

### **1. Title of Dataset:**

Supplementary figures and tables to manuscript entitled “Low Progesterone and Low Estradiol Levels Associate with Abdominal Aortic Aneurysms in Men”

### **2. Author Information:**

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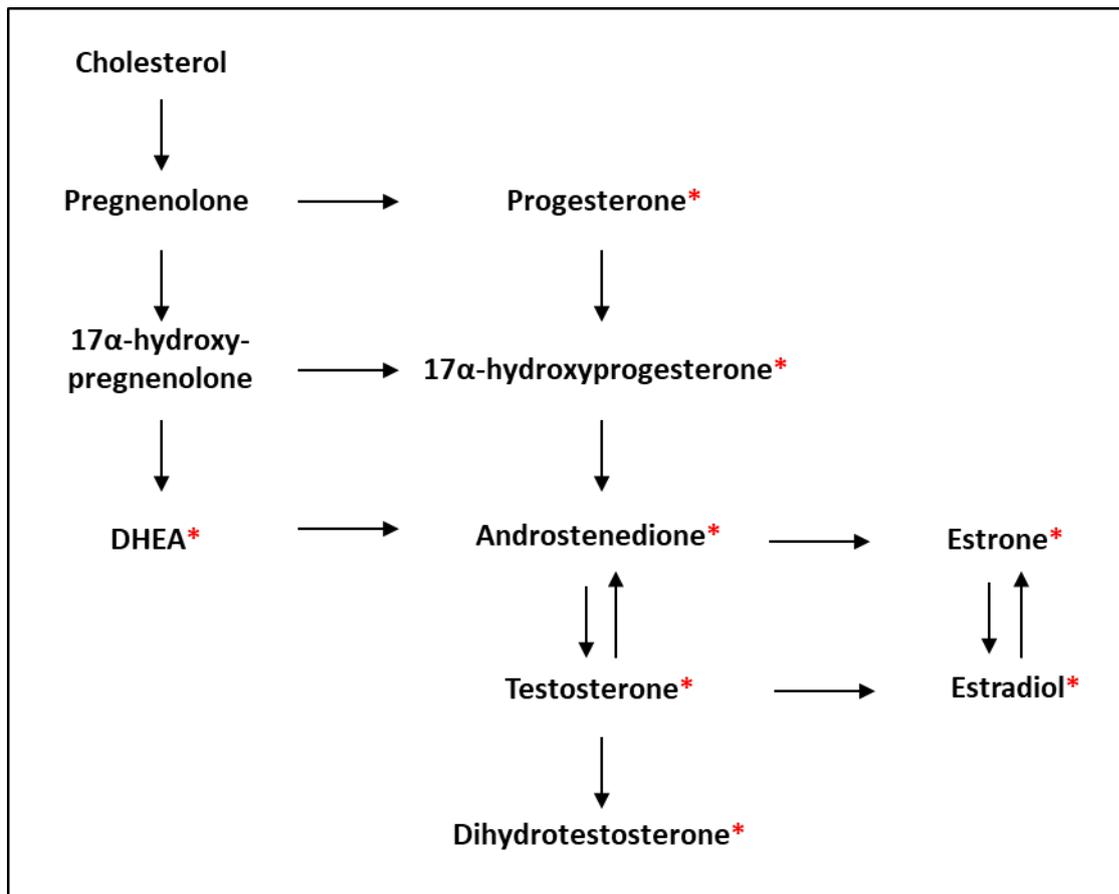
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### **4. Date:**

