Lab Assessments – Male Patients

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President, Precision Analytical, Inc.

1

Monitoring TRT

2

First, What about DHEA?

If DHEA and T are low,
can I just give DHEA?

3

DHEA Independently Important

- Besides a decline in testosterone levels, there is also a decline seen in dehydroepiandrosterone (DHEA) in aging males.
- Several studies have shown that **restoring DHEA to youthful levels in older adults increases both physical and mental well being**.
- In a randomized, placebo-controlled trial of 50mg of DHEA given every night for six months, **both male and female patients (aged 40 – 70) who took DHEA had statistically significant improvements in their energy levels, quality of sleep, mood, and ability to handle stress**.

References: Morales A, Nolan J, Nelson J, Yes S. Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. The Journal of Clinical Endocrinology And Metabolist [serial online]. June 1994;78(6):1360-1367

4

DHEA Independently Important in CAD

STUDY:

- 2,614 men age 69-80 in Gothenburg, Uppsala and Malmö for five years – assessed DHEA levels.
- The findings demonstrated that the lower the DHEA level at the study start, the greater the risk of coronary heart disease events during the five-year follow-up.

Men with low levels of DHEA in the blood run an increased risk of developing coronary heart disease events

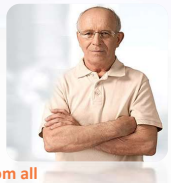
References: Åsa Tivesten, et at. (2014). Dehydroepiandrosterone and its Sulfate Predict the 5-Year Risk of Coronary Heart Disease Events in Elderly Men. *Journal of American College of Cardiology*, 28 October 2014

5

Low Serum DHEA Sulfate Predicts All – Cause and Cardiovascular Mortality

STUDY - MAIN OUTCOME MEASURES:

- Association between low DHEA-S and CVD death remained after adjustment for C-reactive protein and circulating estradiol and testosterone levels.



Conclusions:

Low serum levels of DHEA(-S) predict death from all causes, CVD, and ischemic heart disease in older men.

References: Ohlsson, C. et al. (2010). Low serum levels of dehydroepiandrosterone sulfate predict all-cause and cardiovascular mortality in elderly Swedish men. *J Clin Endocrinol Meta*. Sep;95(9):4406-14. doi: 10.1210/jc.2013-0760

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DHEA Therapy in Men

- **Starting Doses: 10 to 25 mg**
- **Common Maintenance Dose: 25 to 75 mg**
- **High Dosages: 100 to 200 mg**

Monitor:

- **Initial, then 2-3 months after initial TX, then q 6 to 12 months**
- DRE, DHEA-S, Free & Total Testosterone, Estradiol, DHT, SHBG, PSA, CBC with Diff, Ferritin. (same labs as for TRT!)

(Don't forget to first address excess alcohol, exercise, body composition, insulin, stress and sleep issues that can affect the P450 enzymes involved in androgen production)

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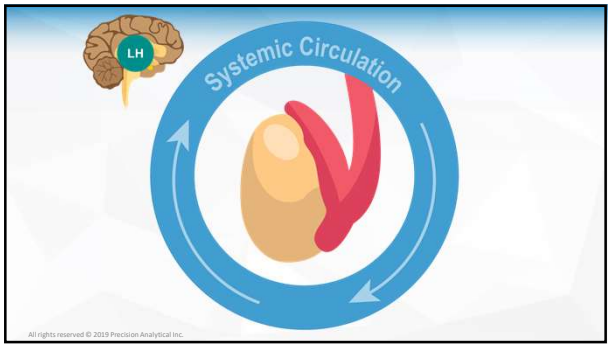
DHEA Supplementation

- NOT TRT!!!!
- Remember proper physiology
  - The testes do NOT make T from circulating DHEA.
- DHEA is for DHEA…TRT is for T
  - But both can get turned into estrogen!
- DHEA can be a good way to increase levels of T in women

8



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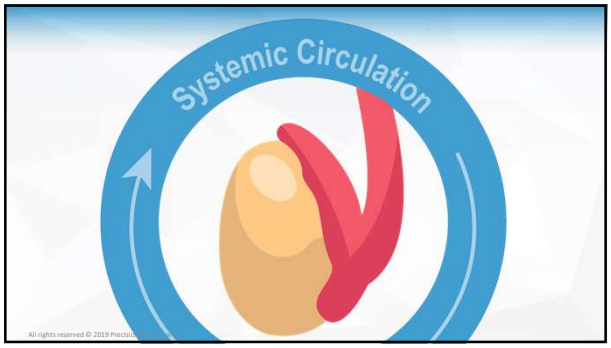
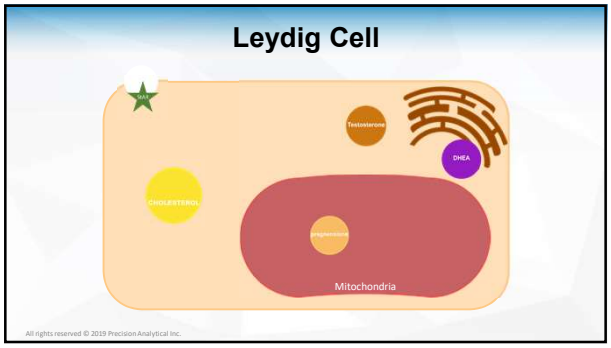
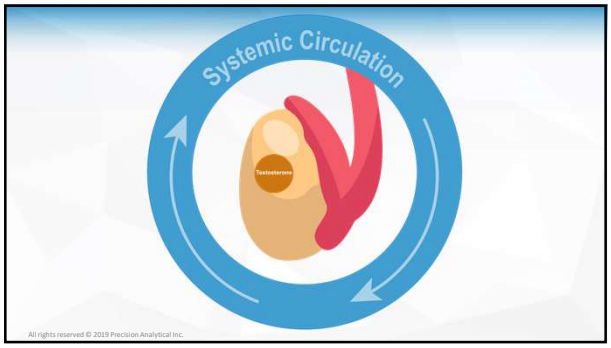

