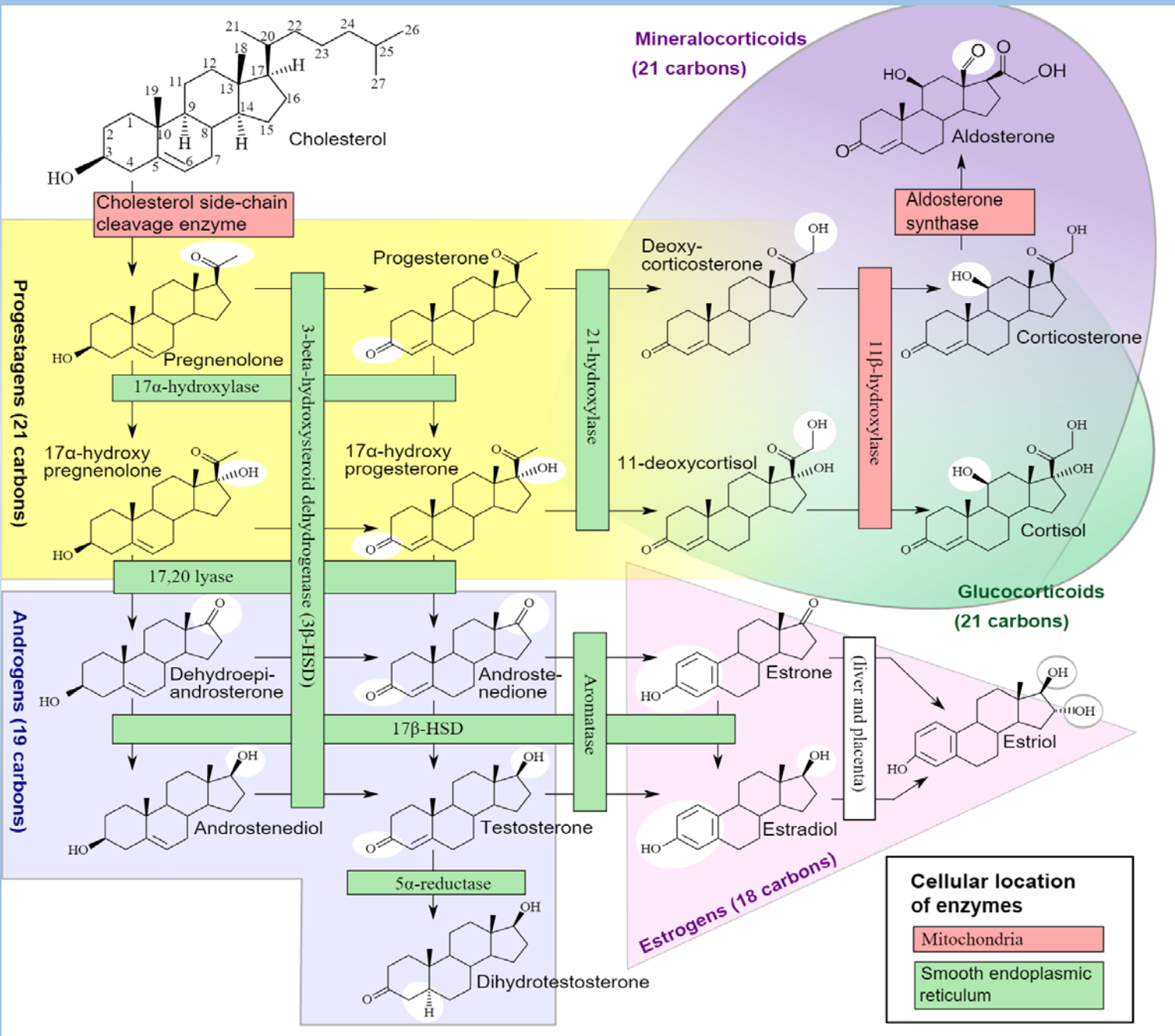


Sanis Biomedical

Capillary Sampling & Hormone Monitoring



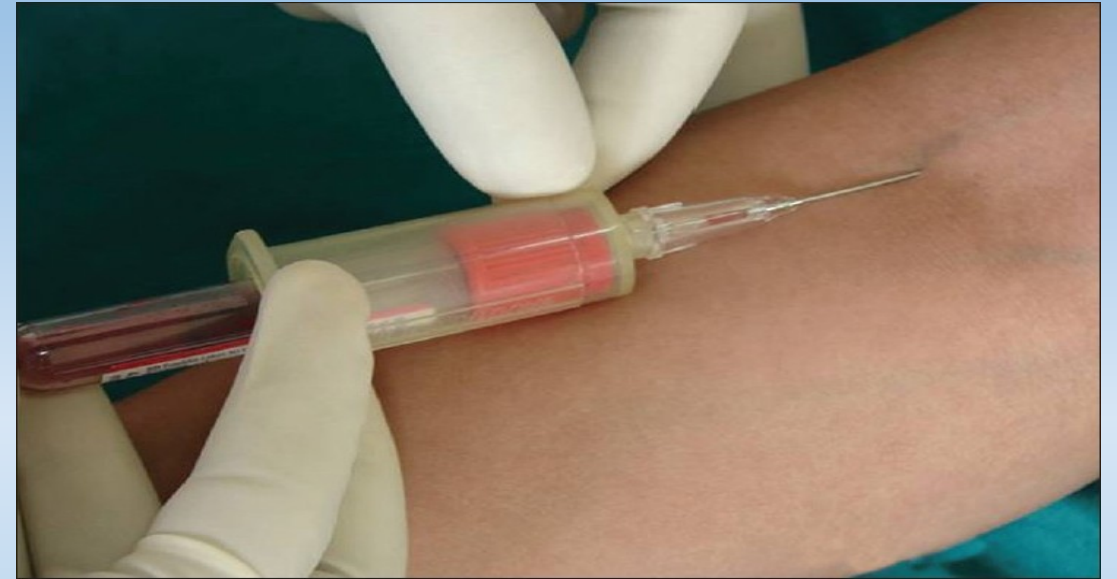
Professional Background

- 5 years Assay & Method development at Merck pharmaceutical
 - Drug Metabolism
 - Metabolic Disease (Januvia & Janumet)
 - Infectious Disease (Isentress)
- 3 years method development in agricultural testing (food products, GMOs, metals and fatty acid profiling of food products & Nutritional Supplements)
- 3 years Lab buildout & design of Clinical Diagnostic Test in multiple states including New Jersey, California, Louisiana and Florida
- Developed novel IP protected supplements utilizing structurally modified amino acids and B-vitamins that increased absorption across the blood brain barrier
- Board Certified ASCP Specialist in Chemistry
- Certified nutritionist & Personal Trainer
- 5 years working with U.S. Olympic Team competing and/or training high level athletes

Sanis vs. Traditional Methods



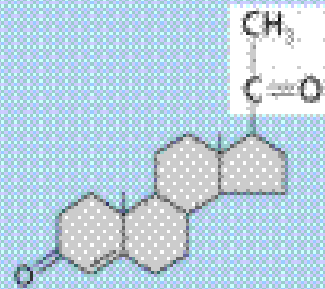
- 350 uL sample is used to screen for 13 different hormones.
- Capillary puncture is safe, simple and gives reliable results.
- No need for phlebotomist, nurse, etc.
- Easily transferable to DTC model



- ▶ Traditional methods use venous puncture and focus on only a few hormones.
- ▶ Turn around time varies.
- ▶ Larger volume & Medical Professional prevent direct to consumer (DTC)

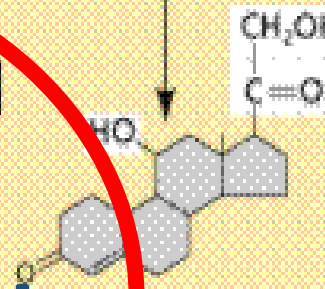
3 Classes of Steroids

Sex steroid



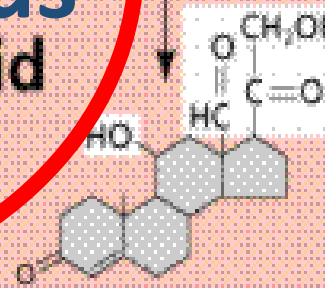
Progesterone

Glucocorticoid



Corticosterone

Corticosteroids
Mineralocorticoid

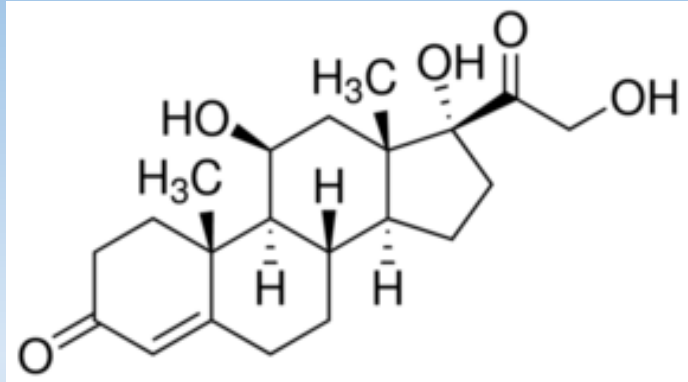


Aldosterone

- **Sex steroids:** involved in a wide variety of processes ranging from muscle development to cardiovascular health.
- **Glucocorticoids:** play role in regulating metabolism, anti-inflammatory response, and stress response amongst others.
- **Mineralocorticoids:** play role in salt and water balance.

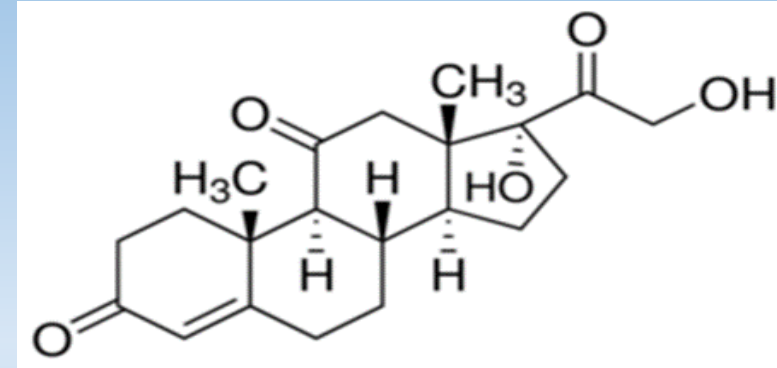
Major Corticosteroids

Cortisol



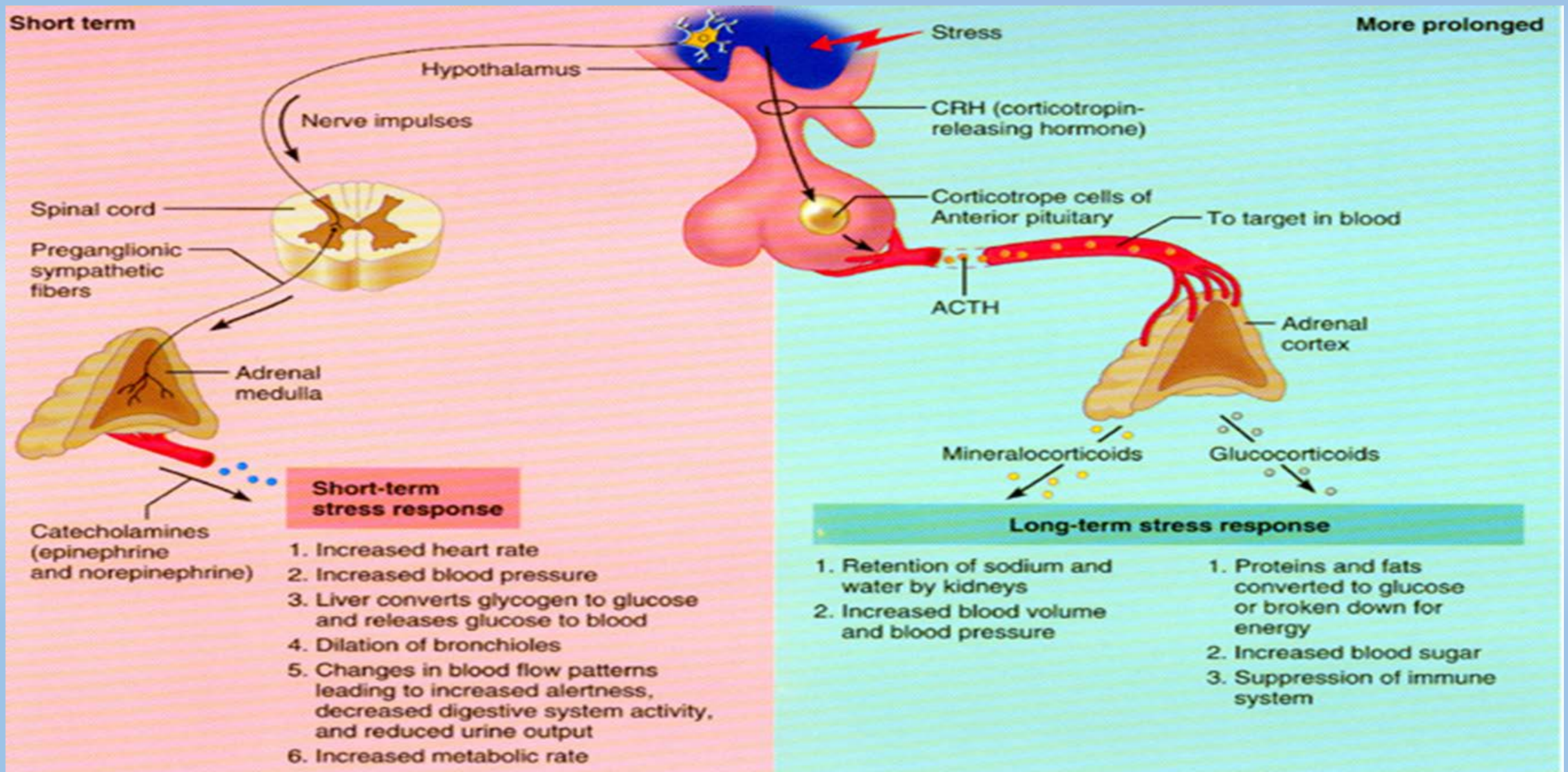
- the “stress” hormone – important in blood sugar levels; suppresses immune system, metabolism.
- Excess cortisol problematic:
 - Reduced muscle growth (protein breakdown)
 - Reduced immune response
 - Increased abdominal fat
 - Decrease in bone growth/repair
- Used medicinally (hydrocortisone)

