SUPPLEMENTAL MATERIALS

The Roles of Insulin Resistance, Hyperinsulinemia, and LH in Peripubertal Obesity-Associated Hyperandrogenemia

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Hormone Assays

Characteristics of the total testosterone, SHBG, LH, and insulin assays are described in the main text.

FSH, insulin-like growth factor 1 (IGF-1), dehydroepiandrosterone sulfate, and growth hormone were measured by chemiluminescence (Immulite 2000, Siemens, Los Angeles, CA; sensitivity 0.1 IU/L, 25 ng/mL, 7 µg/dL, 0.01 ng/mL; intra-assay CVs 2.5-4.8%; inter-assay CVs 4.4-7.9%).

Estradiol, progesterone, androstenedione, and 17-hydroxyprogesterone were measured by radioimmunoassay (Siemens; sensitivities 10 pg/mL, 0.1 ng/mL, 0.1 ng/mL, and 10 ng/dL; intra-assay CVs 4.3-6.1%; inter-assay CVs 7.1-8.1%).

DHEA was measured by ELISA (ALPCO, Salem, NH; sensitivity 0.4 ng/mL; intra-assay CV 5.7%; inter-assay CV 8.0%).

Glucose levels during insulin clamp were measured by glucose oxidase method via the YSI Life Sciences 2300 glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH; intra-assay and inter-assay precision <2%, limit of detection = 1 mg/dL).

Hemoglobin A1c was measured by high-performance liquid chromatography (Tosoh G7 HPLC Analyzer, Tosoh Bioscience, Inc., South San Francisco, CA; intra-assay precision 1.5% at HbA1c 7-8%; inter-assay precision 3%).

To convert metric units to SI units: 17-hydroxyprogesterone (ng/dL) x 0.030257 (pmol/L); androstenedione (ng/mL) x 3.4916 (pmol/L); dehydroepiandrosterone (ng/mL) x 3.4674 (pmol/L); dehydroepiandrosterone sulfate (µg/dL) x 27.174 (nmol/L); estradiol (pg/mL) x 3.6711 (pmol/L); glucose (mg/dL) x 0.0555 (mmol/L); growth hormone (ng/mL) x 1 (µg/L); insulin (µIU/mL) x 7.1750 (pmol/L); IGF-1 (ng/mL) x 0.1307 (nmol/L); progesterone (ng/mL) x 3.1797 (pmol/L); testosterone, total (ng/mL) x 3.4674 (pmol/L); testosterone, free (pg/mL) x 3.4674 (pmol/L).

Supplemental Table 1. Effect of different methods for determining insulin sensitivity index (ISI) on primary analysis. Our *a priori* primary analysis: Partial Spearman rank (nonparametric) correlation to examine the relationship between morning free T (dependent variable) and insulin sensitivity index (independent variable), controlling for estimated 24-hour LH and Tanner stage (covariates). **Panel A:** Average ISI (mean ± SD) according to method used. **Panel B:** Correlations between ISI and testosterone (T)—while simultaneously correcting for differences in estimated 24-hour LH and Tanner stage—when using different methods for correcting ISI for subject size.

A

Subject size variable used for correcting ISI	ISI (mean ± SD)
Total mass (kg)	2.23 ± 1.09
Fat free mass (kg)	3.57 ± 1.62
Body surface area (m^2) [a]	0.94 ± 0.43

B

		Free T (RIA)		Free T (LC-MS/MS)		Total T (RIA)		Total T (LC-MS/MS)	
		r_s	p	r_s	p	r_s	p	r_s	p
ISI using the following variable to correct for subject size	Total mass (kg)	0.68	0.046	0.78	0.023	0.65	0.060	0.74	0.036
	Fat free mass (kg)	0.62	0.072	0.7	0.054	0.63	0.064	0.73	0.040
	Body surface area (m^2) [a]	0.70	0.035	0.79	0.019	0.70	0.034	0.80	0.017

Footnote: [a] Body surface area = √(height [cm] * weight [kg]/3600).