Systemic Circulation

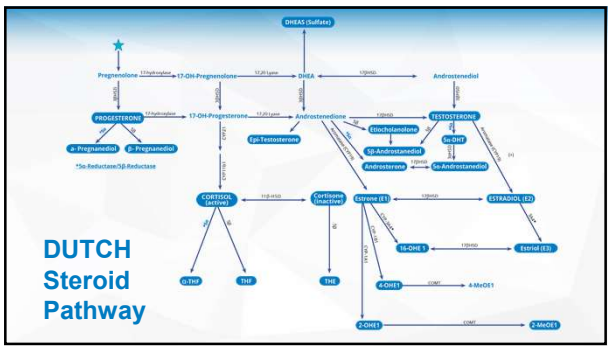
10


Leydig Cell
Testosterone
DHEA
CHOLESTEROL
Mitochondria

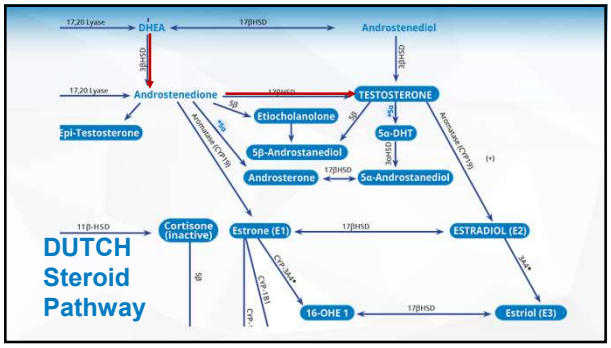
11


Systemic Circulation
Testosterone

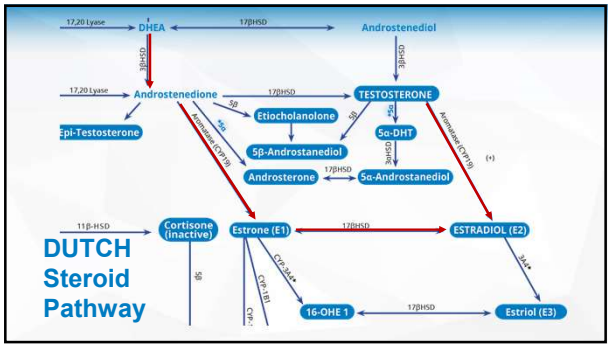
12


DUTCH
Steroid
Pathway

13


DHEA
Androstenediol
Androstenedione
TESTOSTERONE
Epi-Testosterone
Etiocholanolone
5β-Androstanediol
5α-DHT
Androsterone
5α-Androstanediol
Cortisone (inactive)
Estrone (E1)
ESTRADIOL (E2)
16-OHE 1
Estriol (E3)
DUTCH
Steroid
Pathway

14


DHEA
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Cortisone (inactive)
Estrone (E1)
ESTRADIOL (E2)
16-OHE 1
Estriol (E3)
DUTCH
Steroid
Pathway

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Monitoring TRT

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Oral Progesterone	Patch, Pellet, Injection	Transdermal Estrogen	Transdermal Testosterone	Transdermal Progesterone	Vaginal or Anal Mucosa	Oral Estrogen	Sublingual
✓ DUTCH	✓ DUTCH	✓ DUTCH	✓ DUTCH	✗ DUTCH	✓ DUTCH (E/T)	✗ DUTCH	✗ DUTCH
The DUTCH test provides useful feedback when using oral progesterone to aid sleep disturbance related to menopause. 5a (more active) and 5b (less active) metabolites are measured to individualize doses of oral progesterone. Much of the clinical impact is from the effects of the 5a-metabolites.	Values increase intuitively with dosing. For estrogen patches, see Transdermal Estrogen comments. Pellets and injections also increase levels intuitively, but the increase may exceed what is seen in serum testing. DUTCH allows for monitoring both the proper dosing of hormones as well as metabolic patterns.	Target values between the top of the postmenopausal range and the lower third of the premenopausal range correlate with patient clinical improvement (bone density, hot flash relief, etc.). Doses that push levels to the middle of the premenopausal range and beyond may be excessive. DUTCH is preferred over serum due to the inclusion of metabolites.	Levels generally parallel measurable clinical outcomes (increased lean body mass, decreased LH values in men). Epi-testosterone values can also be used to assess gonadal suppression due to TRT (levels decrease as TRT increases and are <10 ng/mg with complete suppression).	Creams and gels cannot be effectively monitored with any lab testing. Values increase only slightly with dosing. Because of the uncertainty of tissue levels, take caution to use concurrently with estrogen therapy without endometrium surveillance (ultrasound or biopsy).	Special method removes potential contamination and monitoring is helpful with most hormones. Very low doses may impact local tissue without increasing lab values. ✗ DUTCH (Pg) Progesterone is measured indirectly in urine by measuring pregnanediol. This metabolite is underrepresented when taken vaginally.	Cannot be used to effectively monitor dosing due to 1st-pass metabolism. Most of the hormone in urine has not been in circulation as "free" hormone. While dosing is not effectively monitored with DUTCH, metabolite patterns can be effectively assessed.	Lab testing is not effective. DUTCH is confounded by the hormone that is swallowed. While dosing is not effectively monitored with DUTCH, metabolite patterns can be effectively assessed.
✗ SERUM	✓ SERUM	✓ SERUM	✓ SERUM	✗ SERUM	✓ SERUM	✓ SERUM	✗ SERUM
Results go up-and-down quickly. If taken at bedtime, levels return to baseline within a few hours. Results can also be inaccurate due to progesterone metabolites cross-reacting with immunoassay tests.	Serum testing is well suited for use with these types of therapies. Results increase with increased dosing in a fairly linear fashion.	Effective for monitoring estrogen creams and gels similarly to patches. Levels may have an up-and-down pattern throughout the day, unlike when using patches.	Results correlate to clinical symptoms. In men, lean body mass increases only when serum (and likely urine) results increase.	Values do not increase significantly with dosing.	While serum levels likely represent systemic uptake of hormone, interpret with care as you may not know if your value represents a peak or a trough.	Serum testing offers the best feedback on monitoring the actual dose of oral estradiol.	Serum testing is not effective. Results rise and fall too rapidly for useful testing. In many cases, results are back to baseline within a few hours.