Cortisone

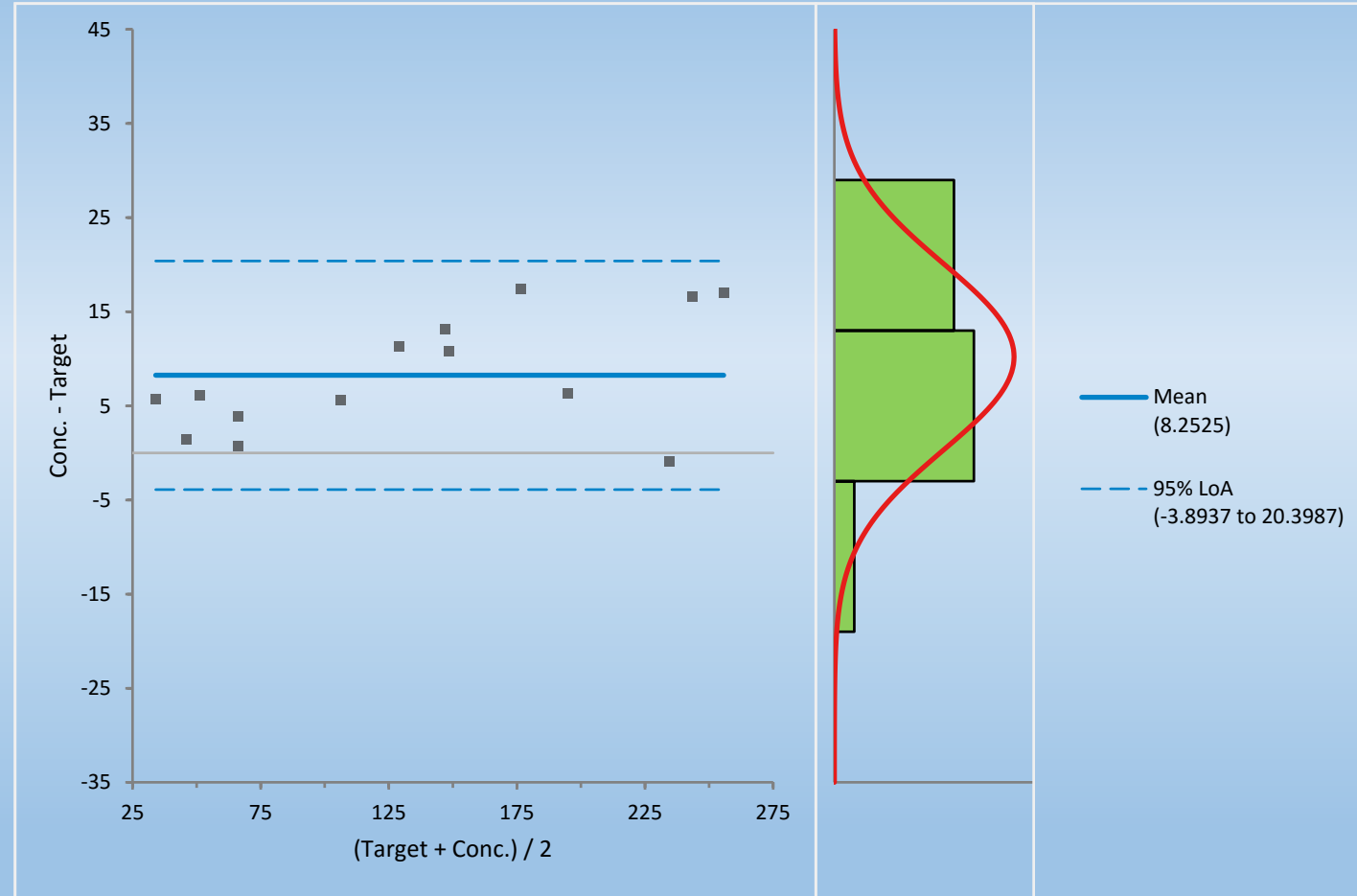
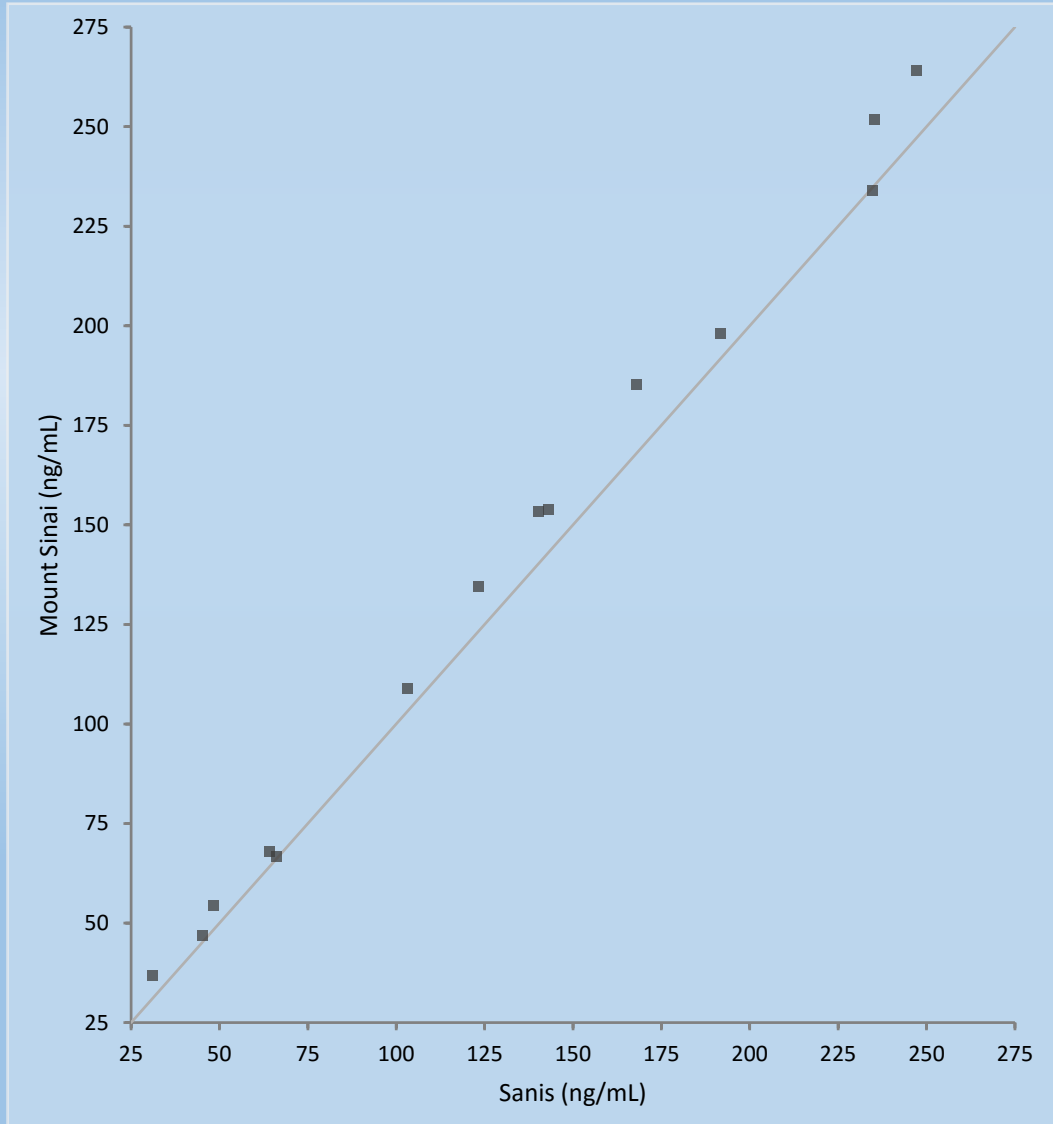


- Breaks protein into carbohydrates
- Plays a role in electrolyte levels in the blood and blood pressure
- High levels of cortisone may indicate health risk – such as Cushing’s disease.
- Used medicinally to suppress inflammation, pain and swelling.

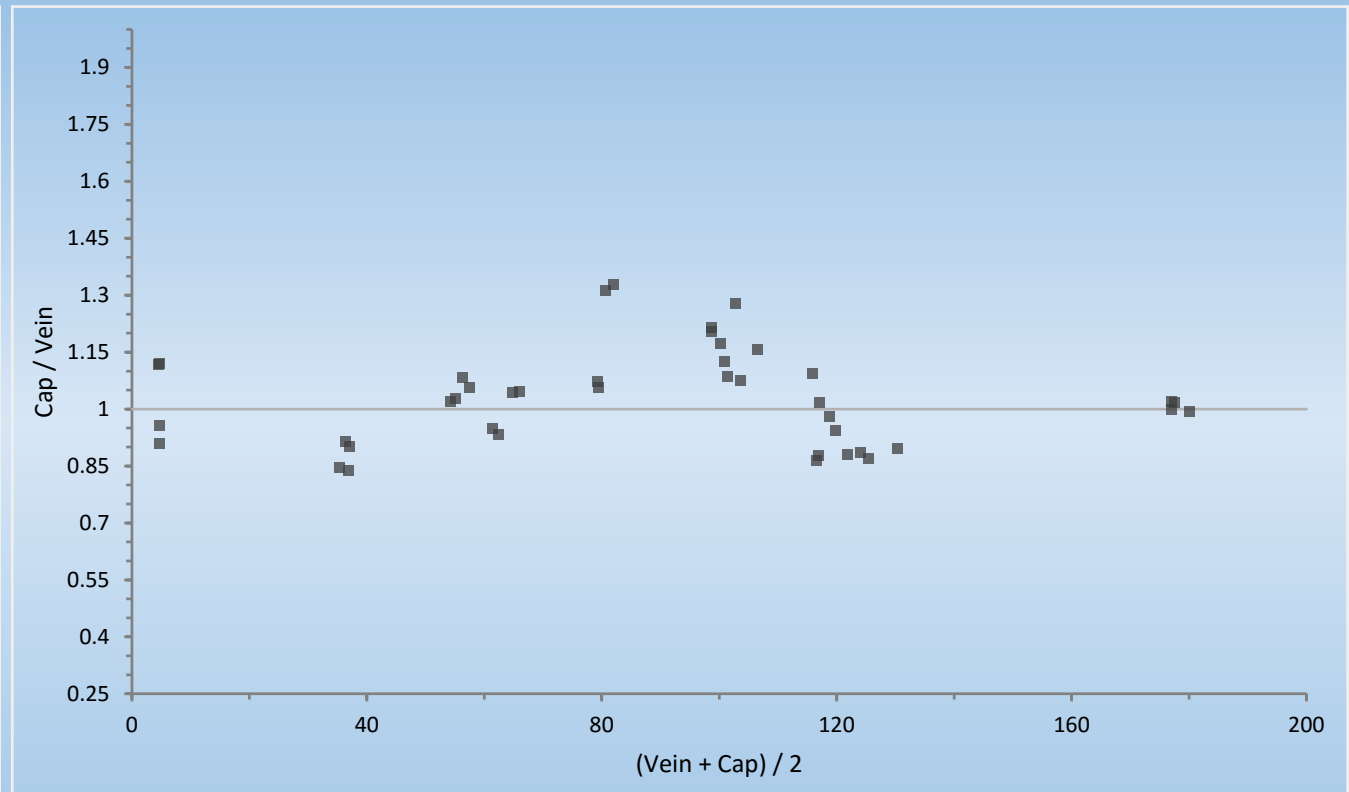
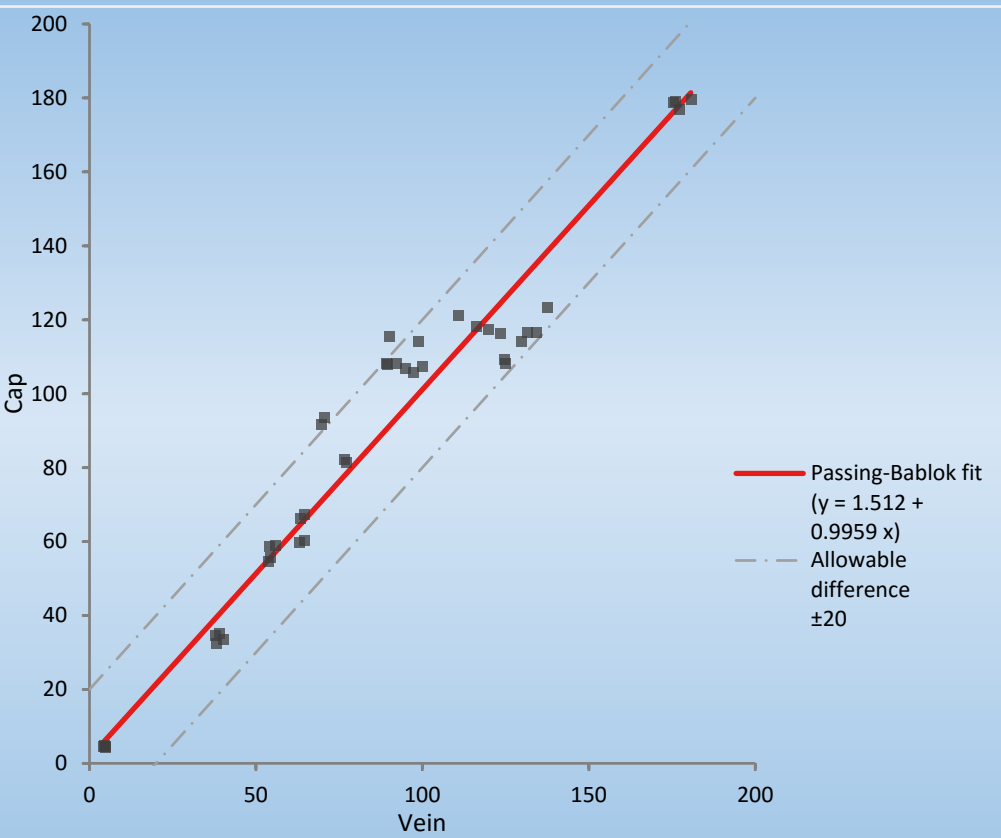
Cortisol Stress Response



