Supplemental File: Text

Methods: Hormone Assays

Hormone assays and safety laboratory tests

The safety laboratory tests were analyzed at the clinical laboratories at each of the study sites. NES concentrations in human serum samples were measured at the Population Council by a specific radioimmunoassay method previously described (27). The NES radioimmunoassay was validated and gave linear results over concentration range between 27 to 864 pmol/L (10 to 320 pg/ml). Intra-assay and inter-assay coefficient of variation varied from 3.4 to 13.9% and 9.3 to 13.5% respectively. All other hormone measurements were measured at the Endocrine and Metabolic Research Laboratory at Los Angeles Biomedical Research Center at Harbor-UCLA Medical Center using assays that have been fully validated and reported. Serum testosterone was measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) (28, 29). Testosterone (>99% pure Sigma Aldrich, St. Louis, MO) was used as a calibration standard. 1, 2 deuterated (D_2)-testosterone (>98% pure, Cambridge Isotope Laboratories, Inc., Andover, MA) was used as the internal standard for T measurements. Calibration standards and test samples were prepared for T using a liquid/liquid extraction twice with 2 mL ethyl acetate: hexanes (3:2 volume: volume). LC-MS/MS runs were performed with a Shimadzu HPLC system (Columbia, MD) attached to an Applied Biosystems API5000 LC-MS/MS (Foster City, CA) equipped with a TurboIon Spray source. The calibration standards showed a linear response from 1 ng/dl (0.35 nmol/liter) to 2000 ng/dL (69.3 nmol/liter) for T. The within and between run precision was less than 5 % and the recovery of samples spiked with the steroids was between 100 to 113% for T. The lower limit of quantification (LLOQ) for T was 2 ng/dl or 0.069 nmol/liter). The reference range for T in adult males is from 265 to 972 ng/dl (9.2 to 33.7 nmol/liter). Free testosterone (T) was measured by equilibrium dialysis using labeled T as described previously (30) and the adult male reference range is from 4.7-18.0 ng/dl (0.16 to

0.62 nmol/liter). Serum sex hormone binding globulin (SHBG) was measured by fluoroimmunometric assays with reagents obtained from Delfia (Wallace, Gaithersburg, MD). The LOQ was determined to be 0.5 nmol/liter. The intra- and inter-assay coefficients were 2.3% and 6.1%, respectively. The adult male range for this assay is 10.8 to 46.6 nmol/liter. Serum LH and FSH levels were measured by ultra-sensitive and highly specific fluoroimmunometric assays with reagents from Delfia (Pharmacia, Sweden). Both assays have a LLOQ of 0.2 IU/liter. The LH assay has a sensitivity of 0.2 IU/liter of 2nd IRP-hMG, the intraassay variation is 5.1% and inter-assay variation is 9.9%. The cross reactivity with TSH, FSH and free alpha subunit is < 1%. The sensitivity of the FSH assay is 0.2 IU/liter of 2nd IRP. The intra-assay variation is 5.8% and the cross reactivities in the LH, TSH and free alpha subunit are all < 1% (31-34).

Supplemental Table 1. Percentage of men with sperm concentrations =0, ≤1 and ≤ 3 million/ml or serum gonadotropins suppressed to ≤0.5 or 1 IU/liter at 20-24 weeks

	Percentage of Men			Lower 95% Confidence Bound on Percentage of Men		
	0 mg NES	8 mg NES	12 mg NES	0 mg NES	8 mg NES	12 mg NES
Sperm Concentration:						
≤ 1 million/ml	5/22 = 22.7%	16/18 = 88.9%	14/16 = 87.5%	7.8%	65.3%	61.7%
≤ 3 million/ml	5/22 = 22.7%	16/18 = 88.9%	14/16 = 87.5%	7.8%	65.3%	61.7%
0	5/22 = 22.7%	14/18 = 77.8%	11/16 = 68.8%	9.4%	52.4%	41.3%
Gonadotropins:						
LH and FSH < 0.5 IU/liter	4/22 = 18.2%	11/17 = 64.7%	11/15 = 73.3%	5.2%	38.3%	44.9%
LH and FSH < 1.0 IU/liter	7/22 = 31.8%	13/17 = 76.5%	12/15 = 80.0%	13.9%	50.1%	51.9%