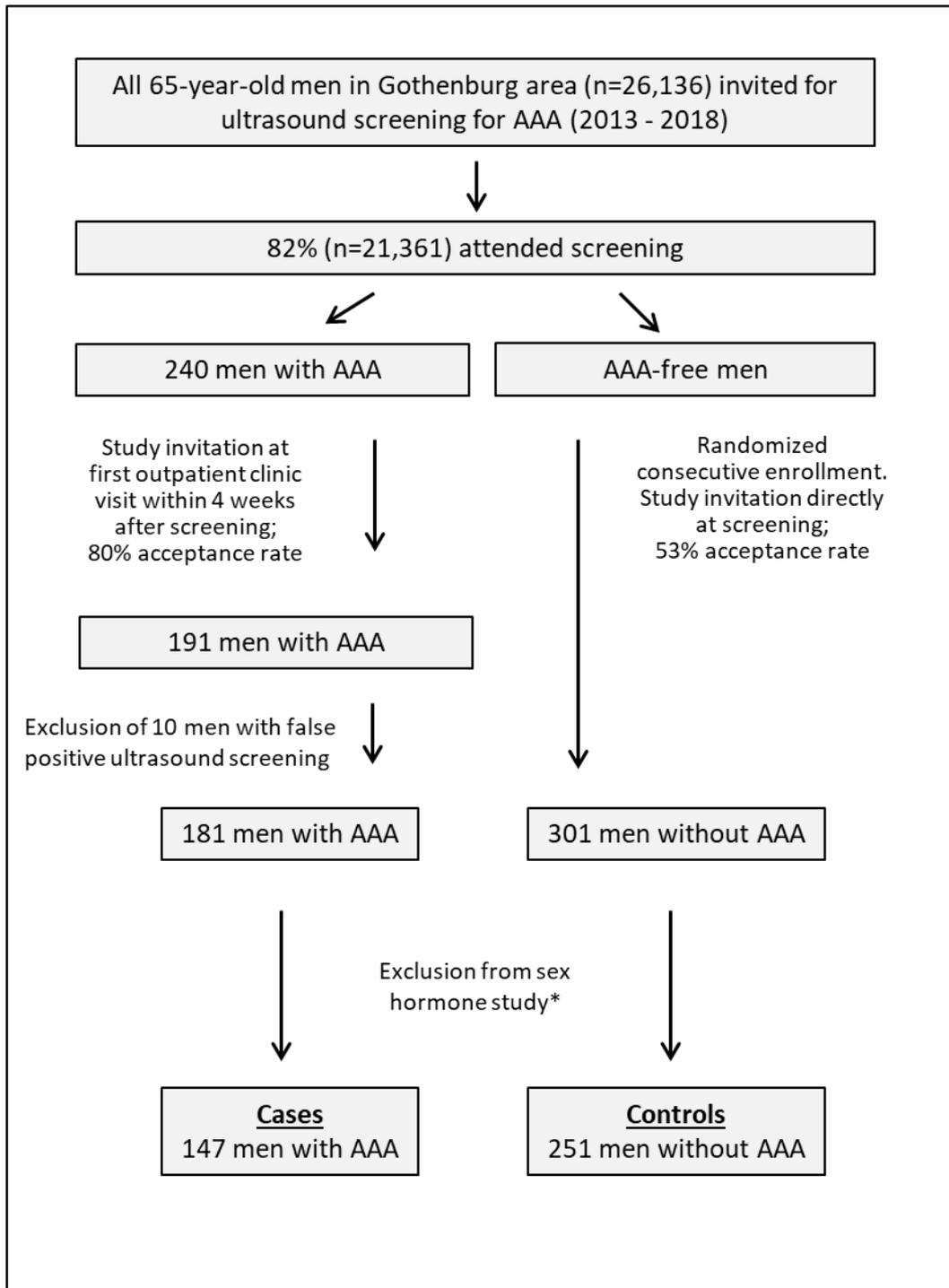
November 18, 2021



**Supplementary Figure 1.** Overview of sex steroid biosynthesis.

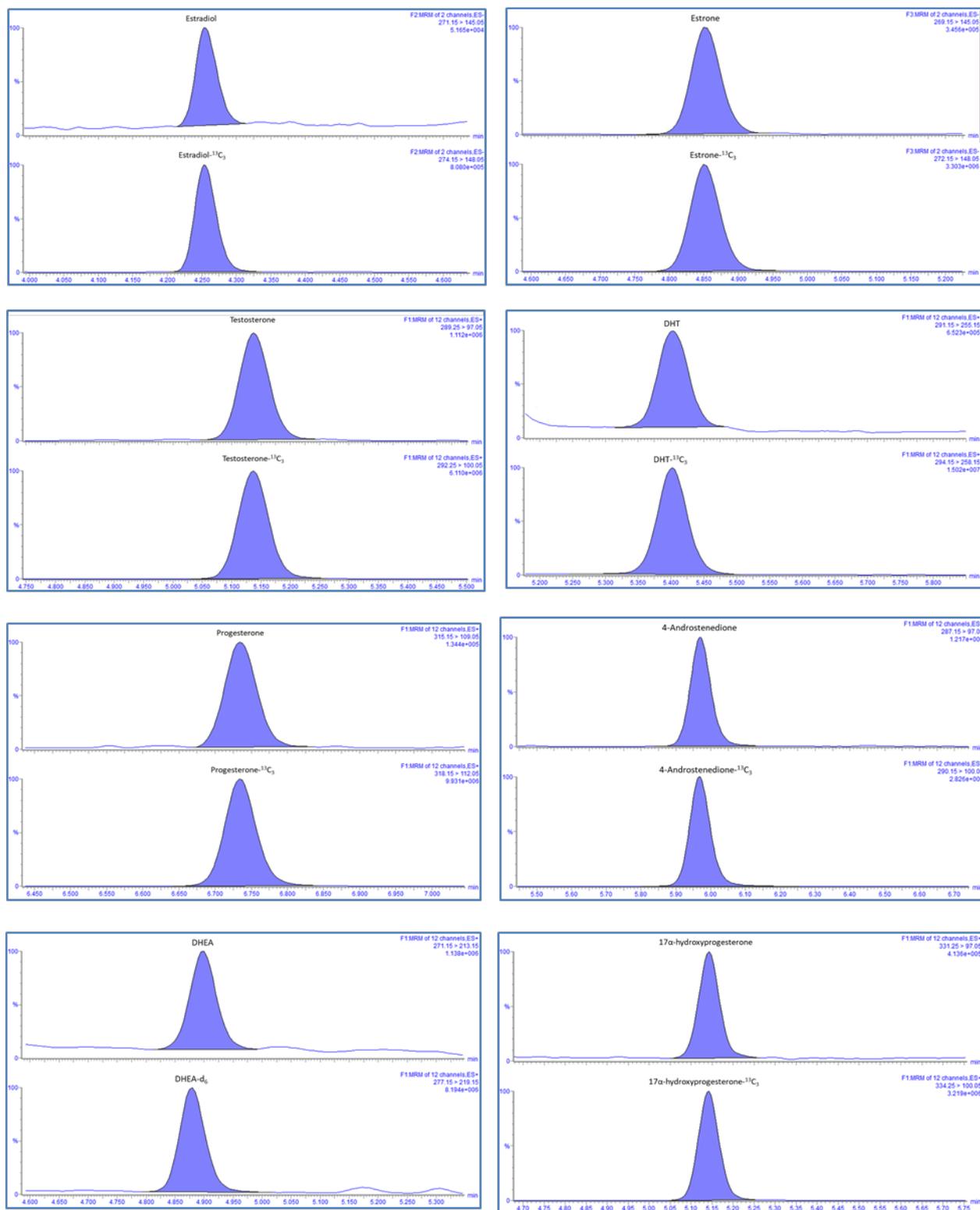
DHEA; dehydroepiandrosterone.

\*Sex steroids included in the sex hormone profile by liquid chromatography-tandem mass spectrometry (LC-MS/MS).



**Supplementary Figure 2.** Recruitment of men with AAA and AAA-free controls to the study.

\*Men who were taking androgens or drugs suppressing testosterone production, metabolism or action (8 AAA, 11 controls) or men with missing serum samples (26 AAA, 39 controls; mainly due to freezer breakdown) were excluded from the sex hormone study.