Correlation Study Mt. Sinai



Cortisol (Vein vs. Capillary)

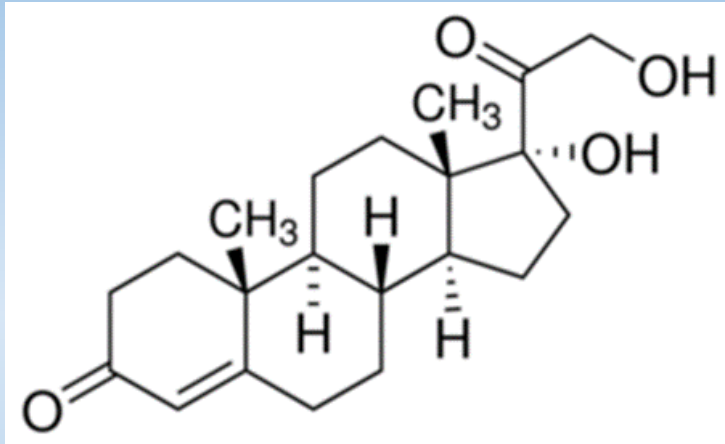


N	42	
	Minimum	Maximum
Vein	4.2170	180.6270
Cap	4.4260	179.5080
Correlation - r	0.973	

Fit Y on X		
Passing-Bablok fit		
Equation	Cap = 1.512 + 0.9959 Vein	
Parameter	Estimate	95% CI
Intercept	1.512	-1.933 to 9.628
Slope	0.9959	0.8942 to 1.070

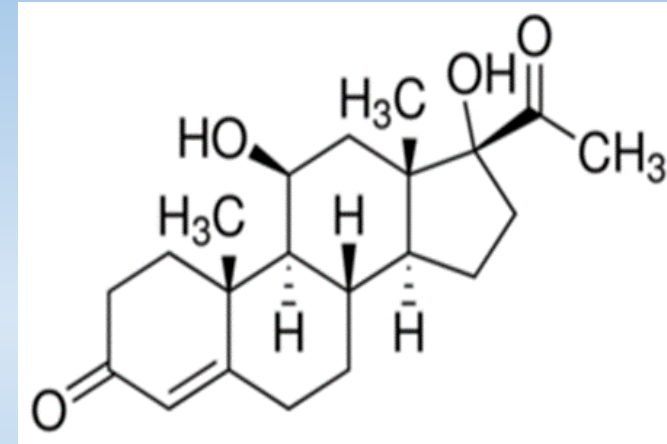
Corticosteroids

➤ 11-deoxycortisol:



- **Is directly converted to cortisol**
- Has many of the same effects as cortisol
- Levels of this hormone may be used to indicate (or monitor) congenital adrenal hyperplasia, or to differentiate primary vs secondary adrenal insufficiency.

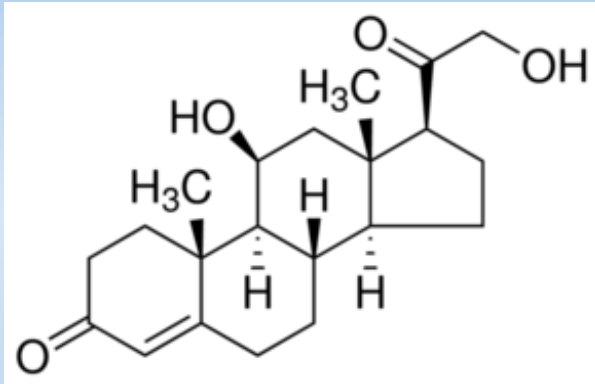
➤ 21-deoxycortisol:



- Used as a biomarker of **late-onset**, or **virilizing adrenal hyperplasia** (the 21-hydroxylase deficiency subtype of CAH).

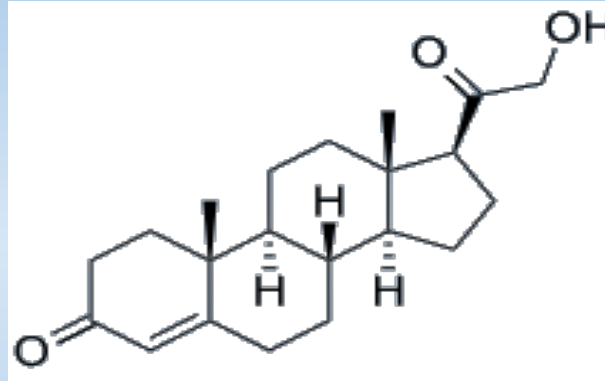
Minor Corticosteroids

➤ Corticosterone:



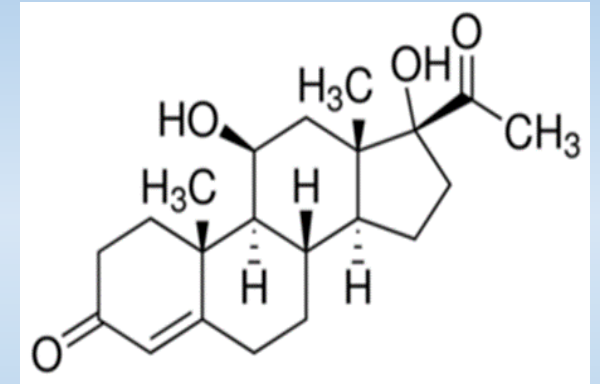
- Precursor to aldosterone, less potent corticosteroid.
- May be useful for athletes heading towards contests/competitions.

➤ 11-Deoxycorticosterone:



- Precursor to corticosterone (and therefore aldosterone).
- Its primary role is to regulate salts and water retention – similar to aldosterone.
- **Plays a role in intramuscular water retention.** (Competition/shows).

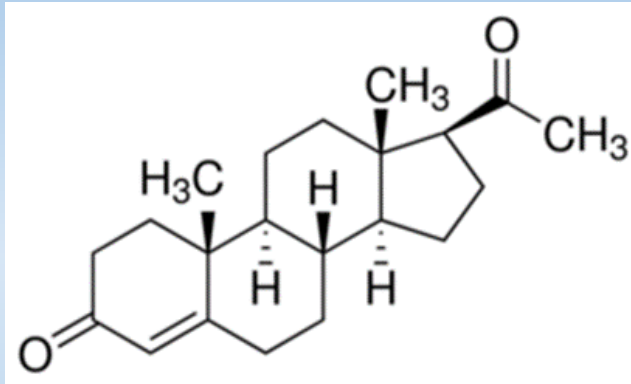
➤ 21-deoxycortisol:



- Used as a biomarker of **late-onset**, or **virilizing adrenal hyperplasia** (the 21-hydroxylase deficiency subtype of CAH).

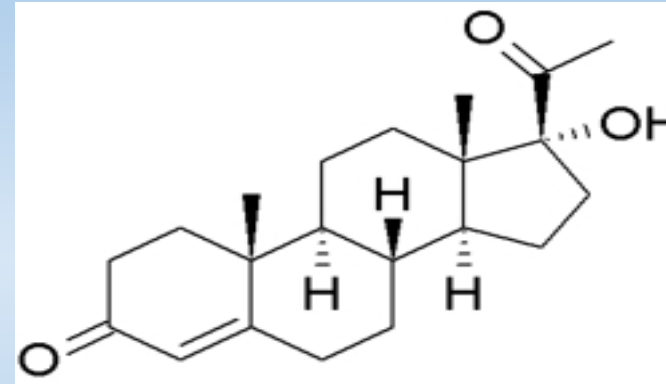
Sex Hormones

➤ Progesterone:



- In women: plays role in menstrual cycle, pregnancy, and sex drive.
- plays a role in brain health.
- Is commonly thought to be an estrogen antagonist.
- Its role in aging is actively being researched.

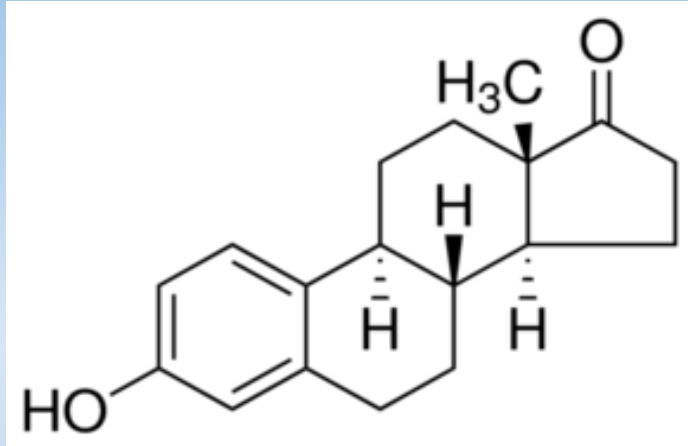
➤ 17-alpha-hydroxyl-Progesterone:



- Acts similar to progesterone, but on a weaker scale.
- **Decreases** activity of corticosteroids.
- Used in part to determine subtype of congenital adrenal hyperplasia
- Also used to reduce pregnancy loss in women.

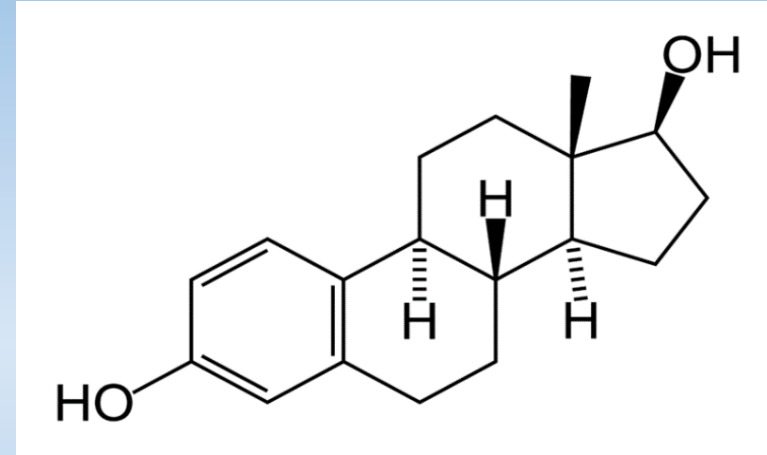
Sex Hormones

➤ Estrone:



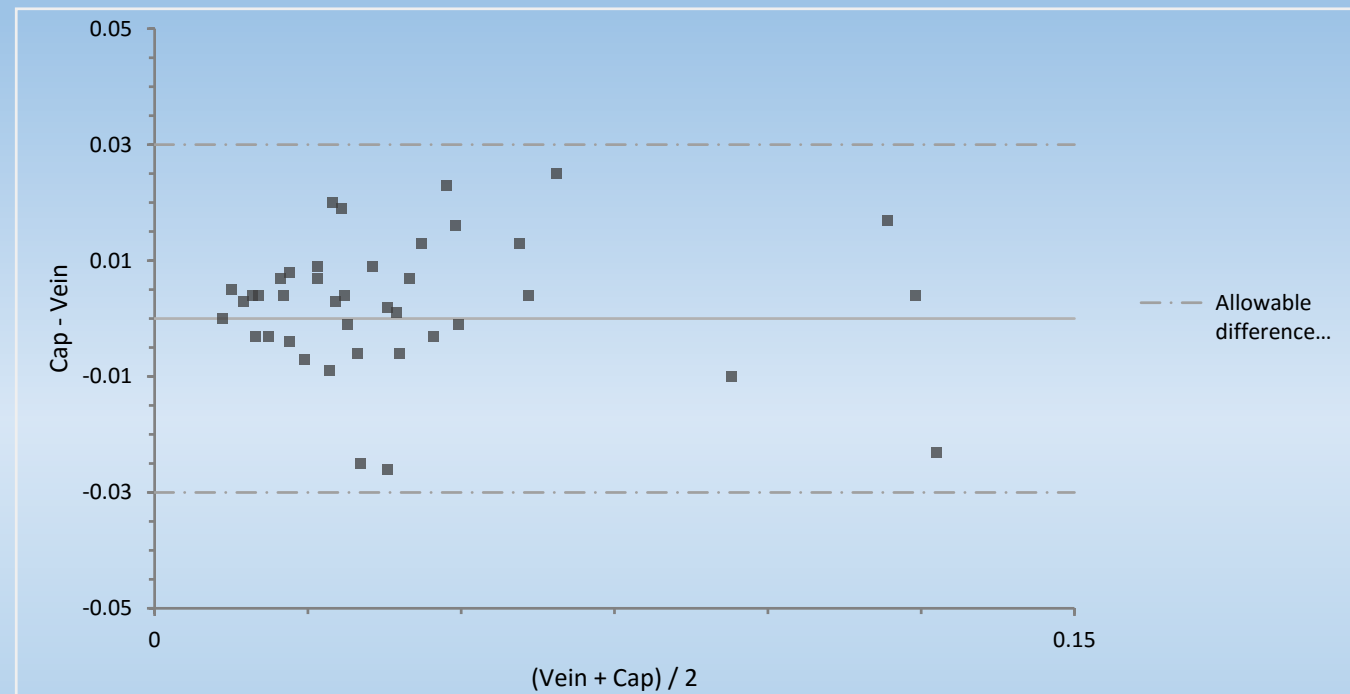
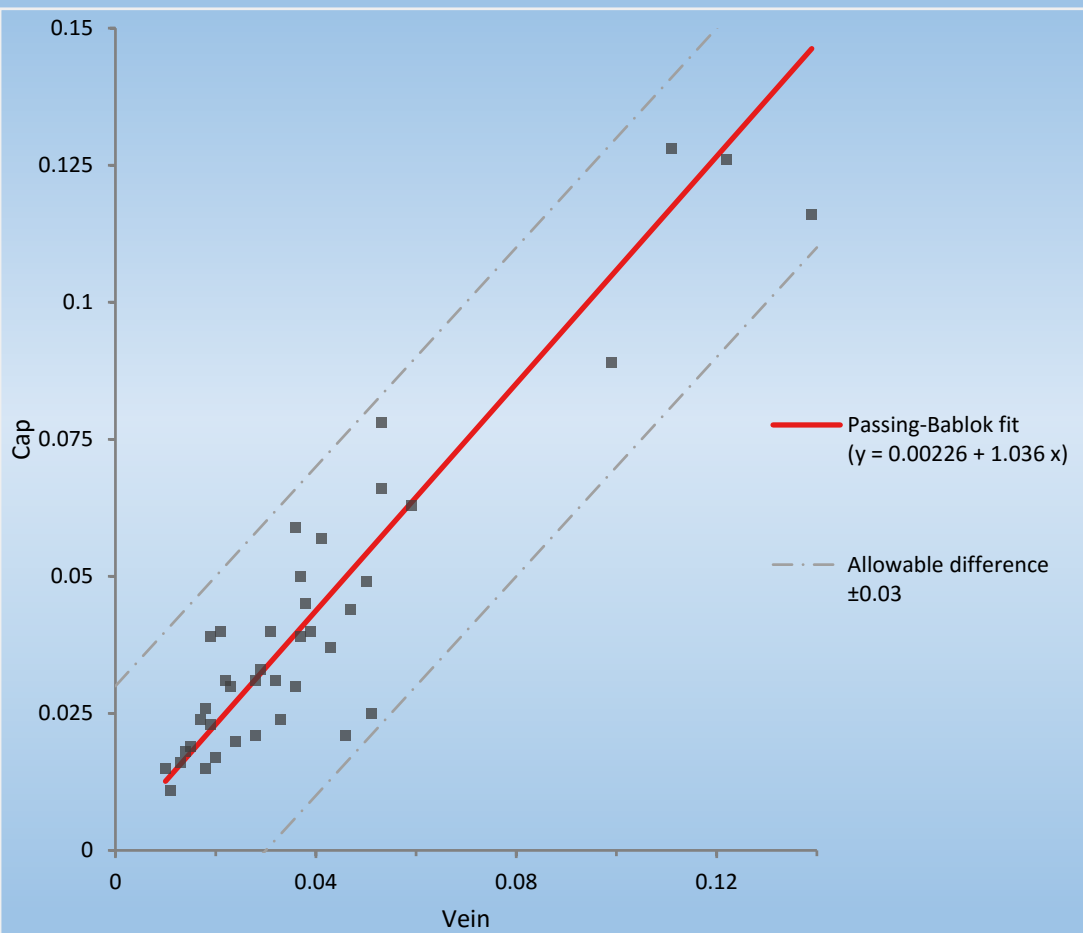
- Least abundant natural estrogen.
- Useful in measuring estrogen levels in menopausal women.
- Change in the ratio between estrone and estradiol may reflect ovarian deterioration or other conditions.
- May also be measured in men and women for cancer of the ovaries, testes and/or adrenal glands.

➤ Estradiol:



- Primary female sex hormone.
- Most potent of 3 estrogens.
- Plays a role in: female physical characteristics, pregnancy, growth, nervous system maturation, bone metabolism, etc.
- Useful in determining fertility in females, puberty disorders, estrogen deficiency in men, fracture risk assessment, etc.

Estrone (Vein vs Capillary)



Passing-Bablok fit

Equation | Cap = 0.00226 + 1.036 Vein

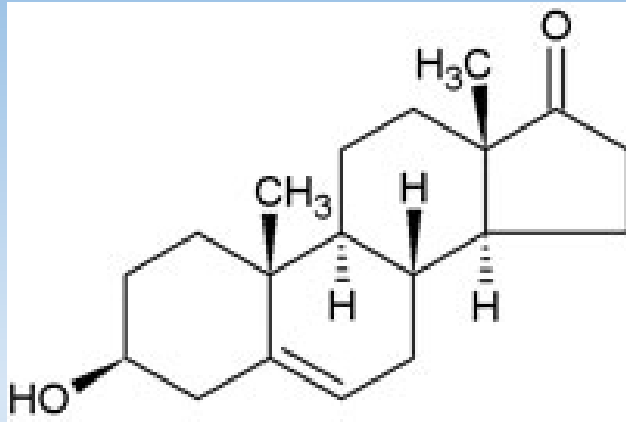
Parameter	Estimate	Bootstrap 95% CI
Intercept	0.002260	-0.004811 to 0.006111
Slope	1.036	0.8682 to 1.285

CI based on 99 bootstrap samples.

N	40	
	Minimum	Maximum
Vein	0.0100	0.1390
Cap	0.0110	0.1280
Correlation - r	0.923	

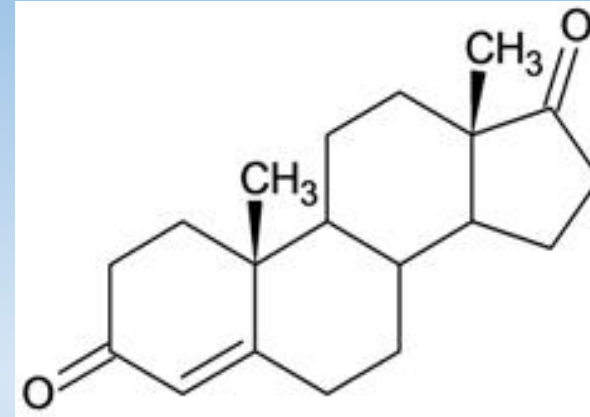
Sex Hormones

➤ DHEA (Dehydroepiandrosterone):



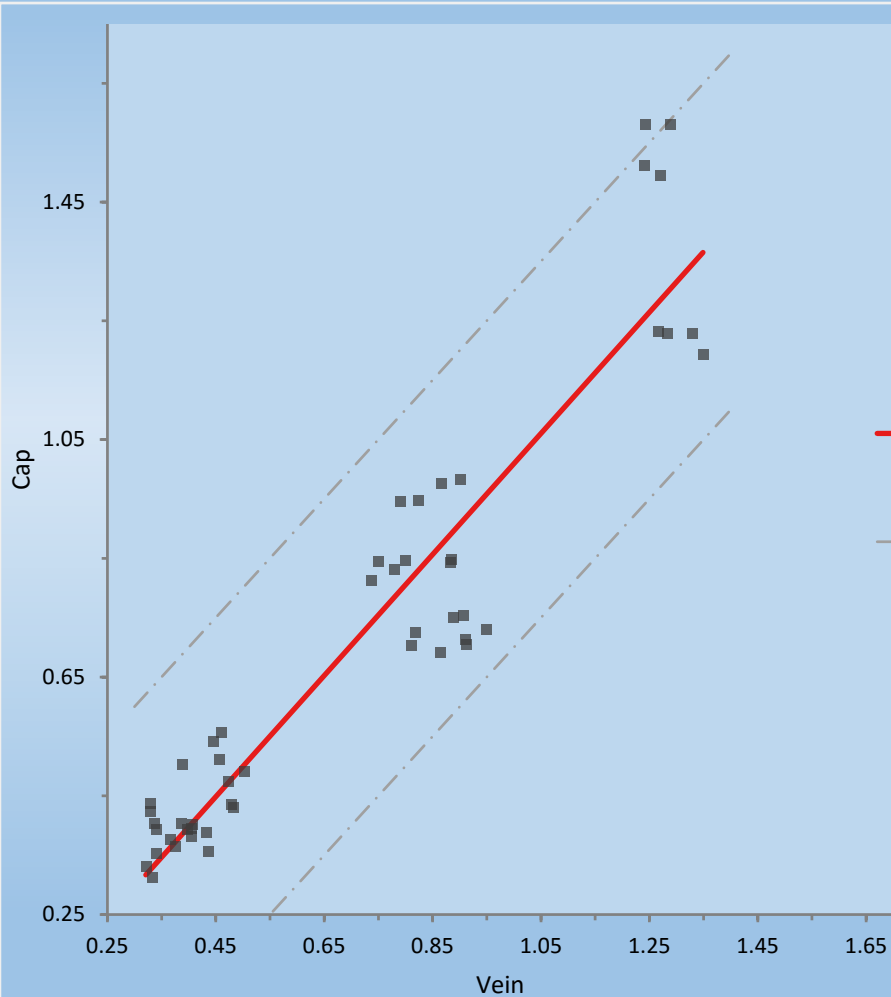
- Secreted by the adrenal gland, gonads, and brain.
- Leads to the production of androgens and estrogens.
- For men and women: DHEA levels start decreasing around age 30.
- Decreased DHEA levels have been measured in various disease/chronic states.
- Elevated DHEA levels do NOT translate into higher Testosterone levels

➤ Androstenedione:



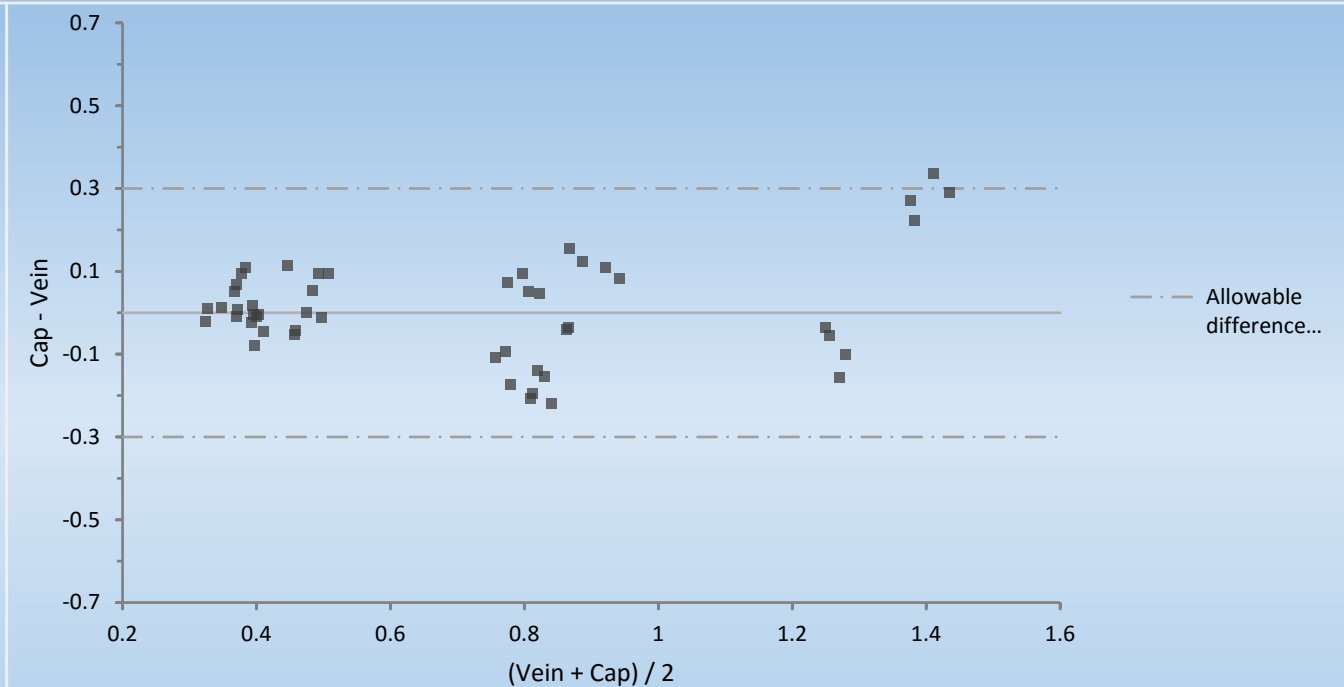
- Weak androgen, precursor of other androgens/estrogens.
- In doses = or > 300 mg/d, androstenedione has been shown to increase testosterone and estradiol concentrations in *some* healthy men.¹