Supplemental Table 2. Effect of different methods for determining periprandial insulin levels (for use in the estimated 24-hour insulin calculation) on primary analysis. Our *a priori* primary analysis: Partial Spearman rank (nonparametric) correlation to examine the relationship between morning free T (dependent variable) and insulin sensitivity index (independent variable), controlling for estimated 24-hour LH and Tanner stage (covariates). **Panel A:** Average periprandial insulin levels (mean ± SD) according to method used. **Panel B:** Correlations between estimated 24-hour insulin and testosterone (T)—while simultaneously correcting for differences in estimated 24-hour LH and Tanner stage—when using different methods for determining periprandial insulin levels (for use in the estimated 24-hour insulin calculation).

A

Method of determining periprandial insulin concentration	Resulting periprandial insulin concentration (mean ± SD)
Mean insulin 1800-2100 h	76.1 ± 34.9
Mean insulin 1800-2200 h	80.0 ± 38.8
Mean insulin 1800-2300 h	75.4 ± 36.8
Mean insulin 1900-2200 h	96.2 ± 49.1
Mean insulin 1900-2300 h	86.5 ± 44.4

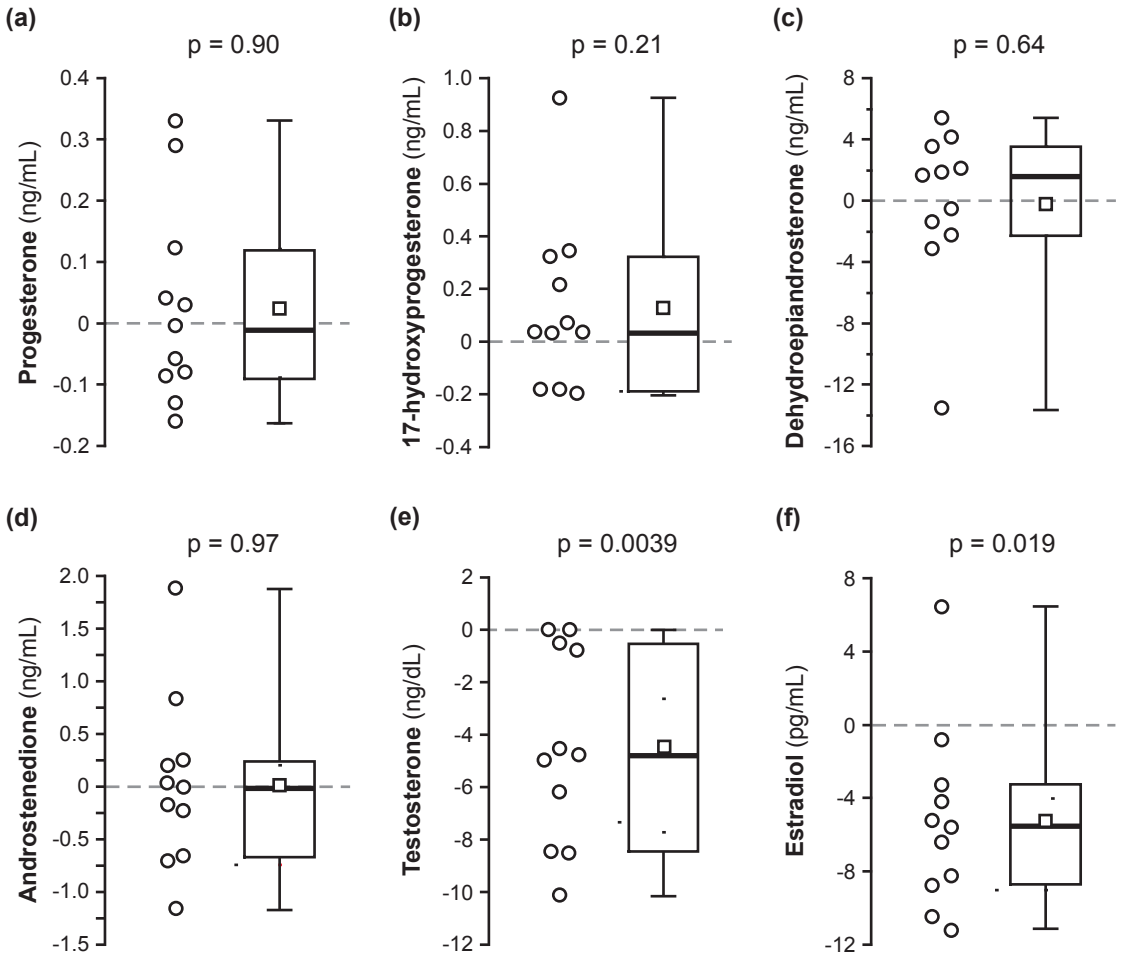
B

		Free T (RIA)		Free T (LC-MS/MS)		Total T (RIA)		Total T (LC-MS/MS)	
		r_s	p	r_s	p	r_s	p	r_s	p
Average 24-hour insulin, calculated using the following periprandial insulin concentration	Mean insulin 1800-2100 h	0.47	0.20	0.36	0.38	0.49	0.18	0.42	0.29
	Mean insulin 1800-2200 h	0.52	0.18	0.43	0.34	0.53	0.18	0.50	0.25
	Mean insulin 1800-2300 h	0.51	0.19	0.43	0.34	0.52	0.18	0.50	0.25
	Mean insulin 1900-2200 h	0.51	0.19	0.43	0.34	0.52	0.18	0.50	0.25
	Mean insulin 1900-2300 h	0.54	0.17	0.49	0.27	0.57	0.14	0.58	0.18

Supplemental Table 3. Steroid changes with insulin clamp

	Summary data			Subject number										
	Mean	SD	Median	1	2	3	4	5	6	7	8	9	10	11