P-values:

	0 vs. 8 mg Nes	0 vs. 12 mg Nes	8 vs. 12 mg Nes
Concentration ≤ 1	<0.0001	0.0002	>0.99
Concentration ≤ 3	<0.0001	0.0002	>0.99
Concentration = 0	0.001	0.008	0.70
LH and FSH < 0.5 IU/liter	0.007	0.002	0.71
LH and FSH < 1.0 IU/liter	0.01	0.01	>0.99

Supplemental Table 2: Parameters and Hormone Concentrations at 24 weeks and Percent Change of Semen (Median, 95% CI in parenthesis)

* among subjects with detected sperm.

	Week 24 Median (95% CI)			Median % Change From Week 0 to Week 24 (95% CI)		
	0 mg NES (n=22)	8 mg NES (n=18)	12 mg NES (n=16)	0 mg NES	8 mg NES	12 mg NES
Total Sperm ($X10^6$/ ejaculate)	53.5 (22.2,116.7)	0.0 (0.0,0.0)	0.0 (0.0,0.2)	-59.8 (-82.4, -23.8)	-100 (-100, -100)	-100 (-100, -99.7)
Sperm Concentration ($X10^6$/ml)	20.7 (15.8,44.1)	0.0 (0.0,0.0)	0.0 (0.0,0.1)	-50.1 (-67.1, 7.3)	-100 (-100, -100)	-100 (-100, -99.9)
% Motile Sperm*	64.0 (58.0,68.0)	47.0 (0.0,100.0)	41.0 (23.0,100.0)	0.1 (-6.0, 11.5)	-26.6 (-100, 42.9)	17.1 (-61.7, 220)
% Sperm with Normal Morphology*	11.0 (10.0,15.0)	10.0 (2.0,12.0)	10.0 (0.0,11.0)	-6.3 (-28.6, 14.3)	-47.4 (-75.0, -36.8)	-35.2 (-100.0, -8.3)
Testosterone (nmol/liter)	26.2 (19.2,37.8)	18.5 (10.7,32.7)	26.4 (18.7,40.6)	20.4 (-4.6, 135.6)	3.2 (-34.4, 108.3)	24.8 (-13.7, 201.1)
Free Testosterone (nmol/liter)	0.36 (0.32,0.55)	0.32 (0.16,0.61)	0.33 (0.26,0.94)	16.8 (-15.92, 210.3)	25.6 (-29.2, 167.7)	33.5 (14.0, 306.8)
SHBG (nmol/liter)	37.4 (24.7,43.9)	29.7 (19.5,45.8)	34.2 (26.1,42.4)	-11.2 (-18.4, 1.6)	-22.7 (-29.5, 8.8)	-6.0 (-28.8, 3.9)
LH (IU/liter)	3.21 (0.74,3.99)	0.12 (0.12,0.19)	0.12 (0.12,0.17)	-23.5 (-77.2, 35.1)	-95.9 (-97.6, -92.9)	-96.8 (-97.6, -93.8)
FSH (IU/liter)	1.99 (1.15,4.26)	0.12 (0.10,0.98)	0.22 (0.10,0.74)	-16.5 (-75.3, -1.5)	-94.3 (-98.1, -68.6)	-96.5 (-97.6, -68.8)
NES (pmol/liter)		372 (162,653)	632 (367,885)			