Androstenedione



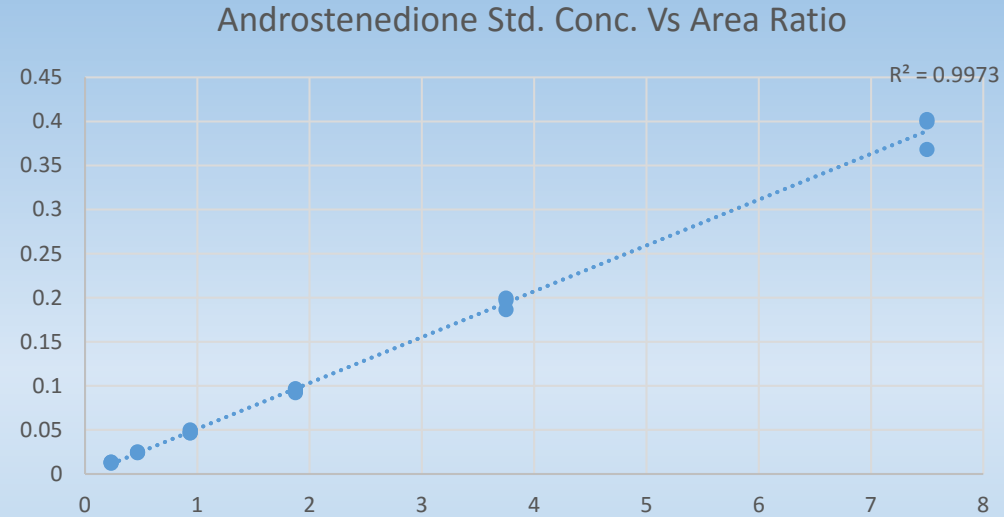
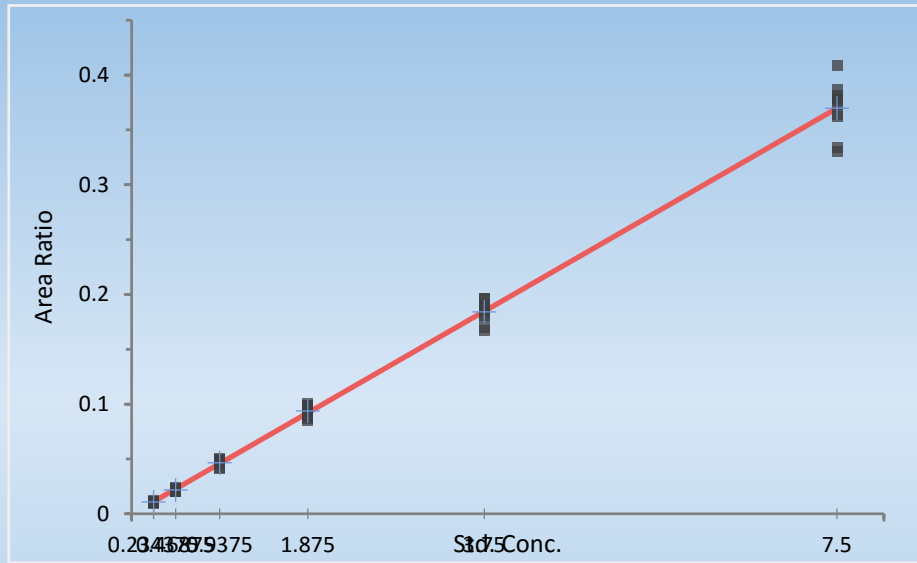
Passing-Bablok fit
($y = -0.0106 + 1.02x$)

Allowable difference
 ± 0.3



N		50	Passing-Bablok fit		
			Equation	Cap = -0.0106 + 1.02 Vein	
	Minimum	Maximum	Parameter	Estimate	Bootstrap 95% CI
Vein	0.3210	1.3490	Intercept	-0.01060	-0.1184 to 0.06257
Cap	0.3130	1.5810	Slope	1.020	0.8810 to 1.267
Correlation - r		0.940	CI based on 99 bootstrap samples.		

Androstenedione Linearity

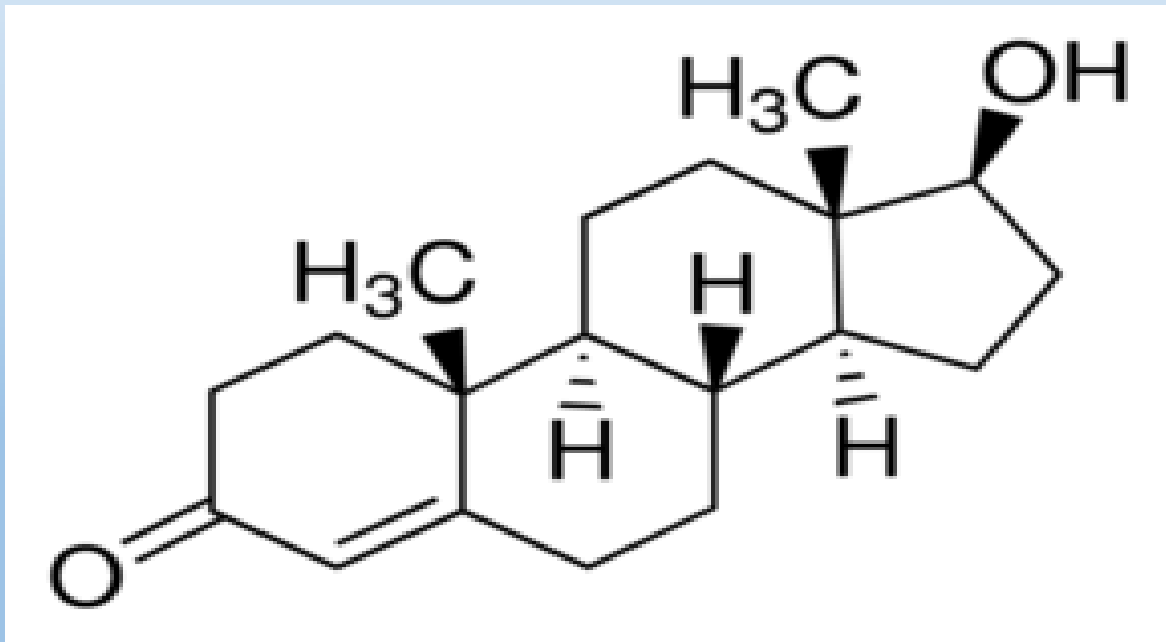


Measuring interval | 0.23437500 to 7.50000000

Std. Conc.	N	Mean	Mean SE	Recovery	SD
0.234375	10	0.01071737	0.000180004	4.6%	0.00056922
0.46875	10	0.02173708	0.000327889	4.6%	0.00103688
0.9375	10	0.04646030	0.000967319	5.0%	0.00305893
1.875	10	0.09378464	0.001655540	5.0%	0.00523528
3.75	10	0.18398780	0.003142871	4.9%	0.00993863
7.5	10	0.36967970	0.007503894	4.9%	0.02372940

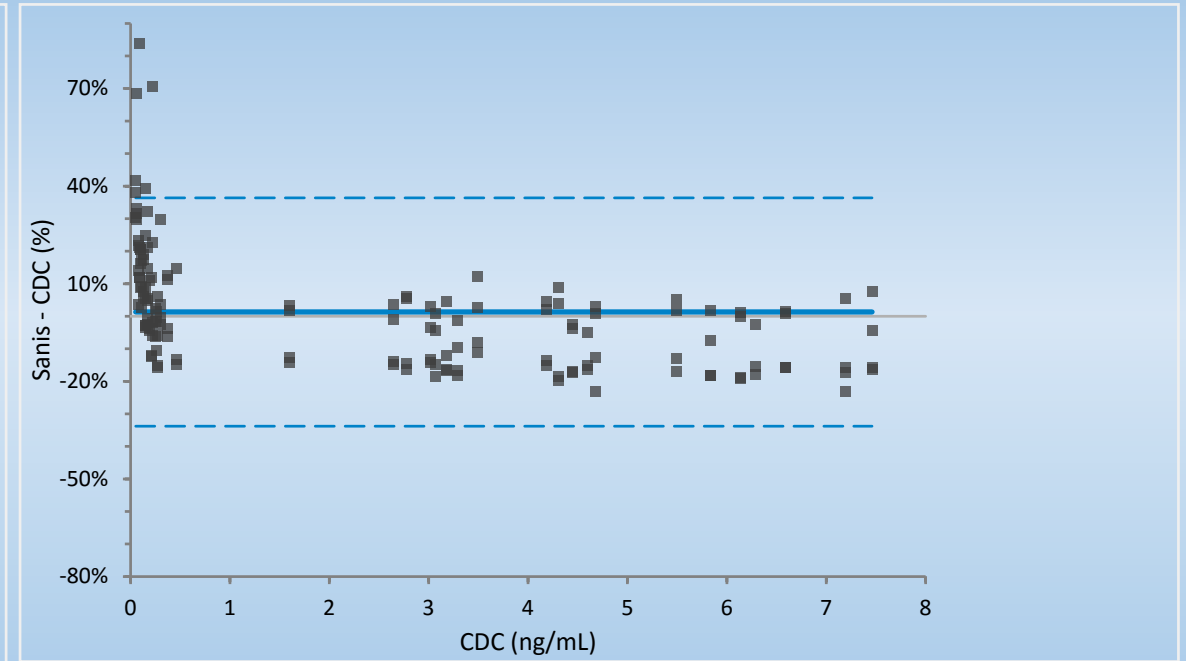
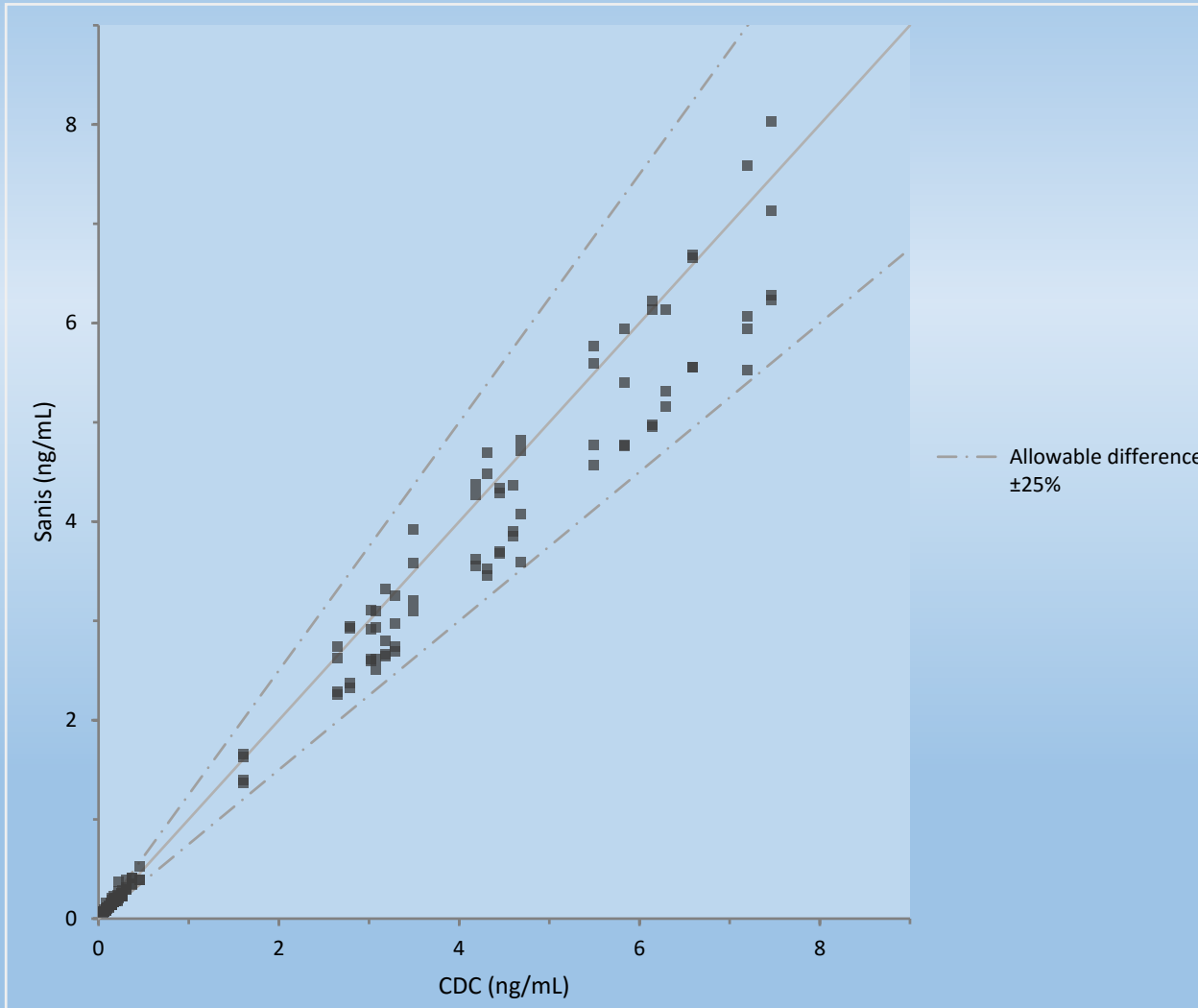
Sex Hormones (Testosterone)

- Primary androgen.
- Starts decreasing after approximately age 40 in men.
- Responsible for male physical characteristics, maintenance of muscle bulk, cardiovascular health, bone growth and numerous other functions.
- Low testosterone can due to: hypogonadism, metabolic disorders, infection, malnutrition, *excessive exercise*, tumors, etc