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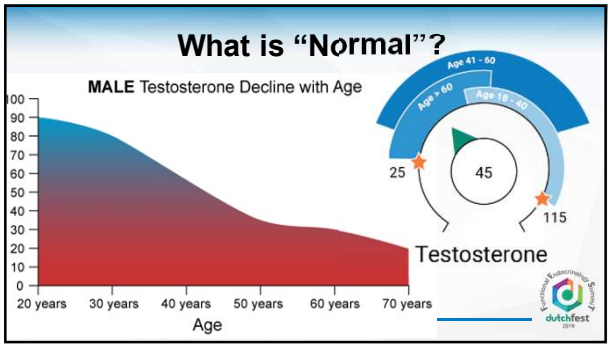

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First, let's start with a good baseline

- Serum Total with SHBG is a must
- Why not just use urine?
  - Urine-serum correlation is great for E2/Pg
    - We consider DUTCH as a great primary tool for HRT in women
  - Urine-serum correlation is not as good for T
    - We consider DUTCH a complimentary tool for men

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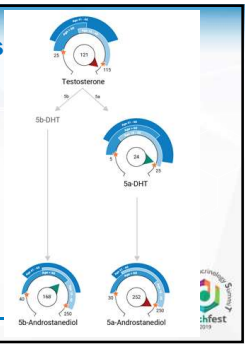
Useful Urine Meas

- Testosterone and metabolites



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Useful Urine Measurements

- Testosterone and metabolites
- Estrone (E1) and Estradiol (E2)
  - Phase I Metabolites (2-OH, 4-OH, 16-OH)
  - Methylation

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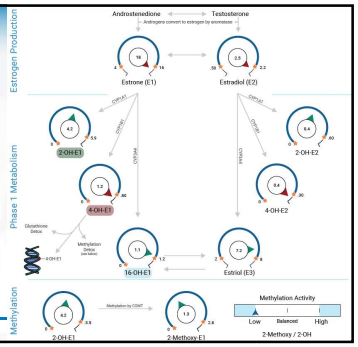
TRT Case Study

- Too much E1/E2?
- Phase I
- Methylation



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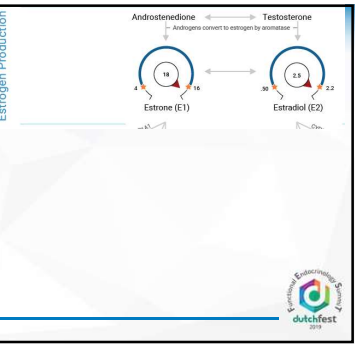
TRT Case Study

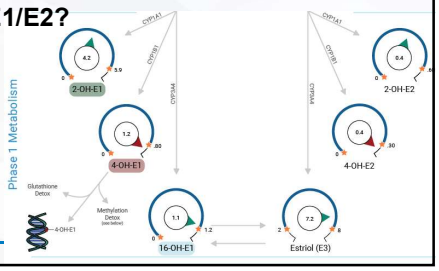
- Too much E1/E2?
- Phase I
- Methylation



23

TRT Case Study

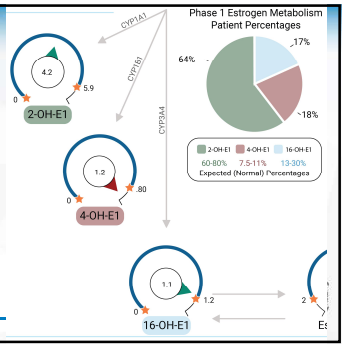
- Too much E1/E2?
- Phase I
- Methylation



24

TRT Case Study

- Too much E1/E2?
- Phase I
- Methylation



25

TRT Case Study

- Too much E1/E2?
- Phase I
- Methylation
  >2:1 ratio

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Useful Urine Measurements

- Testosterone and metabolites
- Estrone (E1) and Estradiol (E2)
  - Phase I Metabolites (2-OH, 4-OH, 16-OH)
  - Methylation
- Epitestosterone

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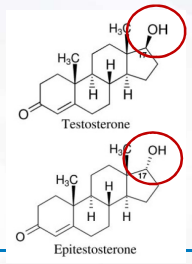
Epitestosterone, A Useful Tool



- EpiT is also made by the testes
- EpiT is NOT androgenic
- Made in similar concentrations

- A marker of approximate testicular androgen production

A. T. Kicman et al.

© 1999 Blackwell Science Ltd. *Clinical Endocrinology*, **50**, 661–668

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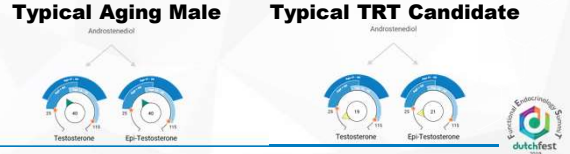
Epitestosterone with TRT

- Before TRT, EpiT is similar to T (usually)
- With TRT, EpiT will go down to the extent that LH and gonadal androgen production is suppressed

Typical Aging Male | **Typical TRT Candidate**



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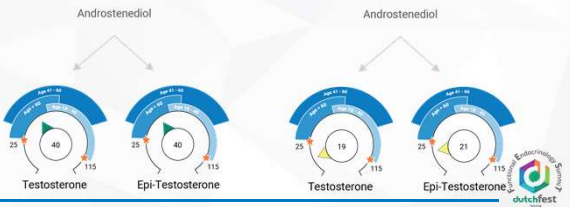
Epitestosterone with TRT