Supplemental Table 2 (footnote): Efficacy Changes at Week 24 P-values

Week 24:	0 vs. 8 mg NES	0 vs. 12 mg NES	8 vs. 12 mg NES
Total Sperm	0.0001	0.001	0.53
Sperm Concentration	<0.0001	0.002	0.51
% Motile Sperm	0.30	0.047	0.90
% Normal Sperm	0.26	0.20	0.72
Testosterone	0.12	0.87	0.16
Free Testosterone	0.32	0.72	0.36
SHBG	0.58	0.87	0.62
LH	0.001	0.0006	>0.99
FSH	0.011	0.022	0.46
Nestorone			0.16

% Changes:	0 vs. 8 mg NES	0 vs. 12 mg NES	8 vs. 12 mg NES
Total Sperm	<0.0001	0.002	0.48
Sperm Concentration	<0.0001	0.002	0.45
% Motile Sperm	0.22	0.80	0.33
% Normal Sperm	0.007	0.06	0.48
Testosterone	0.40	0.77	0.47
Free Testosterone	0.81	0.45	0.58
SHBG	0.28	0.82	0.66
LH	0.001	0.0003	0.28
FSH	0.004	0.004	0.84

Supplemental Table 2a: Percent Change from Week 0 to Final Evaluation for Sperm Count and Concentration

	Final Evaluation Median (95% CI)			Median % Change From Week 0 to Final Evaluation (95% CI)		
	0 mg NES (n=22)	8 mg NES (n=18)	12 mg NES (n=16)	0 mg NES	8 mg NES	12 mg NES
Total Sperm ($X10^6$/ ejaculate)	149.9 (97.2,196.0)	150.8 (101.3,240.7)	86.5 (54.6,149.6)	-8.9 (-41.2, 28.7)	-0.1 (-36.2, 52.4)	-1.6 (-50.0, 41.6)
Sperm Concentration ($X10^6$/ml)	41.5 (29.3,51.4)	60.0 (30.5,89.0)	39.5 (24.5,68.0)	0.75 (-24.2, 29.2)	-6.91 (-23.9, 31.1)	19.54 (-26.9, 55.1)

Note: Final evaluation is the value at the end of the trial for each subject for a given parameter.

P-values

Final Evaluation:	0 vs. 8 mg NES	0 vs. 12 mg NES	8 vs. 12 mg NES
Total Sperm	0.935	0.092	0.073
Sperm Concentration	0.704	0.391	0.458

% Change to Final Evaluation:	0 vs. 8 mg NES	0 vs. 12 mg NES	8 vs. 12 mg NES
Total Sperm	0.532	0.768	0.629
Sperm Concentration	0.935	0.574	0.558

Supplemental Table 2b: Sperm Concentration Time to Recovery

	Sperm Concentration Median Time to Recovery (95% CI)		
	0 mg NES (n=22)	8 mg NES (n=18)	12 mg NES (n=16)
Sperm Concentration Time to Recovery (days)	1.0 (0,6)	114.5 (105,141)	115.5 (67,122)

Note: Sperm concentration time to recovery is defined as the time from date of last dose to the first sperm concentration ≥ 15 million/ml.

Supplemental Table 3. Changes in Psychosexual Questionnaire Score During Treatment

	Week 24 Minus Week 0 Changes (Median, 95% CI in parenthesis)			
	All Subjects	0 mg NES	8 mg NES	12 mg NES
Sexual Desire	0.29 (-0.06, 0.86)	0.29 (-0.03, 1.27)	0.22 (-0.17, 1.21)	0.43 (-1.15, 1.15)
Sexual Enjoyment with Partner	0.51 (-0.47, 1.59)	0.07 Not Estimable	-0.245 (-2.27, 0.22)	1.58 Not Estimable
Sexual Enjoyment without partner	-0.10 (-0.68, 0.29)	-0.1 (-0.57, 0.79)	-0.57 (-3.43, 2.75)	0.00 (-0.71, 0.90)
Sexual Activity	-0.01 (-0.03, 0.07)	-0.01 (-0.06, 0.07)	0.045 (-0.03, 0.13)	-0.05 (-0.12, 0.11)
Satisfaction with erection	0.11 (0.04, 0.78)	-0.035 (-0.27, 0.95)	0.40 (-0.02, 1.15)	0.20 (-0.4, 1.92)
Percent erection	2.00 (-0.24, 8.57)	2.17 (-4.15, 8.84)	4.05 (-1.86, 12.74)	0.00 (-34.67, 32.86)
Positive mood scores	-0.18 (-0.42, 0.18)	-0.18 (-0.52, 0.56)	0.25 (-0.2, 0.52)	-0.48 (-1.55, 0.06)
Negative mood scores	0.11 (-0.1, 0.46)	0.31 (-0.17, 0.69)	-0.17 (-0.82, 0.4)	0.2 (-0.01, 1.08)