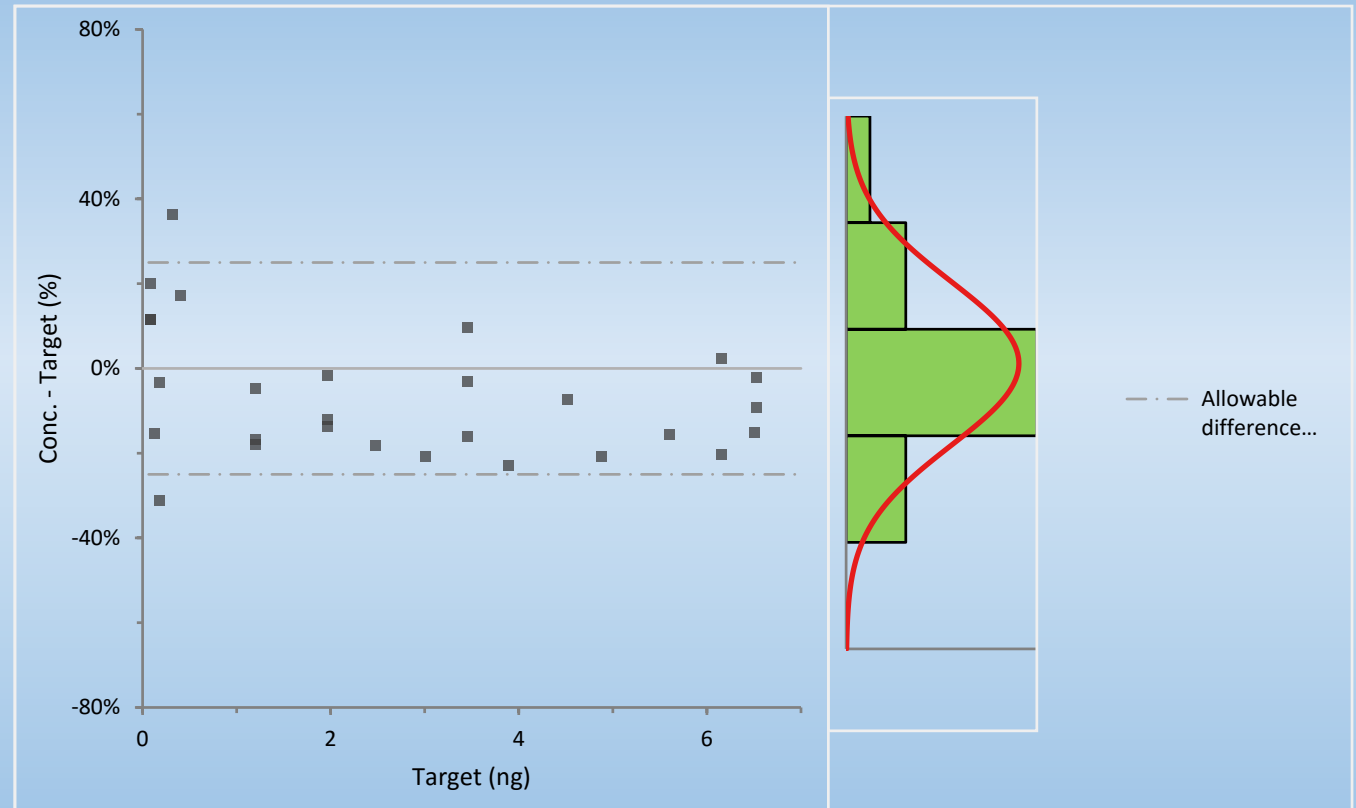
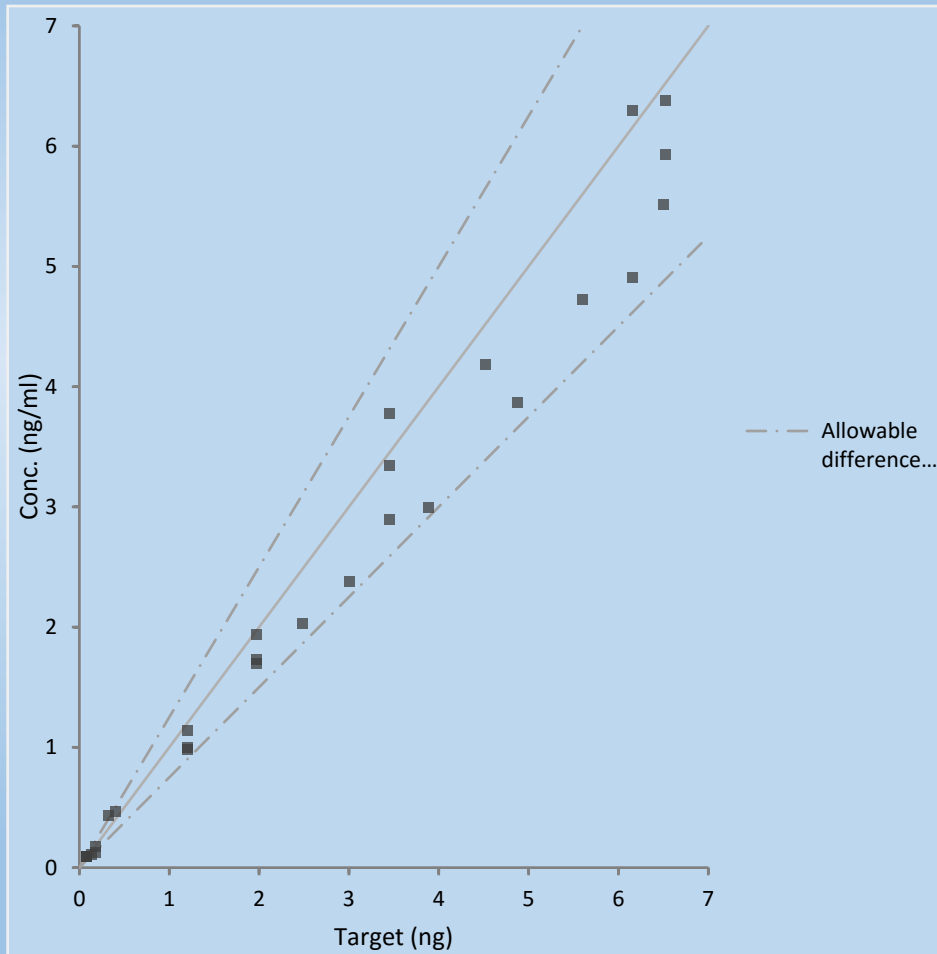
Protein	Steroid(s) Bound	Binding Affinity	Binding Capacity
Albumin	All steroids	Low	High
alpha1-Acid glycoprotein (orosomucoi d)	Progesterone	Low	High
Sex hormone-binding globulin (SHBG)	Dihydrotestostero ne, testosterone, estradiol	High	Low
Corticosteroi d-binding globulin (CBG)	Corticosteroids, progesterone, 17α-hydroxyprogester one	High	Low

CDC Testosterone Correlation Studies



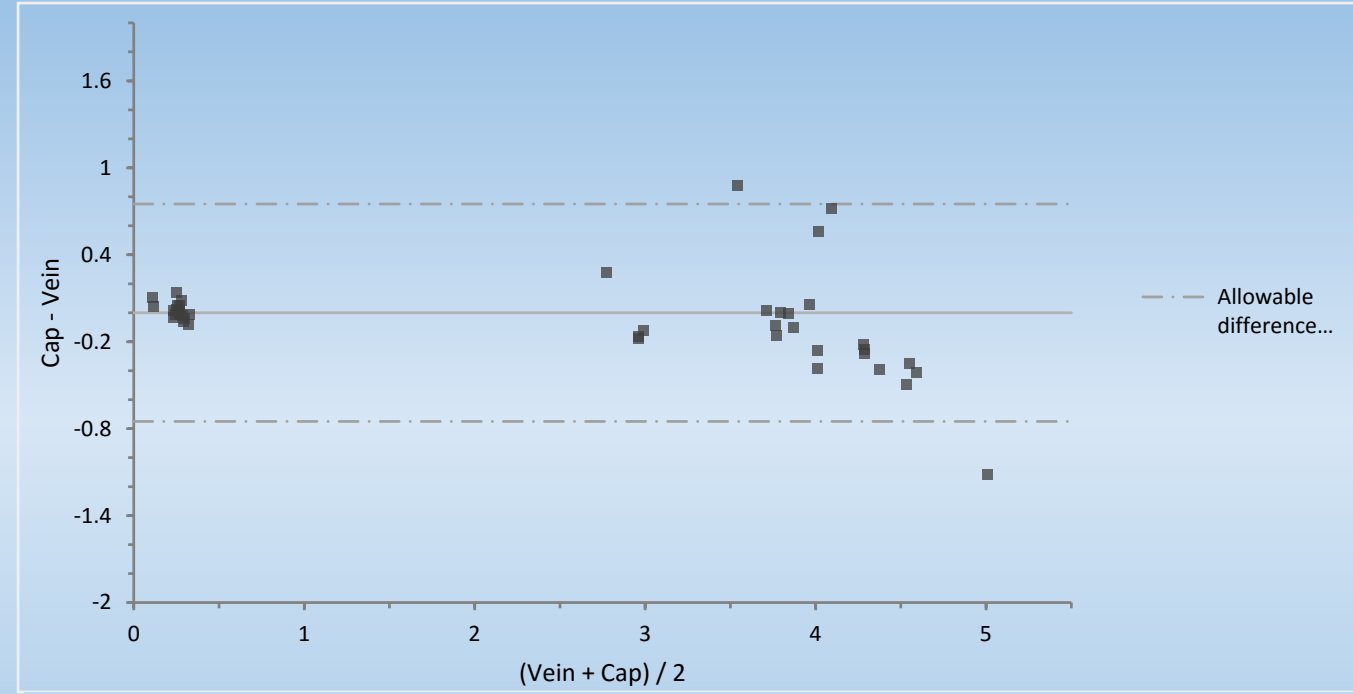
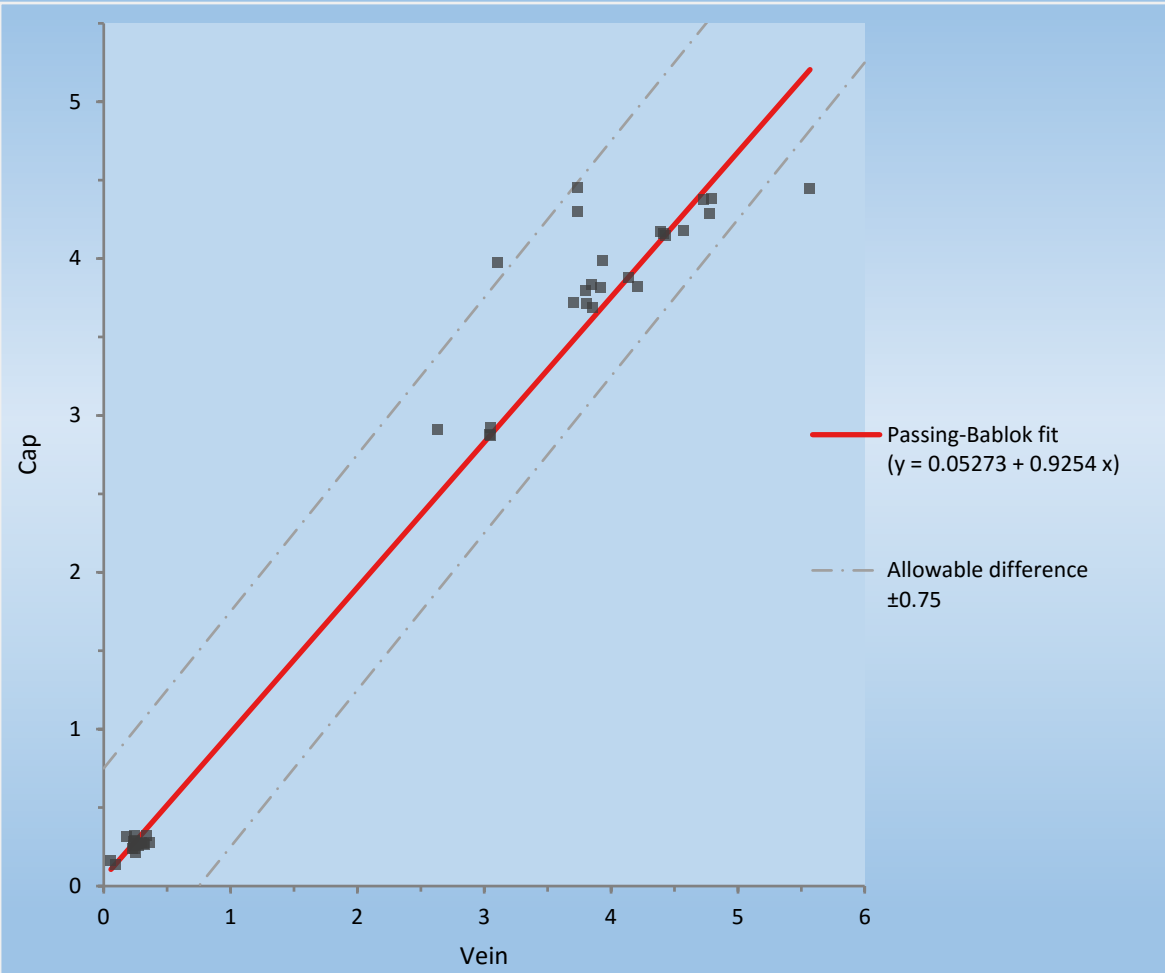
- The only outliers occurred at concentrations less than 100 pg/mL
- 1 of only 4 labs in US submitting to CDC

Mount Sinai Correlation for Testosterone



Demonstrates a high degree of correlation between Hospital & Sanis Biomedical

Testosterone (Vein vs. Capillary)



Passing-Bablok fit

Equation | $\text{Cap} = 0.05273 + 0.9254 \text{ Vein}$

Parameter	Estimate	Bootstrap 99% CI
Intercept	0.05273	0.004135 to 0.1402
Slope	0.9254	0.8884 to 0.9596

CI based on 99 bootstrap samples.