Typical Aging Male | **Typical TRT Candidate**



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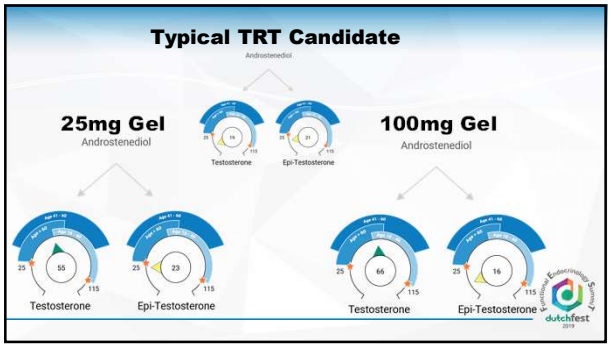
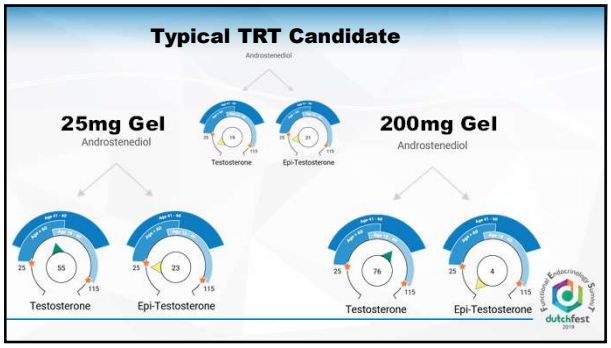
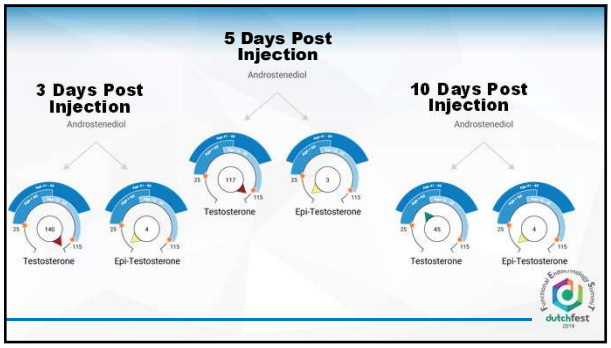

Typical TRT Candidate
Androstenediol
25mg Gel
Androstenediol
Testosterone
Epi-Testosterone
100mg Gel
Androstenediol
Testosterone
Epi-Testosterone
Testosterone
Epi-Testosterone
55
23
66
16
dutchfest

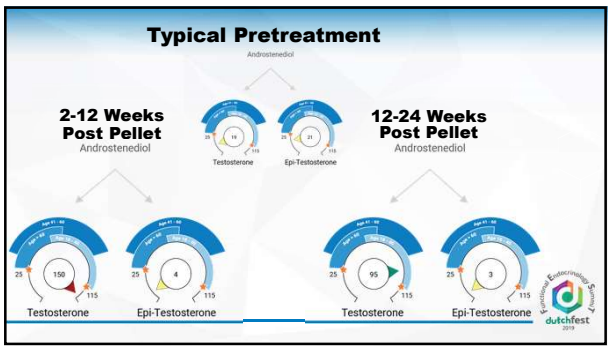
31


Typical TRT Candidate
Androstenediol
25mg Gel
Androstenediol
Testosterone
Epi-Testosterone
200mg Gel
Androstenediol
Testosterone
Epi-Testosterone
Testosterone
Epi-Testosterone
55
23
76
4
dutchfest

32


5 Days Post Injection
Androstenediol
3 Days Post Injection
Androstenediol
Testosterone
Epi-Testosterone
10 Days Post Injection
Androstenediol
Testosterone
Epi-Testosterone
Testosterone
Epi-Testosterone
140
4
117
3
45
4
dutchfest

33



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Useful Urine Measurements

- Testosterone and metabolites
- Estrone (E1) and Estradiol (E2)
  - Phase I Metabolites (2-OH, 4-OH, 16-OH)
  - Methylation
- Epitestosterone
- HPA Axis, OATs, 8-OHdG, Melatonin

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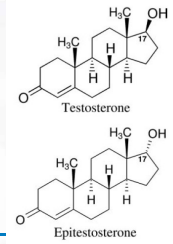
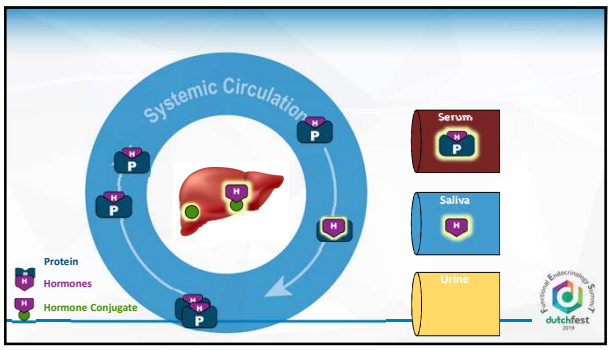
Epitestosterone, A Useful Tool



- EpiT is also made by the testes
- EpiT is NOT androgenic
- Made in similar concentrations

- Exists in urine as a conjugate
  - Epitestosterone-glucuronide

36


Systemic Circulation
Serum
Saliva
Urine
Protein
Hormones
Hormone Conjugate

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Urine Test = Testosterone-Conjugate
Protein-bound (SHBG, Albumin)
Free (unbound) Hormone
Hormone Conjugate (water-soluble)
Serum
Saliva
Urine

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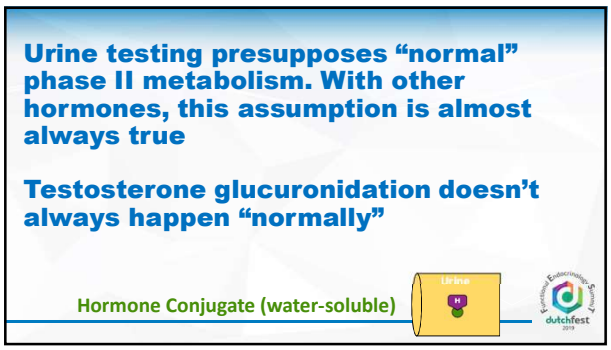
Urine testing presupposes “normal” phase II metabolism. With other hormones, this assumption is almost always true
Testosterone glucuronidation doesn’t always happen “normally”
Hormone Conjugate (water-soluble)
Urine

39

Urine t
phase
hormo
always
Testos
always
al”
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Horr

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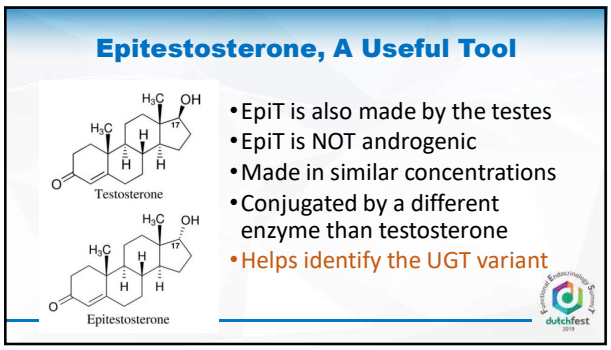
Epitestosterone, A Useful Tool
Testosterone
Epitestosterone
•EpiT is also made by the testes
•EpiT is NOT androgenic
•Made in similar concentrations
•Conjugated by a different enzyme than testosterone
•Helps identify the UGT variant