P-values:	0 vs. 8 mg	0 vs. 12 mg	8 vs. 12 mg
Sexual Desire	0.69	0.79	0.75
Sexual Enjoyment with Partner	0.80	0.08	0.29
Sexual Enjoyment without partner	0.21	0.93	0.21
Sexual Activity	0.44	0.73	0.44
Satisfaction with erection	0.23	0.26	0.48
Percent erection	0.47	0.86	0.80
Positive mood scores	0.27	0.17	0.048
Negative mood scores	0.08	0.54	0.037

Supplemental Table 4. Median levels of Weight, Blood Pressure, blood Counts and Clinical Chemistry and IPPS Scores at 24 weeks

	Week 24 (Median, 95% CI in parenthesis)				p-value		
	All Subjects	0 mg NES	8 mg NES	12 mg NES	0 vs. 8	0 vs. 12	8 vs. 12
Weight (kg)	82.6 (78.9,84.8)	79.9 (73.5,83.9)	81.2 (74.9,84.8)	86.4 (83.9,91.6)	0.81	0.02	0.02
SBP (mm/Hg)	122 (118,128)	122 (117,132)	121 (115,131)	123 (117,130)	0.85	0.86	0.87
DBP (mm/Hg)	69 (66,71)	69 (65,73)	69 (65,74)	69 (66,71)	0.85	0.76	0.75
Total Cholesterol (mg/dl)	166 (162,182)	182 (153,208)	164 (157,175)	166 (147,187)	0.35	0.47	0.89
HDL (mg/dl)	40 (38,44)	42 (39,45)	42 (36,47)	38 (34,43)	0.99	0.16	0.28
LDL (mg/dl)	106 (95,113)	109 (89,136)	1030 (92,115)	106 (89,115)	0.63	0.95	0.81
HDL/LDL (ratio)	0.39 (0.34,0.44)	0.41 (0.32,0.47)	0.41 (0.32,0.50)	0.36 (0.31,0.44)	0.76	0.49	0.30
Triglycerides (mg/dl)	86 (59,103)	107 (54,125)	88 (53,122)	69 (49,98)	0.60	0.41	0.54
Glucose (mg/dl)	90.0 (88, 94)	90.0 (87, 93)	92.5 (84, 94)	90.0 (85, 94)	0.89	0.88	0.88
ALP (U/liter)	60 (56,66)	60 (54,73)	63 (51,67)	60 (49,73)	0.80	0.95	0.87
ALT (U/liter)	25 (22,30)	25 (18,30)	30 (21,38)	22 (18,38)	0.13	0.81	0.42
AST (U/liter)	25 (22,25)	24 (21,27)	25 (22,28)	25 (18,27)	0.60	0.71	0.56
PSA (ng/ml)	0.70 (0.59,0.79)	0.70 (0.50,0.80)	0.61 (0.49,1.00)	0.76 (0.52,1.20)	0.76	0.28	0.31
Hemoglobin (g/dl)	15.4 (15.1,15.9)	15.4 (15.0,15.9)	15.5 (15.0,16.0)	15.4 (14.7,16.1)	0.75	0.99	0.69
Hematocrit (%)	44.7 (44.0,45.1)	44.8 (42.8,45.9)	44.9 (44.0,46.5)	44.5 (43.8,46.5)	0.38	0.75	0.44
IPSS Score	3.0 (2.0,3.0)	2.0 (0.0,3.0)	4.0 (3.0,7.0)	3.0 (0.0,4.0)	0.03	0.44	0.27