**Supplementary Figure 3.** Representative examples of chromatograms of target steroids and isotope-labeled standards. Steroid concentrations in the samples were calculated as estradiol, 2.97 pg/mL; estrone, 6.41 pg/mL; testosterone, 94.9 pg/mL; DHT, 24.7 pg/mL; progesterone, 12.5 pg/mL; androstenedione, 187 pg/mL; DHEA, 426 pg/mL, and 17 $\alpha$ -OH-progesterone 180 pg/mL.

**Supplementary Table 1.** LC-MS/MS assay: Internal standards and settings for MRM transitions

Analyte	IS	Source IS	Analyte MRM	IS MRM	RT (min)	CE (eV)	Range (pg/mL)	R <sup>2</sup>
Estradiol	Estradiol-2,3,4- <sup>13</sup> C <sub>3</sub>	Cerilliant (Sigma Aldrich)	271.2/145.1	274.2/148.1	4.3	- 40	0.5-1 000	0.999
Estrone	Estrone-2,3,4- <sup>13</sup> C <sub>3</sub>	Cerilliant (Sigma Aldrich)	269.2/145.1	272.2/148.1	4.9	- 40	0.5-1 000	0.999
Testosterone	Testosterone-2,3,4- <sup>13</sup> C <sub>3</sub>	Cerilliant (Sigma Aldrich)	289.2/97.1	292.2/100.1	5.2	60	5-10 000 <sup>a)</sup>	0.999
Dihydrotestosterone	Dihydrotestosterone-2,3,4- <sup>13</sup> C <sub>3</sub>	Cerilliant (Sigma Aldrich)	291.2/255.2	294.2/258.2	5.4	15	13-10 000	0.999
Progesterone	Progesterone-2,3,4- <sup>13</sup> C <sub>3</sub>	Cerilliant (Sigma Aldrich)	315.2/109.1	318.2/112.1	6.8	60	5-10 000	0.999
4-Androstendione	4-Androstenedione-2,3,4- <sup>13</sup> C <sub>3</sub>	Cerilliant (Sigma Aldrich)	287.2/97.1	290.2/100.1	6.0	60	5-5 000	0.999
DHEA	Dehydroepiandrosterone-2,2,3,4,4,6-d <sub>6</sub>	Cerilliant (Sigma Aldrich)	271.2/213.1	277.2/219.1	4.9	16	250-50 000	0.998
17 $\alpha$ -OH-Progesterone	17 $\alpha$ -Hydroxyprogesterone-2,3,4- <sup>13</sup> C <sub>3</sub>	Cerilliant	331.25/97.1	334.24/100.1	5.1	8	20-10 000	0.999

R<sup>2</sup> represents the linearity of the calibration curves.

LC-MS/MS, liquid chromatography-tandem mass spectrometry; DHEA, dehydroepiandrosterone; IS, internal standard; MRM, Multiple Reaction Monitoring; RT, retention time; CE, collision energy

<sup>a)</sup>With dilution 1:1 the upper range for Testosterone is 10 000 pg/mL.

**Supplementary Table 2.** LC-MS/MS assay: Accuracy in human serum

	<b>Baseline value</b>	<b>Spiked amount</b>	<b>Observed value</b>	<b>Accuracy</b>
	pg/mL	pg/mL	pg/mL	(%)
Estradiol				
Low	2.3	5.0	7.4	103
High	2.3	160	165	102
Estrone				
Low	17	5.0	21.6	91
High	17	160	179	102
Testosterone				
Low	175	25	198	94
High	176	1600	1757	99
Dihydrotestosterone				
Low	71	200	249	89
High	69	1600	1516	90
Progesterone				
Low	69	50	120	101
High	70	3200	3340	102
Androstenedione				
Low	583	100	689	106
High	588	1600	2157	98
DHEA				
Low	3889	1000	5035	115
High	3921	16000	20200	102
17 $\alpha$ -hydroxyprogesterone				
Low	227	50	281	106
High	232	3200	3540	103

Accuracy was evaluated on two different concentration levels prepared from human serum with low concentration of steroids spiked with two levels of calibrator standard solution containing the target analytes. Five replicates on each level and five on baseline was analyzed, and the accuracy was calculated as  $\left(\frac{\text{observed value} - \text{baseline value}}{\text{amount spiked}}\right) * 100\%$ .