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This guy has low T!
I can tell by his urine T
Low T

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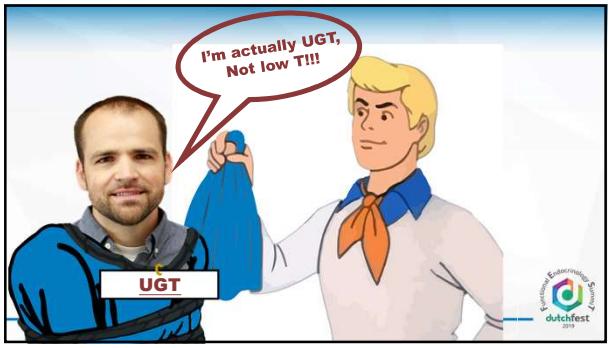

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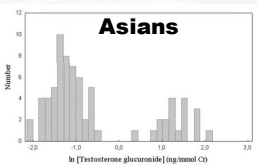
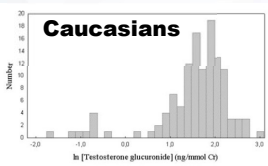
Urine Testing Assumes Proper Conjugation





The Journal of Clinical Endocrinology & Metabolism 91(2):697–701
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Large Differences in Testosterone Excretion in Korean and Swedish Men Are Strongly Associated with a UDP-Glucuronosyl Transferase 2B17 Polymorphism

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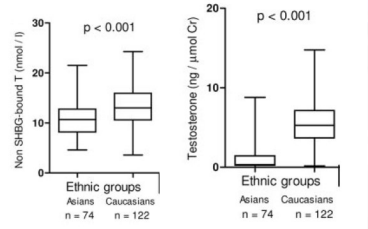
Urine Testing Assumes Proper Conjugation



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UGT Defect – Who has it?

- >60% of people of Asian descent
- <10% of other ethnicities
- Caused by a gene deletion
- No known physiological consequence

The Journal of Clinical Endocrinology & Metabolism 91(2):687–693
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Large Differences in Testosterone Excretion in Korean and Swedish Men Are Strongly Associated with a UDP-Glucuronosyl Transferase 2B17 Polymorphism

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UGT Defect – What does it look like?

- Testosterone (only in urine) is falsely low
- EpiT is "right"
- DHT and 5b-androstanediol are also falsely low
- 5a-androstanediol is "right"

- Why? T, DHT, 5b-Androstanediol are conjugated by the same enzyme (different for epiT)

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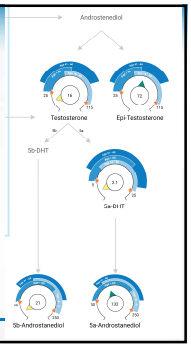
UGT Defect – What does it look like?



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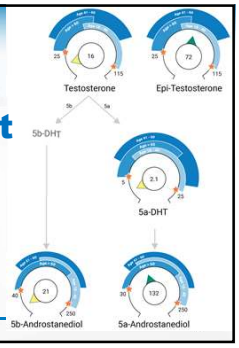
UGT Defect – What does it look like?



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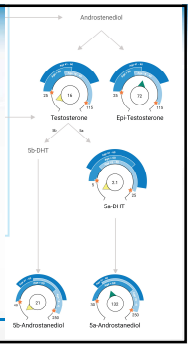
UGT Defect – What does it look like?

50

UGT Defect – What does it look like?



51

Epitestosterone Uses

- Confirming gonadal production for urine T
  - If T is low and EpiT is normal, be sure to test serum T
  - Never trust urine T alone in Asian patients (>60% UGT)
- TRT application
  - When on TRT, testosterone serum and urine levels increase, but how do we know gonadal contribution?
  - **Epitestosterone gives an approximation of gonadal testosterone production/suppression with TRT**

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Common Modes of Delivery for TRT

- Transdermal Cream
- Transdermal Gel
- Injection
- Pellet

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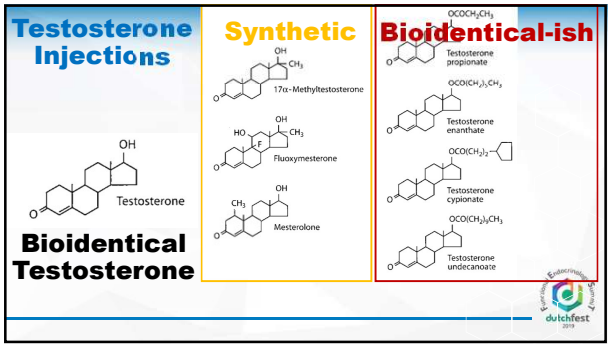
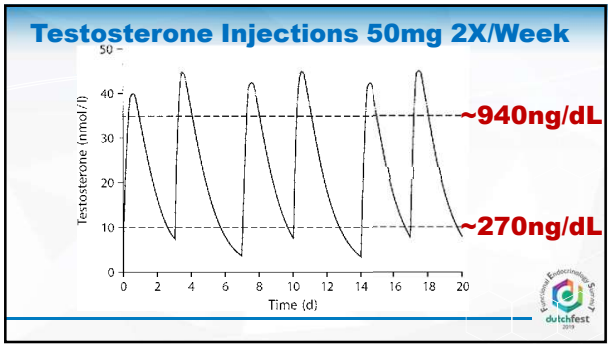
Testosterone Injections, Pellets

- Cypionate (8-day ½ life)
- Enanthate (10.5-day ½ life)
- Propionate (4-5-day ½ life)
- Undeconoate (long lasting)
- Pellets (3-6 months)

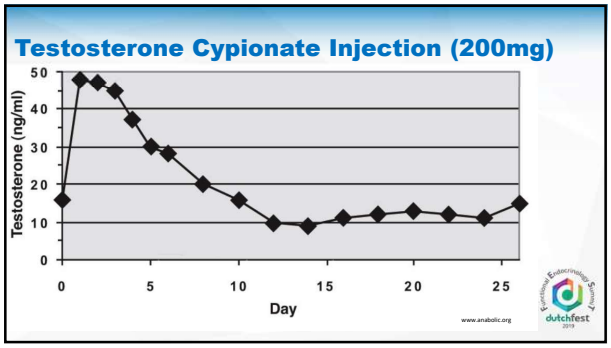
54


Testosterone Injections
Synthetic
Bioidentical-ish
Testosterone
Bioidentical Testosterone
17α-Methyltestosterone
Fluoxymesterone
Mesterolone
Testosterone propionate
Testosterone enanthate
Testosterone cypionate
Testosterone undecanoate