Supplemental Table 5: Changes in Weight, Blood Pressure, blood Counts and Clinical Chemistry and IPPS Scores at 24 weeks

	Week 24 Minus Week 0 Changes (Median, 95% CI in parenthesis)				p-value		
	All Subjects	0 mg NES	8 mg NES	12 mg NES	0 vs. 8	0 vs. 12	8 vs. 12
Weight (Kg)	2.5 (1.8, 3.9)	1.1 (-0.45, 2.5)	5.2 (2.5,6.1)	2.5 (1.6,4.8)	0.004	0.057	0.307
SBP (mmHg)	7 (5,10)	7 (3, 12)	8 (3.5, 14)	4.5 (1,12)	0.76	0.49	0.37
DBP (mmHg)	0.5 (-2.5, 3.0)	-0.5 (-4.5, 5)	-3.0 (-7, 4.5)	3.5 (-1.5, 7)	0.65	0.34	0.17
Total Cholesterol (mg/dl)	3 (-6.5, 4)	1.5 (-7.5, 7)	0.5 (-19.5, 7.5)	4 (-7.5, 8.5)	0.51	0.99	0.46
HDL (mg/dl)	-1 (-2.5, 0.5)	2 (0.5, 5.5)	-3.5 (-5.5, 0)	-4 (-6.5, -1.5)	0.002	<0.0001	0.53
LDL (mg/dl)	-2 (-5.5, 3.5)	-4 (-9, 3)	3.5 (-14.5, 7)	4 (-3.5,10.5)	0.94	0.13	0.39
HDL/LDL (ratio)	-0.00 (-0.024, 0.018)	0.03 (-0.007, 0.078)	-0.01 (-0.052, 0.038)	-0.04 (-0.092, 0.021)	0.044	0.0001	0.09
Triglycerides (mg/dl)	0 (-8, 11.5)	2 (-18, 13)	-0.5 (-11.5, 28)	8 (-22.5, 27)	0.66	0.83	0.81
Glucose (mg/dl)	2.0 -1.0 4.0	0.5 -4.0 4.5	2.0 -6.0 3.5	6.0 3.0 9.0	0.690	0.0561	0.0180
ALP (U/liter)	-3 (-5.5, -0.5)	3 (-1, 5.5)	-5 (-10.5, -1)	-6 (-14.5, -3.5)	0.004	0.0004	0.30
ALT (U/liter)	2 (0, 5)	0 (-4.5,3.5)	8 (4, 10.5)	-1 (-5.5, 7.5)	0.008	0.97	0.047
AST (U/liter)	0 (-1.5, 2.5)	0 (-2.5,3.5)	1 (-1.5, 5)	-1 (-8, 3)	0.54	0.60	0.29
PSA (ng/ml)	0.015 (0.005, 0.10)	0.00 (-0.04, 0.085)	0.03 (0.005, 0.25)	0.6 (0.015, 0.15)	0.27	0.30	0.73
Hemoglobin (g/dl)	0.3 (0.2, 0.55)	0.2 (0.0, 0.5)	0.3 (0.1, 0.7)	0.4 (0.1,0.85)	0.80	0.57	0.99
Hematocrit (%)	1.0 (0.9, 1.65)	1.0 (0.2, 1.3)	0.9 (0.09, 2.95)	1.45 (0.5, 2.5)	0.96	0.52	0.86
IPSS Score	0.0 (0.0, 2.0)	0.0 (-1.5, 3.5)	1.0 (1.0, 3.0)	1.0 (-0.5, 3.5)	0.08	0.23	0.64