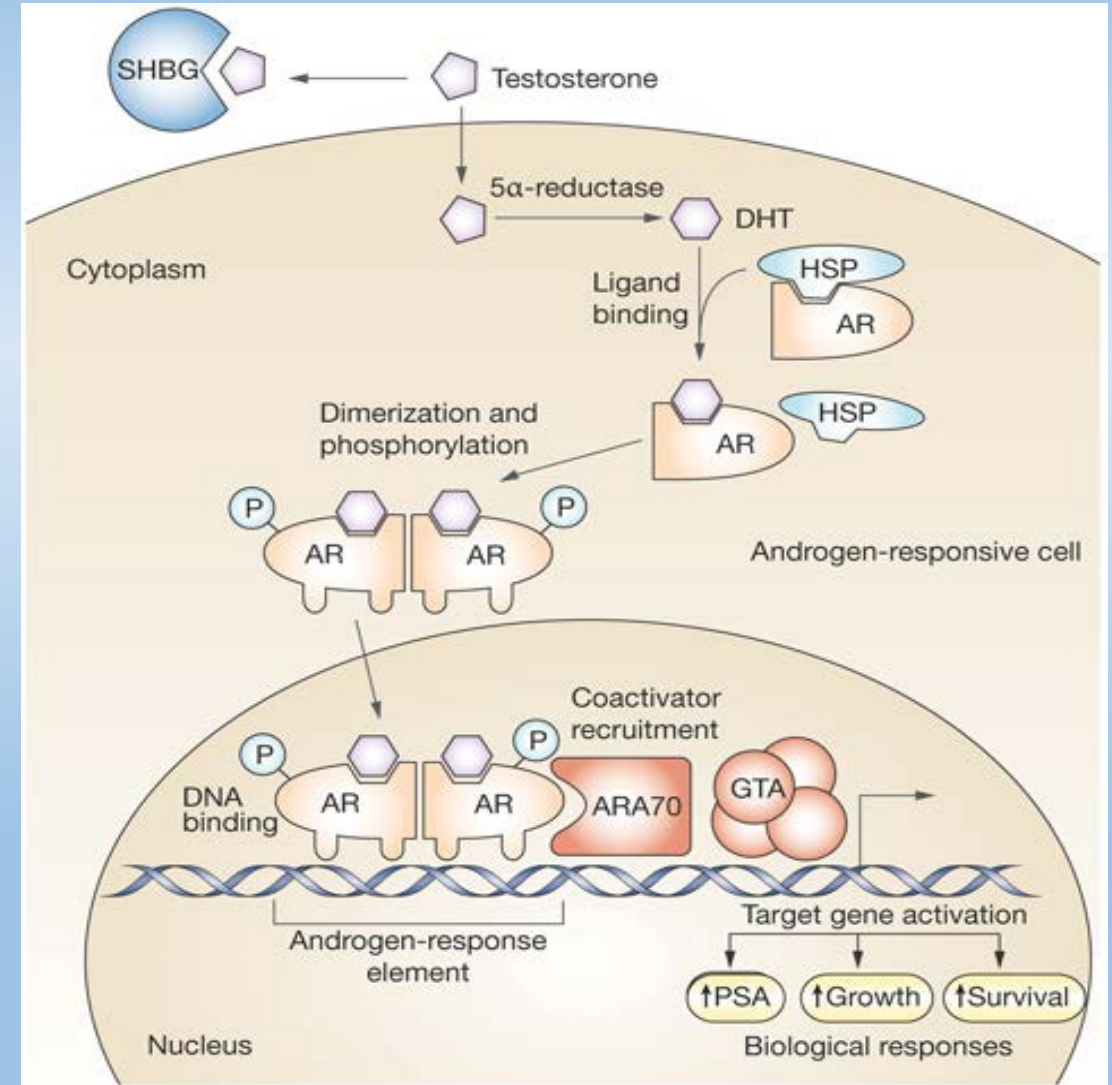
N | 42

	Minimum	Maximum
Vein	0.0570	5.5680
Cap	0.1380	4.4550

Correlation - r | 0.987

Summary of Findings

- CDC demonstrated method's accuracy with greater than 95% confidence to 70 pg/mL.
- Mt. Sinai demonstrates method equivalence to conventional testing utilizing reduce volume of blood in testing with good correlation
- Venous vs Capillary Study demonstrates Blood draw from finger or vein virtually equal
- Mt Sinai also demonstrates good correlation that measurement values are for total testosterone
- Most in depth single shot panel created on LC-MS/MS that utilizes the least amount of required sample
- The statistical data collected from our methodology will accelerate what the medical community understands about the field of endocrinology



Applications with Technology in DTC Marketplace

- Monitoring of Systemic effects of exogenous androgens
- Dietary & Supplementary effects on Hormone levels
- Routine monitoring of endocrine system to improve training and overall performance
- Effects of drugs on the endocrine system

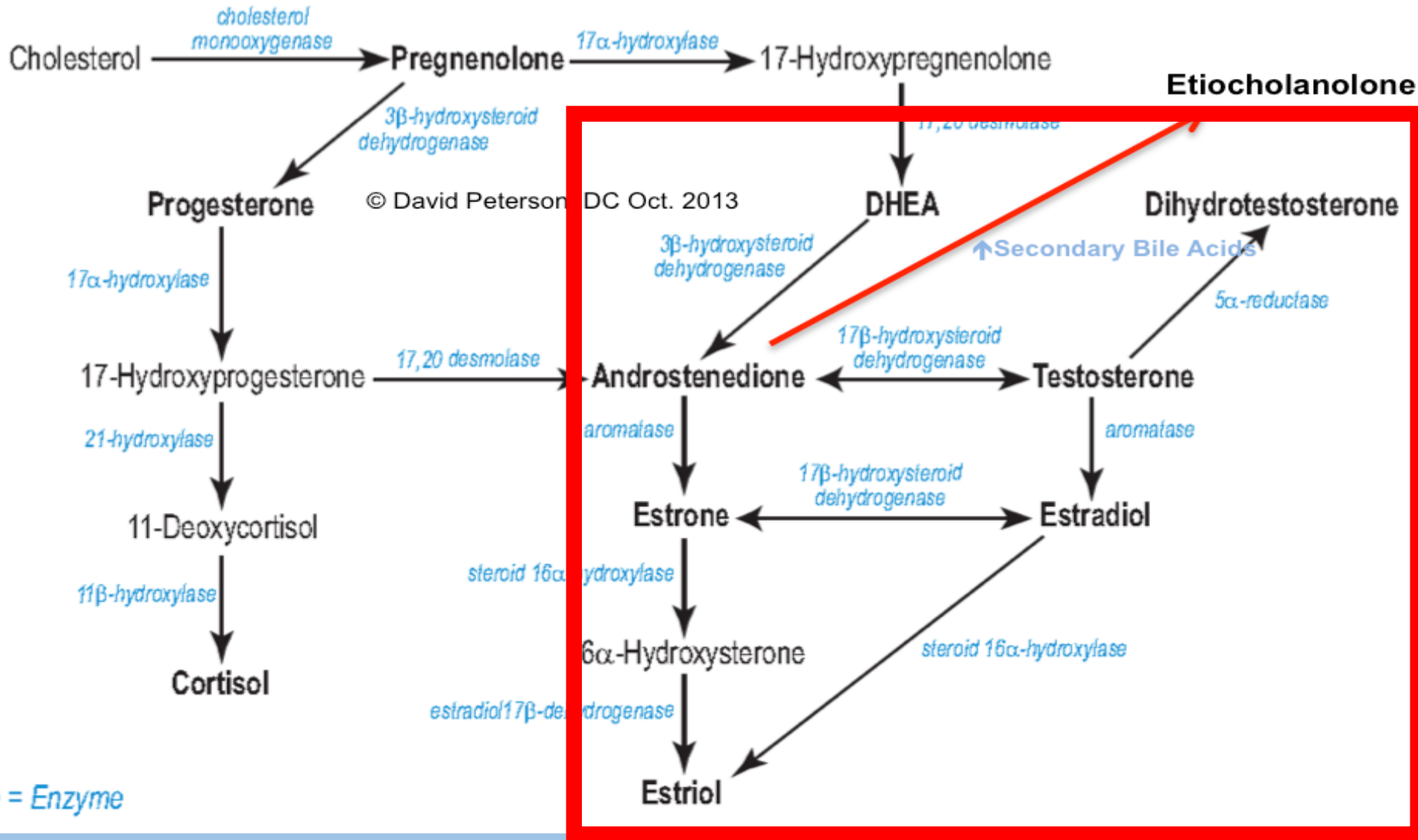
Testosterone Therapy

- Patient receiving 200mg/mL of Testosterone Cypionate per week
- Testosterone in healthy range
- DHEA & Androstenedione fall below normal range due to negative feedback from Testosterone
- DHEA supplementation could slow negative feedback loop of exogenous testosterone supplementation

Hormone	Conc. (ng/ml)	Clinical Range (ng/ml)	Source
Testosterone	8.908	2.4 - 9.5	A
Testosterone	7.899		
Androstenedione	0.547	0.6 - 1.90	B
Androstenedione	0.545		
DHEA	0.719	1.0 - 4.7	C
DHEA	0.64		
Estrone	0.023	≤ 0.068	D
Estrone	0.015		
Estradiol	0.077	≤ 0.029	E
Estradiol	0.065		

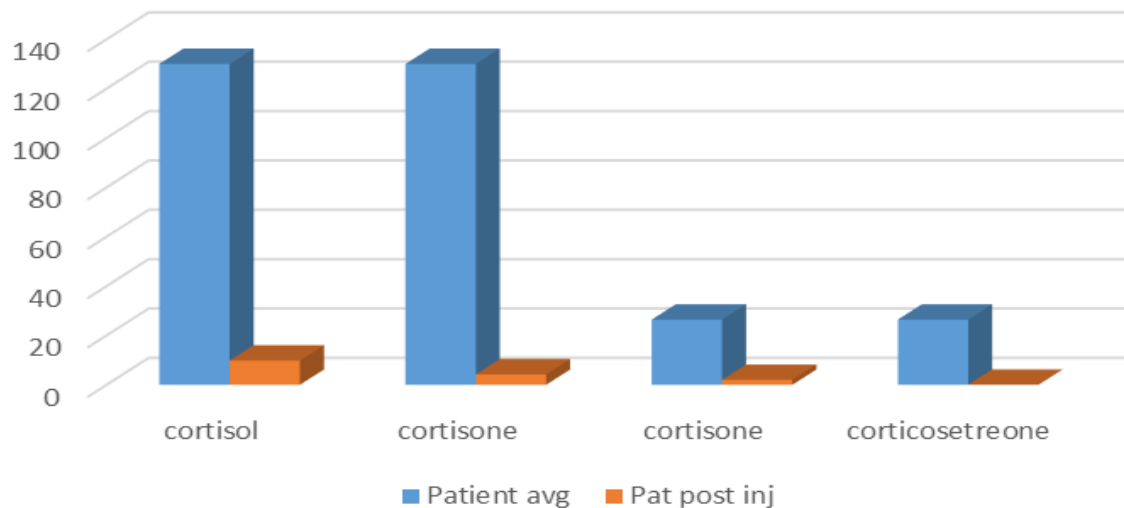
Sources: A. <http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8508> B. <http://emedicine.medscape.com/article/2088804-overview>
 C. <http://emedicine.medscape.com/article/2088870-overview> D. http://www.questdiagnostics.com/hcp/intguide/EndoMetab/EndoManual_AtoZ_PDFs/Estrone_Serum.pdf
 E. <http://www.questdiagnostics.com/testcenter/TestDetail.action?ntc=36169>