0900 h progesterone (ng/mL)	0.36	0.18	0.38	0.28	0.24	0.38	0.41	0.47	0.18	0.71	0.38	0.16	0.20	0.59
1100 h progesterone (ng/mL)	0.37	0.15	0.32	0.31	0.57	0.22	0.32	0.39	0.22	0.58	0.50	0.29	0.19	0.53
0900 h 17-OHP (ng/dL)	89.4	43.0	76.9	55.6	76.9	65.2	116.6	79.0	51.4	179.3	38.5	74.2	99.3	147.1
1100 h 17-OHP (ng/dL)	101.9	38.4	96.4	58.7	169.0	68.3	96.4	113.1	58.3	160.6	70.6	95.4	102.6	128.3
0900 h DHEA (ng/mL)	8.99	11.24	4.37	2.87	4.37	3.99	14.23	3.70	2.97	3.90	11.01	5.36	5.40	41.10
1100 h DHEA (ng/mL)	8.83	7.55	7.30	1.51	7.94	3.48	11.12	7.90	5.14	1.70	16.45	6.98	7.30	27.60
0900 h androstenedione (ng/mL)	2.08	1.42	1.61	1.49	0.83	0.65	3.00	1.61	0.75	5.32	1.30	2.53	1.90	3.49
1100 h androstenedione (ng/mL)	2.10	1.42	2.13	1.31	1.08	0.42	2.30	3.49	0.74	5.35	2.13	2.73	1.24	2.33
0900 h total T (ng/dL)	39.6	27.7	33.1	33.7	10.0	10.0	68.6	33.7	18.4	102.0	33.1	32.8	30.8	62.2
1100 h total T (ng/dL)	35.1	25.7	29.2	29.2	10.0	10.0	63.8	28.7	10.0	91.9	24.6	32.3	30.0	56.0
0900 h estradiol (pg/mL)	24.6	9.2	24.6	24.6	20.7	15.8	39.4	25.4	23.0	32.0	31.8	5.1	21.8	30.9
1100 h estradiol (pg/mL)	19.3	6.5	21.0	16.4	15.4	9.4	28.2	21.2	14.2	28.7	21.3	11.5	21.0	25.3

Abbreviations: 17OHP, 17-hydroxyprogesterone; DHEA, dehydroepiandrosterone; T, testosterone.



Supplemental Figure 1. Acute steroid changes with the hyperinsulinemic-euglycemic clamp. In each panel, the data represent the change in hormone concentration from 0900 h (at start of the hyperinsulinemic-euglycemic clamp) to 1100 h (at the end of the hyperinsulinemic-euglycemic clamp). Circles represent data from individual subjects. These data are also summarized using box-and-whisker plots, which show median (line inside the box); mean (open square); 25th and 75th percentiles (bottom and top of box); minimum and maximum (bottom and top whiskers). P values relate to changes from 0900 h to 1100 h.