55

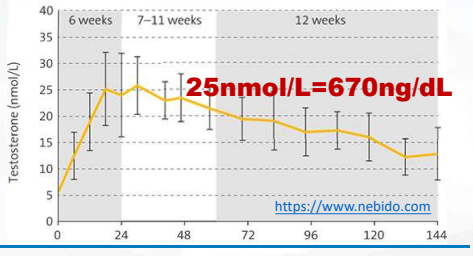

Testosterone Injections 50mg 2X/Week
Testosterone (nmol/l)
Time (d)
~940ng/dL
~270ng/dL

56


Testosterone Cypionate Injection (200mg)
Testosterone (ng/ml)
Day
www.anabolic.org

57

Testosterone Undecanoate Injection



25nmol/L=670ng/dL

https://www.nebido.com

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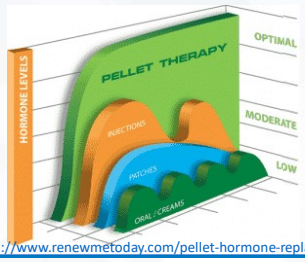
Testosterone Pellets



2000mg Doses? No Thanks!

Injections get as high as >1000ng/dL.

How high are these pellet serum levels?

https://www.renewmetoday.com/pellet-hormone-replacement-therapy/

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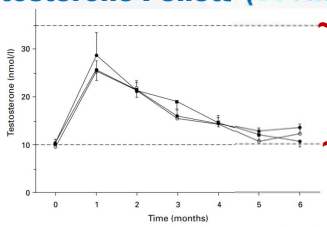
Testosterone Pellets (800mg)



~940ng/dL

~270ng/dL

Fig. 14.15 Blood total testosterone in 43 hypogonadal men receiving four 200 mg pellets (800 mg) implanted either under the skin of the lateral abdominal wall (in 4 tracks [filled circles], n = 9; or in 2 tracks [open circles], n = 16) or in the hip region (filled squares, n = 18) (adapted with permission from Kelleher *et al.* 2001, copyright 2001, Blackwell Publishing).

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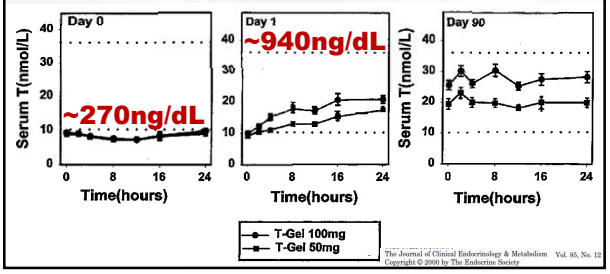
Testosterone Gel



61

How do Creams and Gels Differ?

- Saliva values are similar
- Serum values are generally higher with gels.
  - Dose of cream is generally 2X to achieve similar serum levels.

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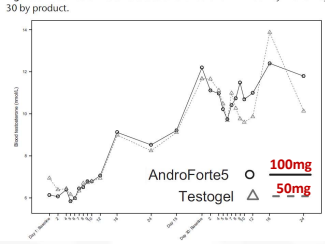
How do Creams and Gels Differ?

- Saliva
- Serum
  - Dose

gels.

ar serum levels.

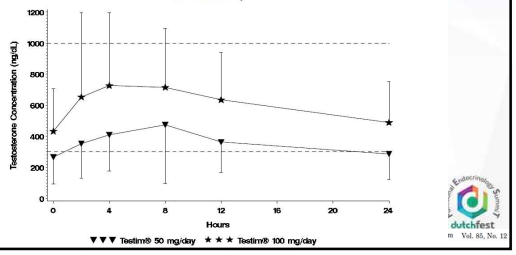
Figure 3 Mean serum total testosterone levels over 24 h at Day 1 and Day 30 by product.



63

Testosterone Gel

Mean Steady-State Serum Testosterone (±SD) (ng/dL) Concentrations on Day 30 in Patients Applying Testim® Once Daily



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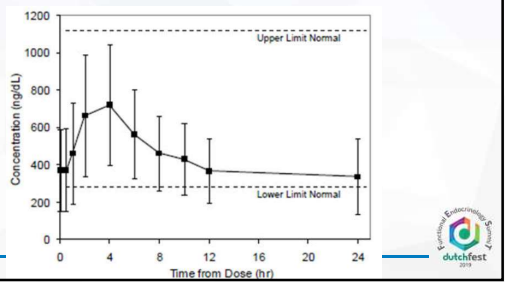
Testosterone Gel (Fortesta)



65

Testosterone Patch

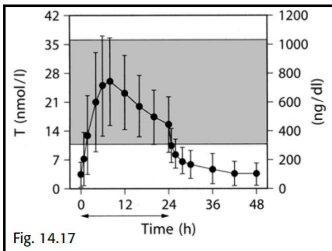


Fig. 14.17
Serum concentrations (mean ± SD) of testosterone during and after nighttime application of two non-scrotal testosterone systems to the backs of 34 hypogonadal men. Shaded area indicates normal range of testosterone (adapted with permission from Meikle *et al.* 1996, copyright 1996, The Endocrine Society).