Testosterone Therapy Negative Feedback Loop

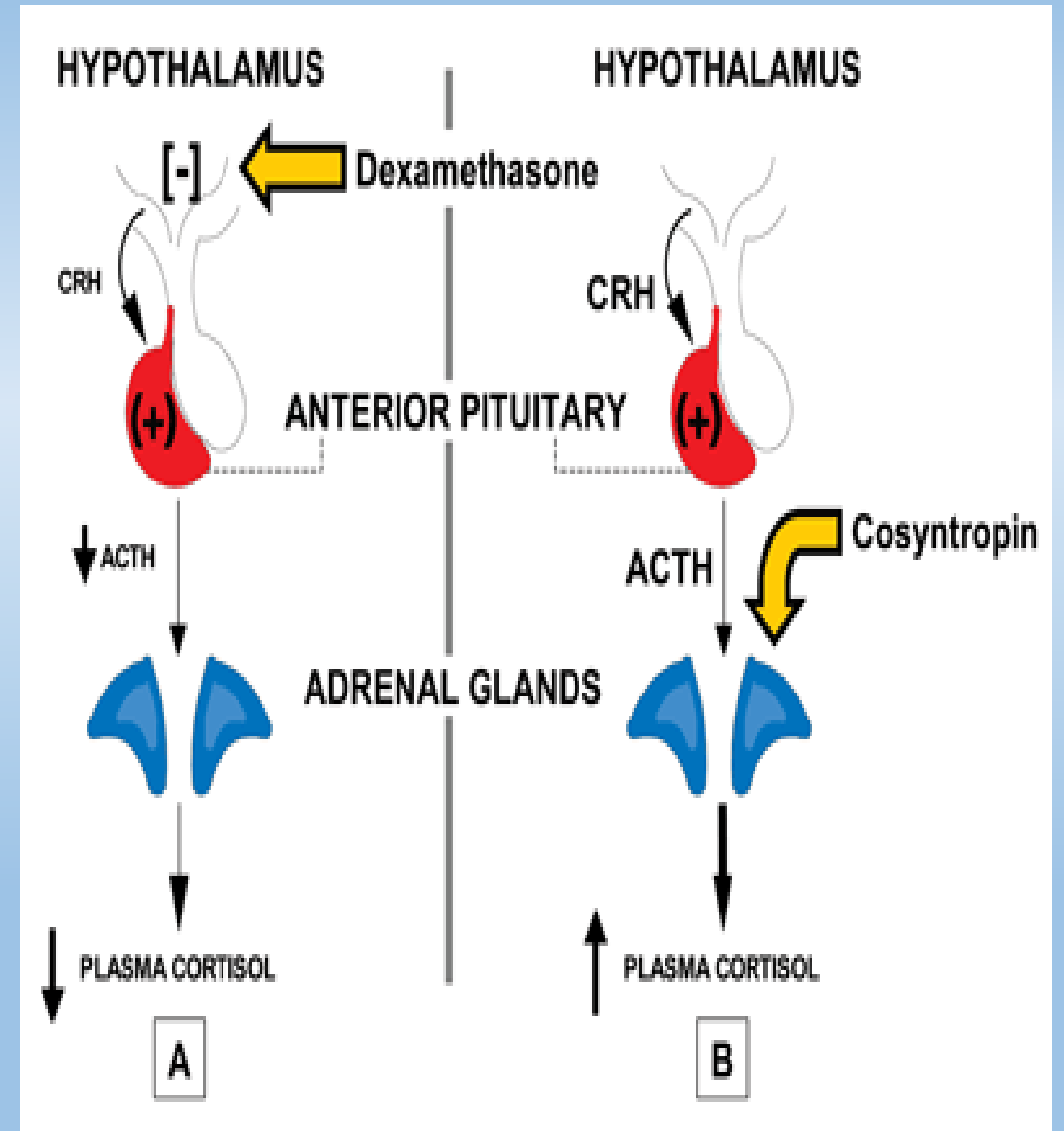


Monitoring Therapeutic Response

Dexamethasone Suppression Effect

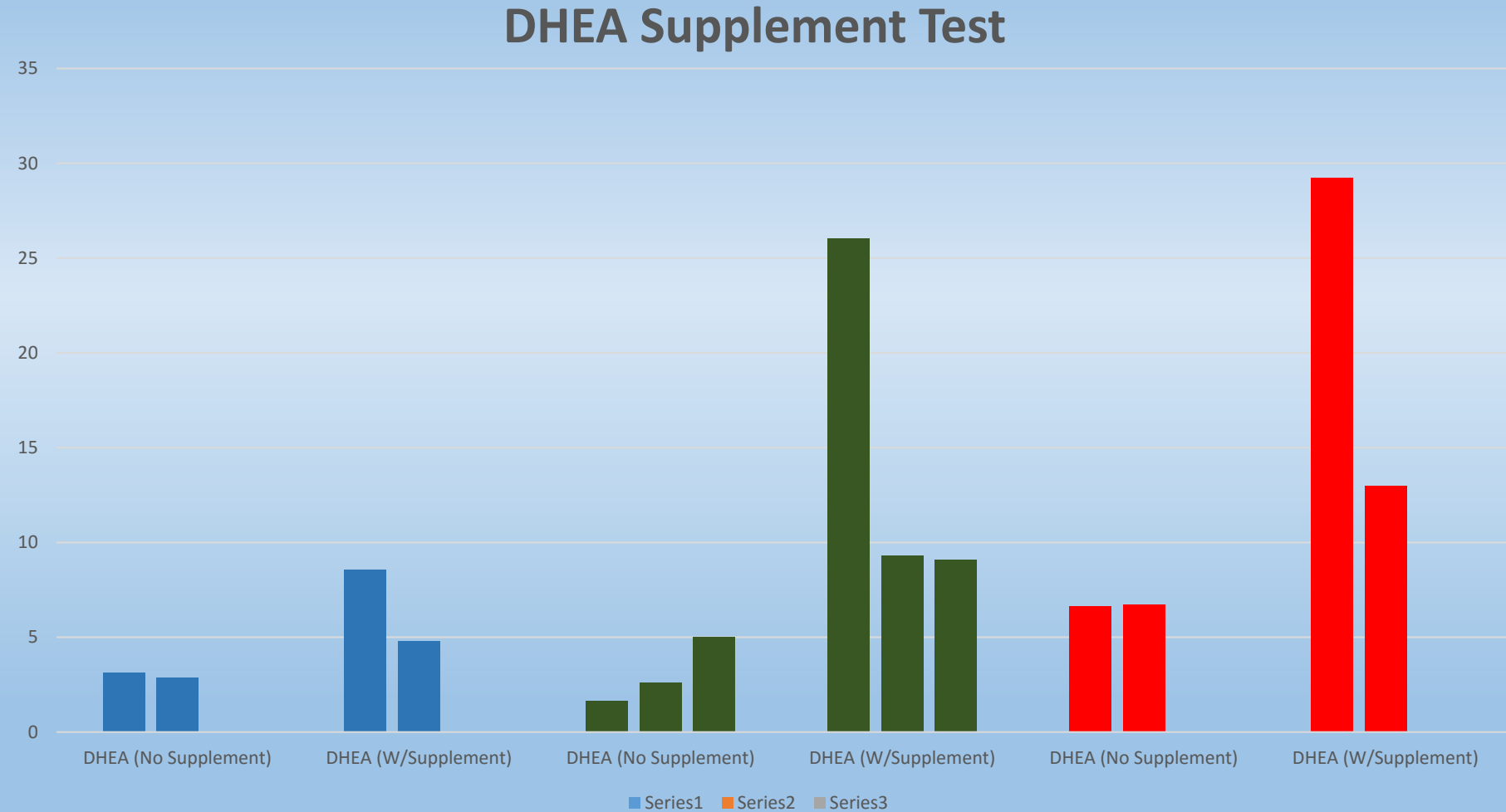


	Conc. (ng/ml)	Average (ng/ml)	Clinical Range (ng/ml)
Patient 1			
Cortisol	9.853	130.00	70 - 280 (a.m.)
Cortisone	2.003	26.40	12 - 35 (a.m.)
Patient 2			
Cortisol	4.21	n/a	70 - 280 (a.m.)
Cortisone	0.35	n/a	12 - 35 (a.m.)



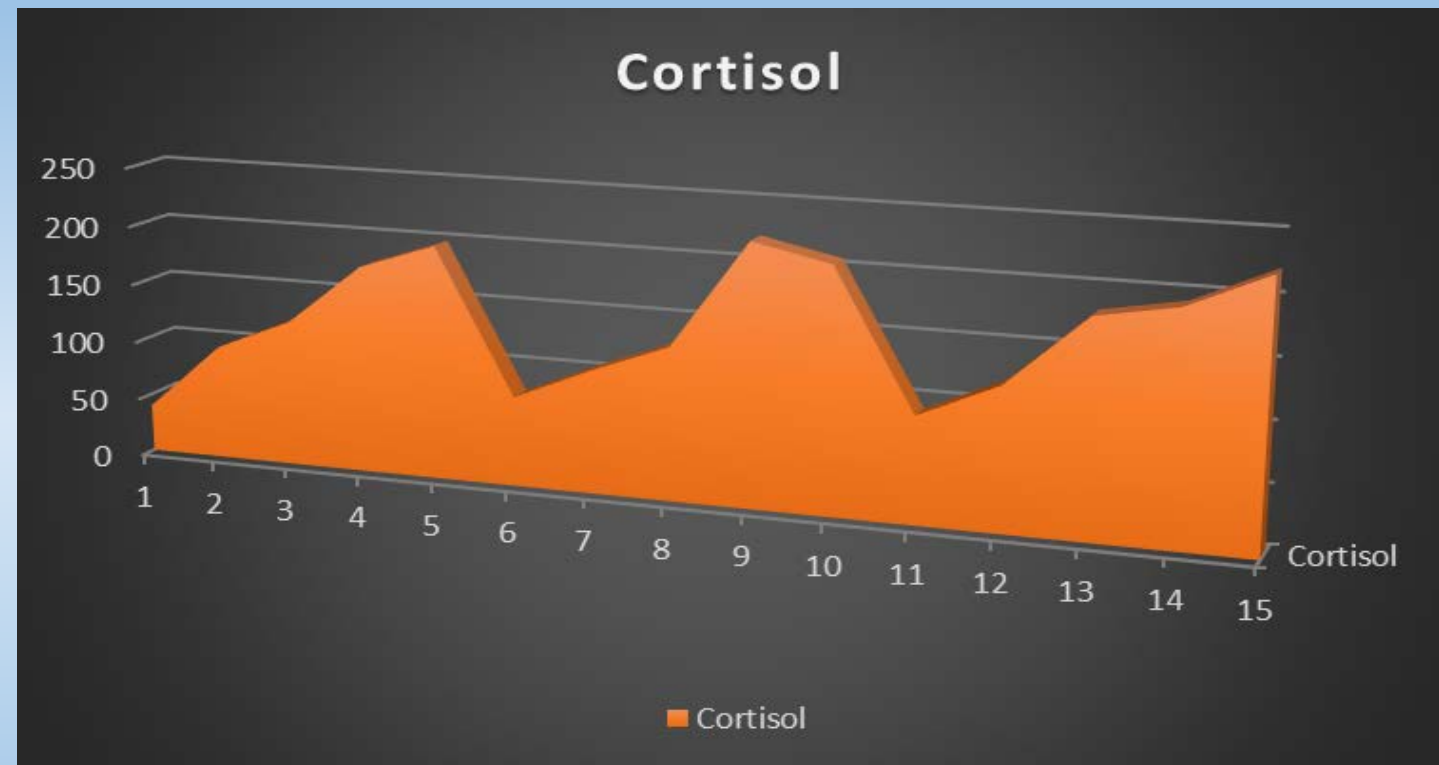
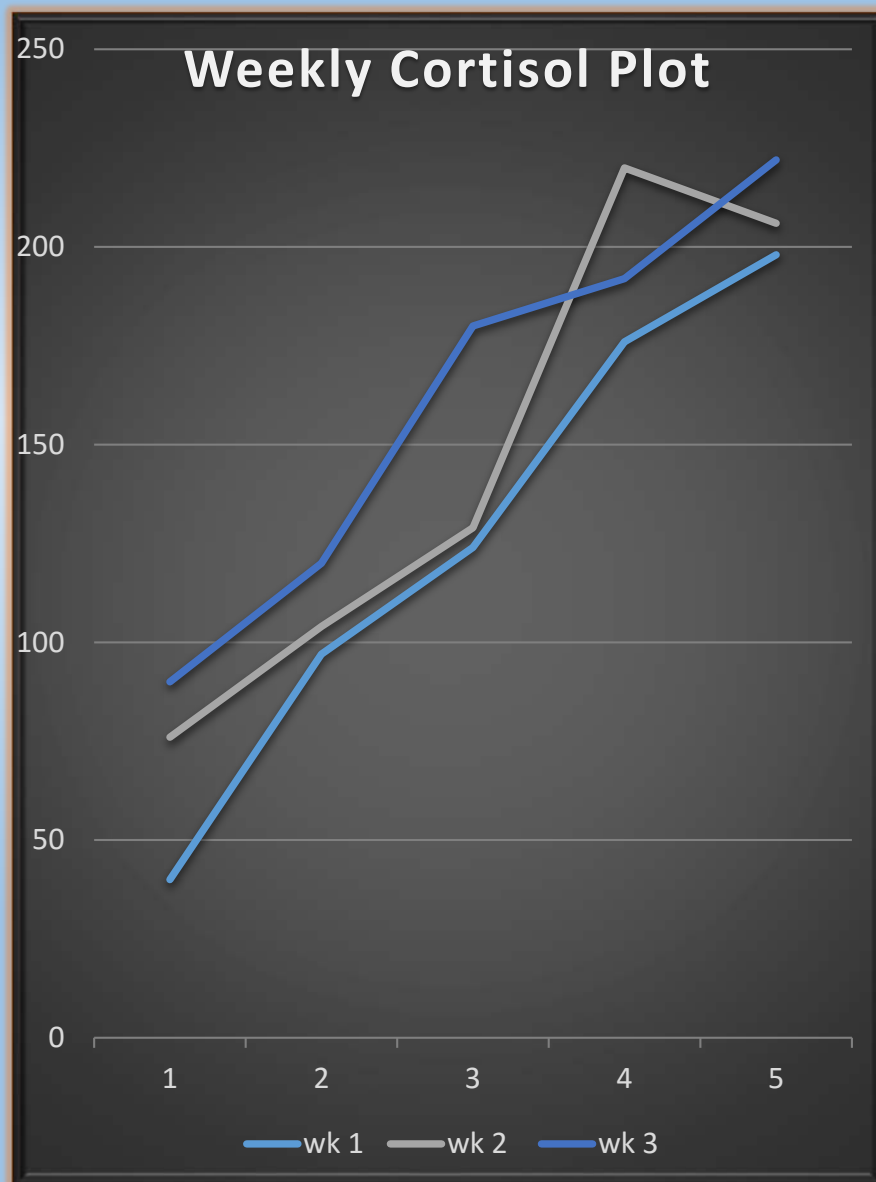
DHEA Supplement Effect on DHEA levels

- 50 mg 2x/day
- Demonstrates Oral Bioavailability of DHEA
- DHEA levels will return to normal rapidly without continual supplementation
- Important clinical in terms of the population receiving testosterone therapy



3 days, 3 patients. Each color represents a different patient;
Blue: (M, 35); Green: (F, 33); Red (M, 40).

Effects of Adrenal Fatigue from Work Week



- the effects of long hours of work or over exhaustion can be quantified
- Marker for overtraining can be established
- Food and supplements that can lower cortisol can be tested
- Pattern Suggest change in schedule to avoid adrenal fatigue by end of week