LC-MS/MS, liquid chromatography-tandem mass spectrometry; DHEA, dehydroepiandrosterone.

**Supplementary Table 3.** LC-MS/MS assay: Extraction recovery

	Low level		High level	
	Concentration (pg/ml)	Extraction recovery (%)	Concentration (pg/ml)	Extraction recovery (%)
Estradiol	50	83	250	81
Estrone	50	89	250	88
Testosterone	250	84	1250	84
Dihydrotestosterone	500	88	2500	87
Progesterone	500	97	2500	93
Androstenedione	250	91	1250	91
DHEA	2500	95	12500	90
17 $\alpha$ -OH-progesterone	500	72	2500	74

The extraction recovery was evaluated on pooled human serum with low levels of steroids spiked with two levels of calibrator standard solution containing the target analytes and calculated as [(value in pool spiked before SLE column extraction – value in corresponding unspiked pool) / (value in pool spiked after SLE column extraction – value in corresponding unspiked pool)] \* 100%. Four replicates of each level and treatment and four of unspiked serum (baseline) were included in the test

LC-MS/MS, liquid chromatography-tandem mass spectrometry; DHEA, dehydroepiandrosterone

**Supplementary Table 4.** LC-MS/MS assay: Matrix effect in serum

	<b>Matrix effect (%)</b>	<b>CV (%)</b>
Estradiol	0	0.8
Estrone	-1	0.9
Testosterone	-48	20
Dihydrotestosterone	-58	19
Progesterone	-24	5.9
Androstenedione	-31	8.4
DHEA	-31	5.3
17 $\alpha$ -hydroxyprogesterone	-22	3.1

The matrix effect was determined using serum samples from six men (healthy blood donors). Three aliquots of each sample were analyzed before and after spiking of the extracted samples. The matrix effect (%) was calculated as  $([\text{peak area in extract after spiking} - \text{peak area in non-spiked extract} - \text{peak area in spiked blank}] / \text{peak area in spiked blank}) \times 100\%$ .

LC-MS/MS, liquid chromatography-tandem mass spectrometry; DHEA, dehydroepiandrosterone.

**Supplementary Table 5.** LC-MS/MS assay: Comparison vs external standards

	<b>Measured concentration (pM)</b>	<b>Vendor target level (pM)</b>	<b>Accuracy (%)</b>	<b>Vendor acceptance range (pM)</b>
Estradiol	299	301	99	211-391
	8834	9170	96	7330-11000
Testosterone	714	697	102	488-906
	27706	27100	102	21700-32500
Dihydrotestosterone	311	286	109	200-372
	4236	3630	117	2900-4350
Progesterone	979	986	99	690-1280
	46395	48200	96	38500-57800
4-Androstenedione	970	967	100	677-1260
	30129	33500	90	26800-40200
DHEA	7523	6970	108	4880-9060
	127045	131000	97	105000-157000
17 $\alpha$ -hydroxyprogesterone	899	908	99	635-1180
	25467	27100	94	21700-32500

Comparison of the assay vs external standards was performed using commercially available control samples (MassCheck® Serum Control Level I and III, Chromsystems). These control samples are traceable to certified reference materials and primary standards. The concentrations were determined in duplicates and were compared to vendor-determined target levels and acceptance ranges. The accuracies were calculated as measured concentration/vendor target level x 100 %.

LC-MS/MS, liquid chromatography-tandem mass spectrometry; DHEA, dehydroepiandrosterone.