Pharmacology of testosterone preparations
H.M. Behre, C. Wang, D.J. Handelsman and E. Nieschlag

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Extra DHT with TD TRT

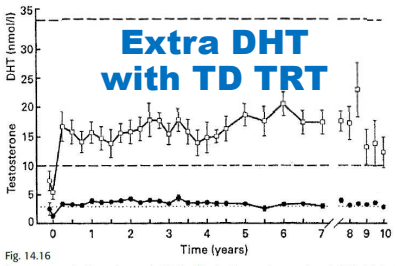

Fig. 14.16
Serum concentrations (mean ± SEM) of testosterone (squares) and DHT (circles) in 11 hypogonadal men before and during treatment with transscrotal testosterone patches. Broken lines indicate normal range of testosterone, dotted line upper normal limit of DHT (adapted with permission from Behre *et al.* 1999b, copyright 1999, Blackwell Publi

Pharmacology of testosterone preparations
H.M. Behre, C. Wang, D.J. Handelsman and E. Nieschlag

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Common Modes of Delivery for TRT

- Transdermal TRT or injections of T-Cyp are the most common approaches
- Serum T levels should be brought into normal levels (~300-1000ng/dL)
- Common Function Medicine approach is ~500-900ng/dL **mid-dosing**
- Lower target values should be used if testing just before the next dose

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Labs for Monitoring TRT

- Serum T, SHBG
- Estradiol, DHT (ADG may be better)
- PSA
- CBC with Differential
- ~~Prolactin, Ferritin~~ (not testing if normal before TRT)

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DUTCH for Monitoring TRT

- Testosterone, Epitestosterone
  - Ratio is usually >3 for transdermal, higher for injections
- Testosterone metabolism
  - DHT production
- Estrogen production and metabolism
  - Phase I (watch out for 4-OH metabolism)
  - Methylation (phase II), especially if phase I poor
- HPA axis function, melatonin, OATs

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TRT Case Study

- **52-year-old man with low T symptoms**
- **Serum T = 272ng/dL (confirmed with repeat)**
- **E2, PSA normal**
- **Serum DHEA-S low**

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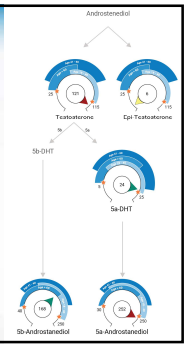
TRT Case Study

- **52-year-old man with low T symptoms**
- **50mg T-Cyp injections M/Th**
- **Serum T follow up on Wed am = 550ng/dL**
- **High 5a metabolism**
- **High E2 production**



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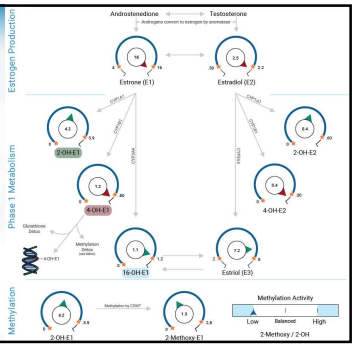
TRT Case Study

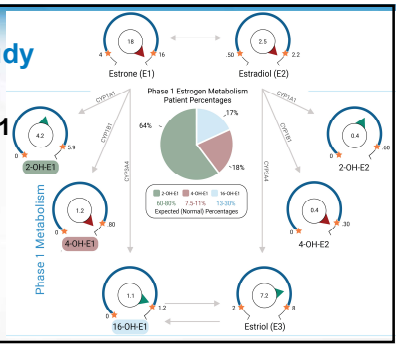
- Too much E1/E2
- Too much 4-OHE1
- Low 2-OHE1
- Methylation poor
- Pyroglutamate?
- Cortisol?



73

TRT Case Study

- Too much E1/E2
- Too much 4-OHE1
- Low 2-OHE1



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TRT Case Study

- Too much E1/E2
- Too much 4-OH
- Low 2-OHE1
- Methylation poor
- Pyroglutamate?
- Cortisol?

75

TRT Case Study

- Consider blocking 5a (PSA doing ok)
  - If considering switch to TD, be cautious
- How to drop estrogen?
  - Chrysin ??
  - Reduce inflammation
- Support phase I metabolism
- Support phase II metabolism
  - Including methylation support

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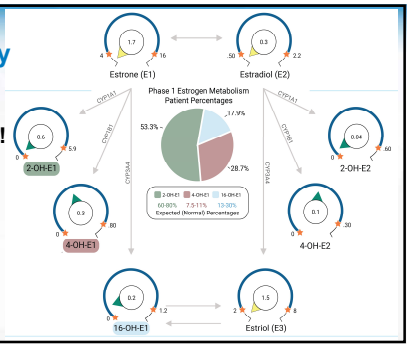
TRT Case Study

- Aromatase inhibitor at work!
- Estrogen metabolites ratios?



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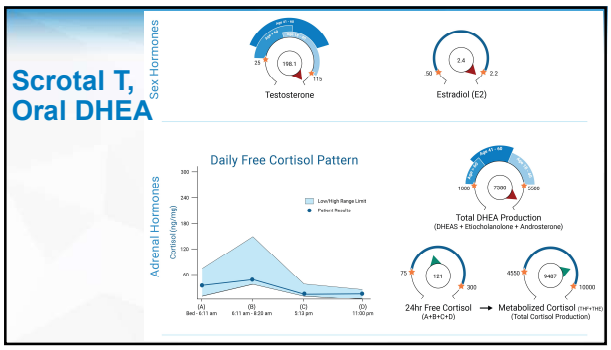
TRT Case Study

- Aromatase inhibitor at work!
- Estrogen metabolites ratios?

78


Scrotal T,
Oral DHEA
Sex Hormones
Testosterone
Estradiol (E2)
Adrenal Hormones
Daily Free Cortisol Pattern
Total DHEA Production
(DHEAS + Etiocholanolone + Androsterone)
24hr Free Cortisol
(A+B+C+D)
Metabolized Cortisol
(Total Cortisol Production)

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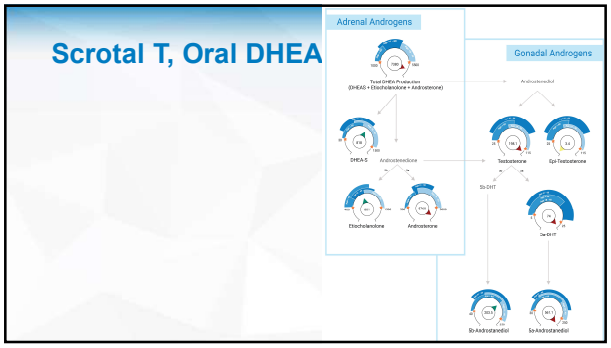
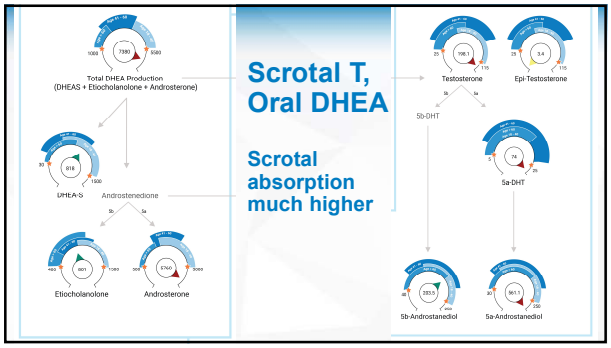

