appendix Figure 1. Conceptual model. Figshare 2019. Deposited 12 February 2019. https://figshare.com/articles/Appendix_Figure_1_tif/7707392.