Scrotal T, Oral DHEA
Adrenal Androgens
Gonadal Androgens

80


Total DHEA Production
(DHEAS + Etiocholanolone + Androsterone)
DHEA-S
Androstenedione
Etiocholanolone
Androsterone
Scrotal T,
Oral DHEA
Scrotal absorption much higher
Testosterone
Epi-Testosterone
5b-DHT
5a-DHT
5b-Androstanediol
5a-Androstanediol

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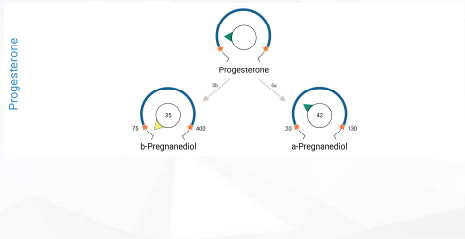
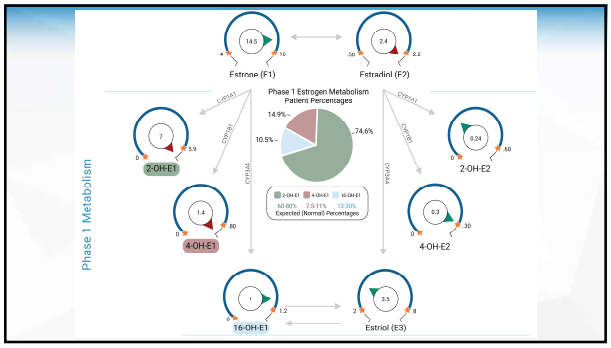
Scrotal T, Oral DHEA



82



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Scrotal T, Oral DHEA

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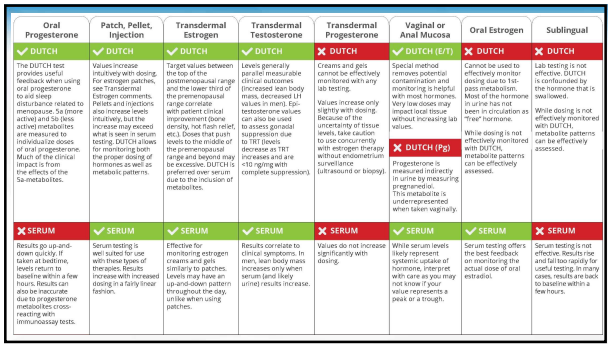

Oral Progesterone	Patch, Pellet, Injection	Transdermal Estrogen	Transdermal Testosterone	Transdermal Progesterone	Vaginal or Anal Mucosa	Oral Estrogen	Sublingual
✓ DUTCH	✓ DUTCH	✓ DUTCH	✓ DUTCH	✗ DUTCH	✓ DUTCH (E/T)	✗ DUTCH	✗ DUTCH
The DUTCH test provides useful feedback when using oral progesterone to aid sleep disturbance related to menopause. 5a (more active) and 5b (less active) metabolites are measured to individualize doses of oral progesterone. Much of the clinical impact is from the effects of the 5a-metabolites.	Values increase intuitively with dosing. For estrogen patches, see Transdermal Estrogen comments. Pellets and injections also increase levels intuitively, but the increase may exceed what is seen in serum testing. DUTCH allows for monitoring both the proper dosing of hormones as well as metabolic patterns.	Target values between the top of the postmenopausal range and the lower third of the premenopausal range correlate with patient clinical improvement (bone density, hot flash relief, etc.). Doses that push levels to the middle of the premenopausal range and beyond may be excessive. DUTCH is preferred over serum due to the inclusion of metabolites.	Levels generally parallel measurable clinical outcomes (increased lean body mass, decreased LH values in men). Epi-testosterone values can also be used to assess gonadal suppression due to TRT (levels decrease as TRT increases and are <10 ng/mg with complete suppression).	Creams and gels cannot be effectively monitored with any lab testing. Values increase only slightly with dosing. Because of the uncertainty of tissue levels, take caution to use concurrently with estrogen therapy without endometrium surveillance (ultrasound or biopsy).	Special method removes potential contamination and monitoring is helpful with most hormones. Very low doses may impact local tissue without increasing lab values. ✗ DUTCH (Pg) Progesterone is measured indirectly in urine by measuring pregnanediol. This metabolite is underrepresented when taken vaginally.	Cannot be used to effectively monitor dosing due to 1st-pass metabolism. Most of the hormone in urine has not been in circulation as "free" hormone. While dosing is not effectively monitored with DUTCH, metabolite patterns can be effectively assessed.	Lab testing is not effective. DUTCH is confounded by the hormone that is swallowed. While dosing is not effectively monitored with DUTCH, metabolite patterns can be effectively assessed.
✗ SERUM	✓ SERUM	✓ SERUM	✓ SERUM	✗ SERUM	✓ SERUM	✓ SERUM	✗ SERUM
Results go up-and-down quickly. If taken at bedtime, levels return to baseline within a few hours. Results can also be inaccurate due to progesterone metabolites cross-reacting with immunoassay tests.	Serum testing is well suited for use with these types of therapies. Results increase with increased dosing in a fairly linear fashion.	Effective for monitoring estrogen creams and gels similarly to patches. Levels may have an up-and-down pattern throughout the day, unlike when using patches.	Results correlate to clinical symptoms. In men, lean body mass increases only when serum (and likely urine) results increase.	Values do not increase significantly with dosing.	While serum levels likely represent systemic uptake of hormone, interpret with care as you may not know if your value represents a peak or a trough.	Serum testing offers the best feedback on monitoring the actual dose of oral estradiol.	Serum testing is not effective. Results rise and fall too rapidly for useful testing. In many cases, results are back to baseline within a few hours.

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Presented by
Mark Newman
mnewman@dutchtest.com for questions

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